Novel Food
Haslberger SS 2022

- Develoment in breeding and biotech
- GVOs, CRISPR
- Cloning and epigenetics
- Foods, microbiota, the I,S. and epigenetics, aging
- Functional foods, pro, pre, syn, post biotics
- Nutraceuticals
- Fermenting foods, meat
- Foods from new technologies
- Ethnic foods
- Nano in food industry
- Regulations, Health claim, functional food,
- Personalised Nutrition

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Dienstag 11.10.2022 15:15 -16:00 UZA2 Hörsaal 4 (Raum 2Z221) 2.OG 2Z221
Donnerstag 20.10.2022 16:45 -19:00 UZA2 Hörsaal 7 (Raum 2Z210) 2.OG 2Z210
Donnerstag 27.10.2022 16:45 -19:00 UZA2 Hörsaal 7 (Raum 2Z210) 2.OG 2Z210
Donnerstag 03.11.2022 16:45 -19:00 UZA2 Hörsaal 7 (Raum 2Z210) 2.OG 2Z210
Donnerstag 10.11.2022 16:45 -19:00 UZA2 Hörsaal 7 (Raum 2Z210) 2.OG 2Z210
Donnerstag 17.11.2022 16:45 -19:00 UZA2 Hörsaal 7 (Raum 2Z210) 2.OG 2Z210
Donnerstag 24.11.2022 16:45 -19:00 UZA2 Hörsaal 7 (Raum 2Z210) 2.OG 2Z210
Donnerstag 01.12.2022 16:45 -19:00 UZA2 Hörsaal 5 (Raum 2Z202) 2.OG 2Z202
Donnerstag 15.12.2022 16:45 -19:00 UZA2 Hörsaal 7 (Raum 2Z210) 2.OG 2Z210
Donnerstag 12.01.2023 16:45 -19:00 UZA2 Hörsaal 7 (Raum 2Z210) 2.OG 2Z210

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Values, beneficiariers loosers needs and technologies

ORF, 20.10.22

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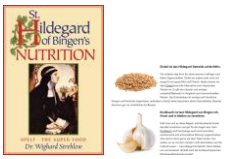
Foods between nutrition and function

Nutrition is the biochemical and physiological process by which an organism uses food to support its life.	Hippocrates, "**Let food be thy medicine, and let medicine be thy food**"



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Foods and function: foods, super food

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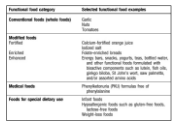
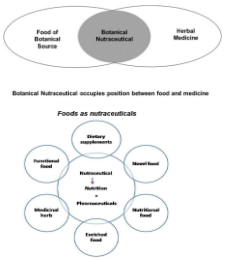
Many faces of foods





- Nahrungsergänzungsmittel
- Lebensmittel für spezielle Gruppen (Ex-*Diätetika*)
- Wasser
- Lebensmittelzusatzstoffe
- Enzyme
- Aromen
- Verarbeitungshilfsstoffe

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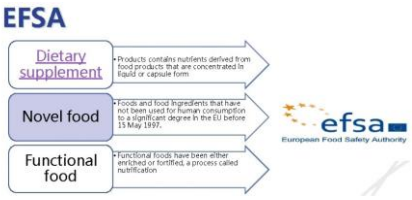
EU, EFSA: novel foods



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Novel food EU

Novel food

- Foods and food ingredients
  - with a new or intentionally modified primary molecular structure (eg, fat substitutes);
  - consisting of microorganisms, fungi or algae, or can be isolated from this (for example, microalgae oil);
  - consisting of plants or isolated (eg phytosterols), and isolated from animals food ingredients.

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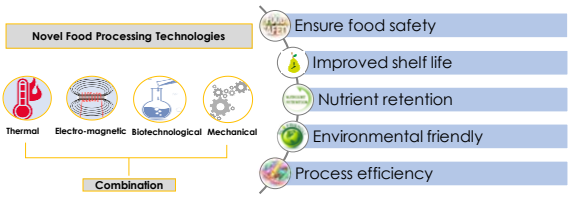
Novel food processing technologies



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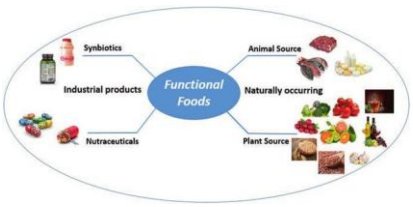
Functional foods



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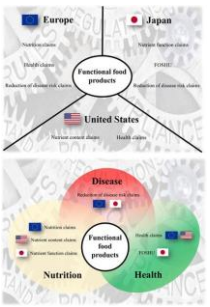
Claims: Health claims

Among the claims that can be used on food and dietary supplement labels are three categories of claims that are defined by statute and/or FDA regulations: health claims, nutrient content claims, and structure/function claims.



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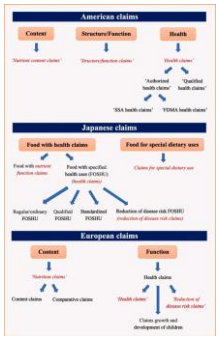

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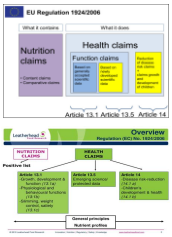
Health claim EU



Does EU health claim regulation work?
No probioticauthorizesd although Cochrane evalution positive, eg antibiotic diarrhea)

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EU examples

Art.14(1) (b)	α-linolenic acid (ALA) & linoleic acid (LA), essential fatty acids	Essential fatty acids are needed for normal growth and development of children.	Information to the consumer that the beneficial effect is obtained with a daily intake of 2 g of α-linolenic acid (ALA) and a daily intake of 10 g of linoleic acid (LA).	Q-2008-079	Commission Regulation (EC) 983/2008 of 21/10/2008. Amended by Commission Regulation (EC) 376/2010 of 03/05/2010	Authorised	N/A
Art.14(1) (b)	'Nutrimune®'	Nutrimune and immune defence against pathogens in the gastrointestinal and upper respiratory tracts	Non-compliance with the Regulation because on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	Q-2018-00727	Commission Regulation (EU) 2022/709 of 06/05/2022	Non-authorised	N/A
Art.13(1)	(Cow's) Milk And dairy products for which milk is the principle ingredient and no sugar has been added – e.g. yogurt, cheese.	(Cow's) Milk products help support dental health. (Cow's) Milk helps support the normal and healthy development of teeth. (Cow's) Milk contributes to dental health.	Non-compliance with the Regulation because on the basis of the scientific evidence assessed, this food is not sufficiently characterised for a scientific assessment of this claimed effect and the claim could not therefore be substantiated.	not validated	2011:9(6):2243	Non-authorised	1141
Art.13(1)	16. *Lactobacillus rhamnosus* LGG®	-helps improve oral health - helps maintain a healthy oral flora -helps maintain oral health -helps improve dental health	Non-compliance with the Regulation because on the basis of the scientific evidence assessed, this claimed effect for this food has not been substantiated.	maintenance of tooth mineralisation	2011:9(6):2233	Non-authorised	3018

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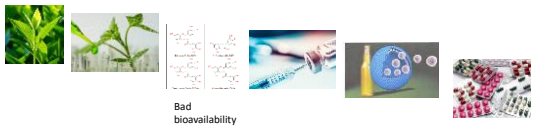

Bad bioavailability

plant	Extract How:could be novel fod	Nutraceutical Additive Novel food	Dosis Tagesdosis Novel food Medicinal	Nanno emulsion. Liposomes ?	Arzneimittel?

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zB Spermidin

Die vielversprechenden Studienergebnisse zu Spermidin haben großes Interesse an entsprechenden Supplementen geweckt. Bestimmt wird die Qualität von Spermidinpräparaten durch Ausgangsmaterial, wobei gekeimter Weizen herausragt,

Für die Einnahme von Spermidin als Nahrungsergänzungsmittel wird in der EU eine Obergrenze von 6 mg pro Tag empfohlen. (34)
Die in Humanstudien verwendete Dosierung von 1,2 bzw. 0,9 mg Spermidin pro Tag entspricht etwa 10 Prozent der alimentären Aufnahme in Industrieländern. (22) Bei einem Körpergewicht von 70 kg entsprechen 1,2 bzw. 0,9 mg pro Tag etwa 0,017 bzw. 0,013 mg/kg KG.
Die Autoren vermuten, dass die Dosis von 0,9 mg pro Tag nicht ausreicht, um Effekte auf kognitiver Ebene zu erzielen. Unter www.clinicaltrials.gov sind derzeit mehrere Studien in der Vorbereitungsphase registriert, in denen auch höhere Dosierungen von Spermidin bis zu 6 mg pro Tag eingesetzt werden.

Discussion: typical maximal daily intake doses (which can be consumed each day) are mostly not sufficient to show health effects of additves (resveratrol, EGCG..) higher daily dosis for a limited time

Stricter authorization (novel food , medicinal food, drug) needs more time, mones,.. Andrsults in monoplitation of big pharm industries (who have to aim for marketimg scuccess primarely)

Now plant extracts (old, no novel food) are mices with eg Zink (with long time ago accepted immune claim) ;-(

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Plant ingredients, additives

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Biotechnology and Agriculture, development

Plant Selection

- Agriculture begins with the collection and planting of seeds from wild plants
- Occurs in 8 locations throughout the world between 7000 -12000 years ago
- Selections were made based on yield, seed size, and taste

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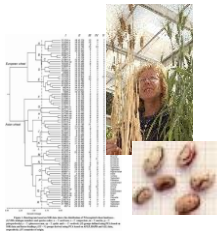
Landraces, Diversity

Refers to the particular kinds of old seed strains and varieties that are farmer-selected in areas where local subsistence agriculture has long prevailed. Landraces are highly adapted to specific locales or groups.

Definition :
modified by native and also immigrant farmers.

The term is usually applied to varieties of corn, squash, and beans that were domesticated by native farmers,



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GREEN Revolution

Term coined by U.S. Agency 1968)
Movement to increase yields by using:
. New crop cultivars
. Irrigation
. Fertilizers
. Pesticides
. Mechanization
A planned international effort funded by: Rockefeller Foundation
Ford Foundation
Many developing country governments
Purposed to eliminated hunger by improving crop performance Norman Borlaug (1970 Nobel price)

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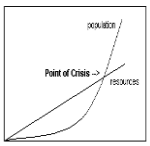
T. Malthus: 1766- 1834
Crisis in food production



Malthus' Basic Theory

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Models for population growth and food security:

Pessimistic or Alarmist Theory

Malthus - 19th century, Coale & Hoover (1958),
Paul Ehrlich (Population Bomb),
Meadows (Limits to Growth) – 1960s and 1970s.
Focus on population policy & fixed, non-renewable resources.

Optimistic Theory
Ester Boserup – 1960s – 70s (agric. Intensification)
Julian Simon – 1970s - 80s (human capital)

Neutralist or Revisionist Theory

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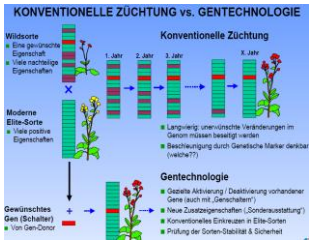
Pflanzenzüchtung
Breeding, yield, time for development



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Klassische Züchtungsmethoden

Auslesezüchtung/Selektionszüchtung
Die Auslesezüchtung fängt mit dem Anbau von Genotypengemischen (vorh. genetische Linien, auch Wildpflanzen) an. Aus dem nach gemeinsamer Abblüte erzeugten Saatgut werden Pflanzen mit vorteilhaften Eigenschaften ausgewählt (Zuchtwahl, Massenauslese).
Kombinationszüchtung
Die Kombinationszüchtung ist eine Kreuzung verschiedener Genotypen (Linien). Es entsteht ein neuer Genotyp

Heterosiszüchtung
In der Heterosiszüchtung werden bei Fremdbefruchtern (Mais, Roggen…) in mehrjähriger Züchtung aus heterozygoten Ausgangspflanzen nahezu homozygote Inzuchtlinien gezüchtet. Kreuzt man zwei solche Linien, tritt bei der F1 Generation oft eine auffallende Mehrleistung gegenüber der Elternformen auf. Dies nennt man „Heterosis-Effekt

Hybridzüchtung
Die Hybridzüchtung ist ein Beispiel für Heterosiszüchtung, zur Erzielung einer hohen markt- oder betriebsgerechten pflanzlichen Produktion durch Bastardwüchsigkeit. So werden bei der Hybridzüchtung geeignete, gesondert gezüchtete Inzuchtlinien einmalig miteinander gekreuzt (Einfachhybride). ⇒ Die Nachkommen der ersten Generation (F1) einer solchen Kreuzung haben gegenüber der Elterngeneration ein üppigeres Wachstum (Heterosiseffekt
Für den Landwirt bedeutet dies jedoch, dass das Saatgut jedes Jahr wieder neu bezogen werden muss, wenn er den Ertragsvorteil gegenüber Nicht-Hybriden weiterhin erhalten will, da der Heterosiseffekt nur in der F1-Generation auftritt und danach wieder verloren geht.

Mutationszüchtung
Bei der Mutationszüchtung werden Samen Röntgen- oder Neutronenstrahlen, Kälte- und Wärmeschocks oder anderen Mutagenen ausgesetzt⇒, um neue Eigenschaften durch Mutation zu erzielen, die einen positiven Effekt aufweisen. Damit wird die Züchtung neuer Sorten erheblich beschleunigt.

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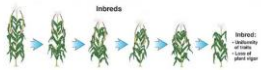
Hybridzüchtung, Heterosis

- Three Main Principles
  - Inbreeding
  - Hybridization
  - Heterosis
    - Main Goals
      - Increase the homozygosity at all or specific loci in the plant genome
      - Produce a plant which breeds true
      - Produce uniform plants



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Hybrid: Heterosis effect

The purpose of crossing is to make use of the heterosis effect partly to improve fertility and partly to combine the different characteristics for which the lines were previously selected. For meat production a desirable quality in the final product is to produce large numbers of rapidly growing individuals. This requires good fertility in the mother combined with good growth rate in the progeny.

The heterosis effect makes the hybrid pigs better than the average of the parents. The traits with the lower heritability show the largest heterosis effect. This is particularly true for fertility, mothering abilities and body structure, which have a low heritability.

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Introducing new traits in a plant family:(Random) Mutation Breeding

Examples of plants that were produced via mutation breeding are given in the table below.

Crop	Cultivar Name	Method Used to Induce Mutation
rice	Calrose 76	gamma rays
wheat	Abbott	sodium azide
	Lewis	thermal neutrons
oats	Avamo-X	X-rays
grapefruit	Rio Red	thermal neutrons
	Star Ruby	thermal neutrons
bumuda grass	Tifgaze	gamma rays
	Tifgreen II	gamma rays
	Tift 94	gamma rays
	Tifway II	gamma rays
lettuce	Ice Cube	ethyl methanesulphonate
	Mini-Green	ethyl methanesulphonate
common bean	Seafarer	X-rays
	Seaway	X-rays
rice	Pyano Petite	thermal neutrons
St. Augustine grass	TXSA 8282	gamma rays
	TXSA 8293	gamma rays

Quite a few flower cultivars have been developed via mutation breeding, among them some of the cultivars of *Alstroemeria*, begonia, carnation, chrysanthemum, dahlia, and snapdragon.

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IAEA

Why Radiation Induced Mutation?

Pierre Lagoda, Head of the FAO/IAEA Plant Breeding and Genetics Section, explains why 'induced mutation breeding' is a practical, sustainable solution to the world's food crisis.

"We offer a very efficient tool to the global agricultural community to broaden the adaptability of crops in the face of climate change, rising prices, and soils that lack fertility or have other major problems," says Lagoda.

Induced mutation: half the time of traditional breeding methods. Routinely, plant breeding requires seven to 10 years of research to produce a promising new variety. A breeder looking for pest resistance, for example, might find the characteristic in a wild variety with poor quality and yield. This wild variety will be crossed with a plant that does have good quality and yield, and any offspring combining the desired traits will then be selected and propagated.

Induced mutation: more options from which breeders can choose. Hybrids, the product of crosses, are only as resilient and productive as the source parents. Over the past century, about 75% of crop biodiversity has been lost and monoculture has diminished plant variety in farmers' fields.

Both conditions limit researchers when crossing strains to create new plants. "This loss in plant genetic diversity endangers food security as resistance to yet latent biotypes of pests and diseases and extreme weather conditions may have become severely weakened," says Lagoda.

There is a solution: using radiation to artificially induce the variations that plant breeders need. Radiation-induced mutation produces millions of variants. Breeders then screen for the desired traits and crossbreed. "Induced mutation breeding is a safe and proven technology. The method does encounter resistance and the public is generally concerned by anything relating to radiation and mutation," Lagoda explains.

"In plant breeding we're not producing anything that's not produced by nature itself. There is no residual radiation left in a plant after mutation induction. Through its Technical Cooperation Programme, the IAEA provides the tool and the expertise, then national agricultural research systems and plant breeders must take the next step, selecting and cross-breeding plants to achieve the desired result," says Lagoda.

Pierre Lagoda, Head of the FAO/IAEA Plant Breeding and Genetics Section. E-mail: P.J.L.Lagoda@iaea.org

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Breeding: Irradiation

Breeding: Irradiation

Irradiator at Institute of Radiation Breeding Ibaraki-ken, JAPAN (http://www.irb.affrc.go.jp/)

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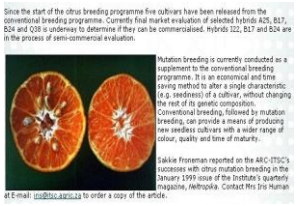
Mutation breeding



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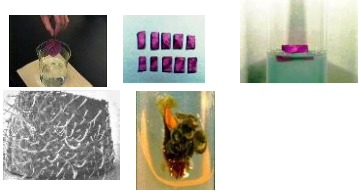
Tissue culture , Clones ?



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Somaclonal variation

- Production of a new variety of japanese butterbur using somaclonal variation.(upper:new variety, lower:native variety)

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Tomoffel

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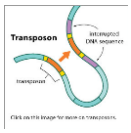
Breeding using transposons

Ein Transposon ist ein DNA-Abschnitt bestimmter Länge im Genom, der seine Position im Genom verändern kann (Transposition). Man unterscheidet Transposons, deren mobile Zwischenstufe von RNA gebildet wird (Retroelemente oder Klasse-I-Transposon), von denjenigen, deren mobile Phase DNA ist (DNA-Transposon oder Klasse-II-Transposon).



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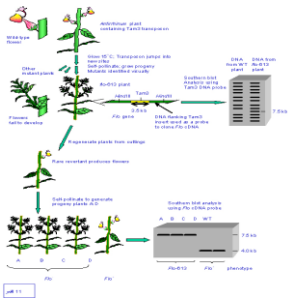

Transposon tagging

The molecular isolation of transposable elements now permits the cloning of genes in which the element resides. The major advantage of this system is that genes whose function is not known can be cloned

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Molecular marker directed breeding

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Bio-Technology ?
INTEGRATED Pest Management: A modern Way of Agriculture

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Cloning, Definition

Cloning is the process of making an identical copy of something

In biology, it collectively refers to processes used to

-- copies of DNA Fragments (molecular cloning)
-- cells (cell cloning)
-- organism

The term also covers when organisms such as bacteria, insects or plants reproduce asexually.

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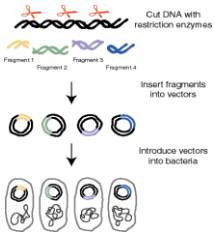
DNA cloning:



To clone a piece of DNA, DNA is cut into fragments using restriction enzymes that recognize specific sequences of bases in DNA. The fragments are pasted into vectors that have been cut by the same restriction enzyme. Vectors (e.g., plasmids or viruses) are needed to transfer and maintain DNA in a host cell.

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Reproductive Cloning

Reproductive cloning is a technology used to generate an animal that has the same nuclear DNA as another currently or previously existing animal. Dolly was created by reproductive cloning technology. In a process called "somatic cell nuclear transfer" (**SCNT**), scientists transfer genetic material from the nucleus of a donor adult cell to an egg whose nucleus has been removed. The reconstructed egg containing the DNA from a donor cell must be treated with chemicals or electric current in order to stimulate cell division. Once the cloned embryo reaches a suitable stage, it is transferred to the uterus of a female host where it continues to develop until birth.

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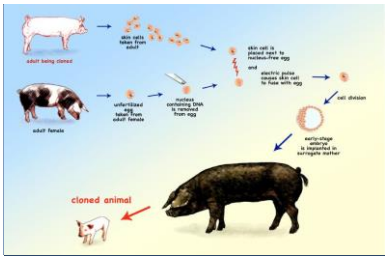
Reproductive Cloning



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Therapeutic Cloning

Therapeutic cloning, also called "embryo cloning," is the production of human embryos for use in research. The goal of this process is not to create cloned human beings, but rather to harvest stem cells that can be used to study human development and to treat disease. Stem cells are extracted from the egg after it has divided for 5 days.
The extraction process destroys the embryo, which raises a variety of ethical concerns. Many researchers hope that one day stem cells can be used to serve as replacement cells to treat heart disease, Alzheimer's, cancer, and other diseases.

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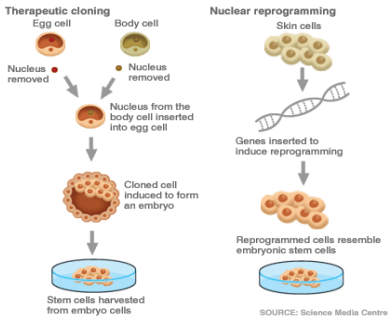

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Horticultural cloning

All plants which are originated from vegetativ reproductions are clones.
They have been derived from a single individual, multiplied by some process other than sexual reproduction.
Examples are bananas, grapes and potatoes.

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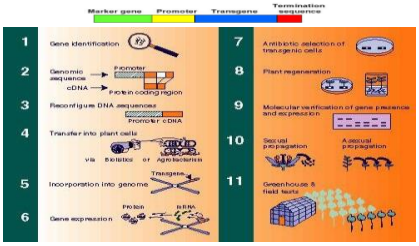
GM plants, Tranferring traits in ways which are not used in nature: GMOs



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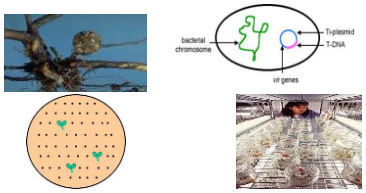
Agrobact. tumefaciens



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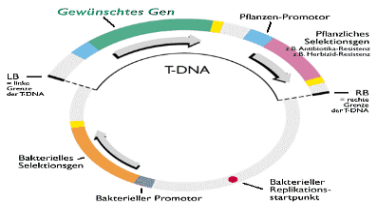
T DNA



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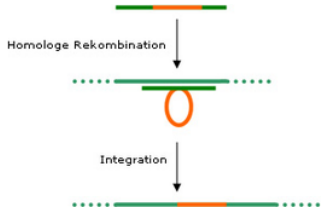
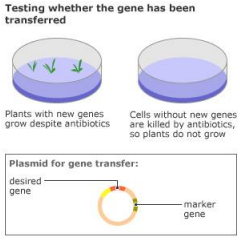
Homolog recombination



49

Antibiotic resistance marker gene



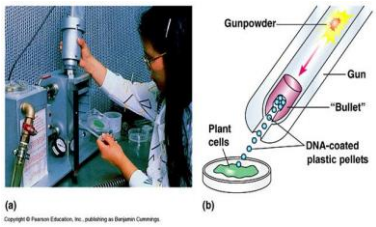
50


An assessment of the risks associated with the use of antibiotic resistance genes in genetically modified plants: report of the Working Party of the British Society for Antimicrobial Chemotherapy

http://www.gmo-compass.org/

51

Gene gun



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Methods, overview

Table 1. Genetic manipulation technologies.

Technology	Definition
Genetic manipulation	Introduction of new genetic material via laboratory methods.
Biolistics	Use of helium-, gunpowder-, or electrical-discharge-mediated force to propel DNA-coated tungsten or gold microprojectiles into cells.
Agrobacterium tumefaciens	Soilborne bacterium causing crown gall disease of fruit trees. Used by biotechnologists to transfer any DNA into plant cells, from which transgenic plants are regenerated.
Agrobacterium rhizogenes	Soilborne bacterium causing hairy root disease. Transfers bipartite piece of bacterial DNA into plant genome, inducing elevated auxin synthesis and auxin sensitivity characterized by fluffy white hairy roots.

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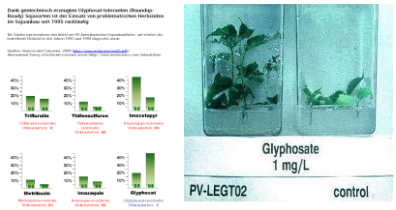
Herbicide tolerance, glyphosate



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Herbicide Resistant Soybean

BEFORE AFTER

HERBICIDE APPLICATION

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Herbicide Resistance: more or less herbicide? depending on local agricultural background

- Roundup Ready Soy, Corn, Canola
- Allows post-emergence herbicide spraying
- Increases yield
- Facilitates no-till farming
- 89% U.S. Soy crop (2006)

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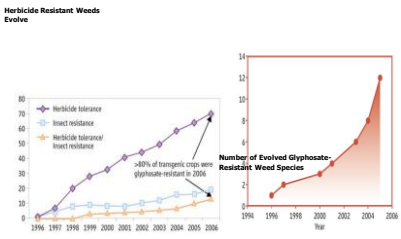
Old and new Problems: Resistance



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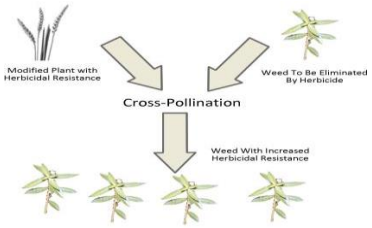
Herbizide resistance, gene transfer



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Gene flow: multiresistant Rape

Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan

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Insect resistance, BT maize

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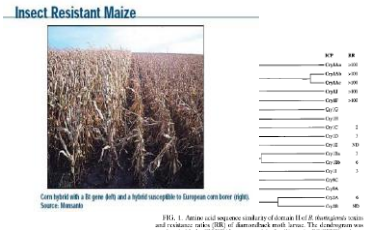
BT resistance: B. thuringiensis proteins



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Roundup ready, Monsanto

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Maiszünsler: wirtschaftlich bedeutendster Maisschädling

Es gibt mehrere Strategien zur Bekämpfung des Maiszünslers:

- **mechanisch durch Zerkleinern und Unterpflügen der auf dem Feld verbliebenen Pflanzenreste**
- **chemisch durch Einsatz von Insektiziden**
- **biologisch mit Hilfe von Trichogramma (Schlupfwespen)**
- **BT Toxin Präparate**
- **gentechnisch vermittelte Insektenresistenz besitzt (Bt-Mais)**

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Bt Corn

- Natural insecticide from *Bacillus thuringiensis*
- Non-toxic to humans
- Target insect: corn borer
- Potential to:
  - reduce insecticide use
  - reduce mycotoxins
- 40% U.S. Corn crop Bt (2006)

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Bt Concerns

- Bt pollen harms non-target species?
- Bt crops select for resistant insects
- Bt pollen can drift to organic fields
- Food system failed to keep BT Starlink corn out of human food products

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Insect Resistant Cotton

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Disease Resistance, viruses

Genetically engineered papaya resistant papaya ringspot virus

- Cantaloupes
- Cucumbers
- Corn
- Rice
- Papaya
- Potatoes
- Soybeans
- Squash
- Tomatoes
- Wheat

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Growth-enhanced fish

Salmon Growth hormone expressed in cold waters & unlinked from seasonal temp.

Auto-transgenic mud loach: β-actin promoter linked to GH gene.

(Devlin et al. 1994)

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GM Salmon

- Probleme der Lachsindustrie
- gv Lachs von Aqua Bounty
- Produktionssteigerung über Ernährung, Krankheitsresistenz

- Gefahr für die Wildlachspopulationen
- Abhängigkeit des Fischfutters
- Umweltverschmutzung durch Lachszucht

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- Atlantischer Lachs von Aqua Bounty
- **Wachstumshormon-Gen** des Chinook Lachs
- **Frostschutz-Protein-Gen**
- bessere Entwicklung in kalten kanadischen Gewässern
- Wachstum über das ganze Jahr
- normales Gewicht in der Hälfte der Zeit erreicht

BELPOND-CURIEUX, O. L et al. : Factors to consider before production and commercialization of aquatic genetically modified organisms: the case of transgenic salmon: Environmental Science & Policy 12: 170–189: 2009.

70

Golden Rice

Goldener Reis,

Unter **Goldenem Reis** (engl. *Golden Rice*) versteht man eine gentechnisch veränderte Reissorte. Es wurden zwei artfremde Gene und damit ein mehrschrittiger Syntheseweg in das Genom eingefügt. Das Phytoensynthase-Gen (*psy*) stammt von der Osterglocke (*Narcissus pseudonarcissus*) und das Carotindesaturase-Gen (*crtI*) von einem Bakterium Namens *Erwinia uredovora* (neuer Name: *Pantoea ananatis*).

Dank dieser zwei Gene kommt es zur Bildung von Beta-Carotin (Provitamin A) im Endosperm der Reiskörner, die deshalb (gold-)gelb / orange gefärbt sind. Das Provitamin wird dann im Körper zu Vitamin A (Retinol) umgewandelt.

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GMO tobacco, expression of human proteins in plants

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GMOs in development:
CLAIMED BREEDING OBJECTIVES

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CLAIMED BREEDING OBJECTIVES

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09.12.2008

Claimed breeding objectives

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BREEDING OBJECTIVES

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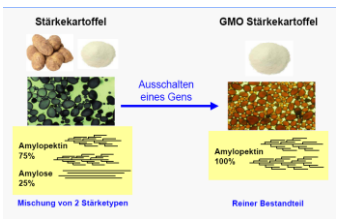
Breeding objectives



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GMO Trees

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GM FLowers

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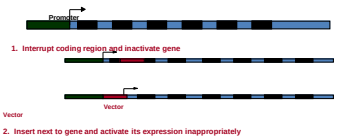
Safety: Random integration, Insertional mutagenesis



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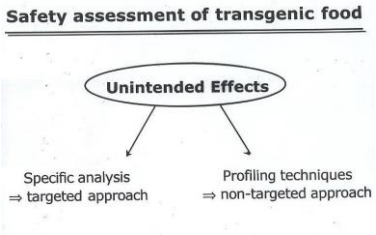

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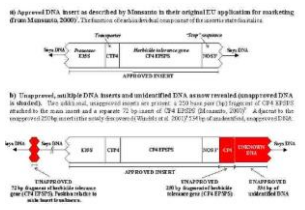
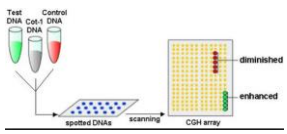


83

Detection of unintended effects in vitro, in vivo



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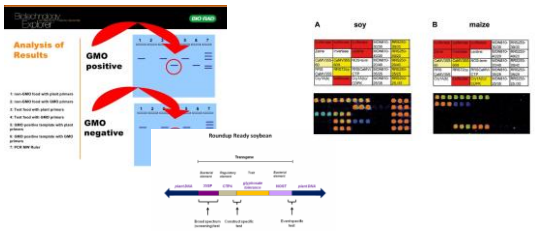
GMO tests: PCR, primers, areas, array



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To consider

Type of modification	Molecul. sequence	Epigenetic	Tox direct	Tox indirect	Environ ment,	Agric-ultural practice
Classic breeding						
Cross breeding						
Random mutation	??????????					
Cell culture, transposons	??????????					
Gene technology(bacteria, plants, animals, vaccines,...)		????????			??????	???????
Cloning, animals				???????	??????	

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Toxicology Asessment: Difficulties Animal Feeding Studies
Whole Foods

- Small doses to be fed (bulk, satiety)
- Nutritional imbalance of the diet
- Many confounding factors
- Small safety margins, if any
- Insufficient sensitivity for specific endpoints

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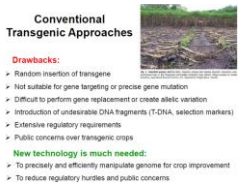
New Objectives



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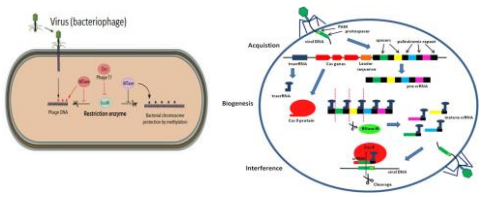
Bacterial immune system against viral DNA, restriction enzymes, Palyndrome repeats (CRISP), cleavage enzyme , CAS9



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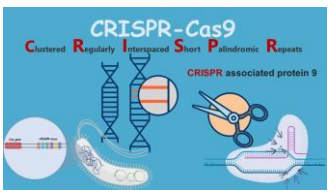

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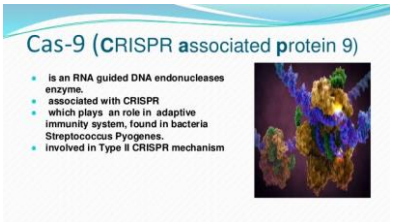

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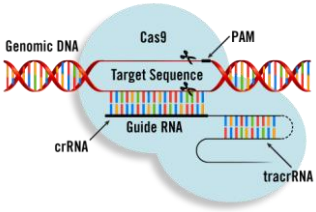

PAM CRISPR
The protospacer adjacent motif (or PAM for short) is a short DNA sequence (usually 2-6 base pairs in length) that follows the DNA region targeted for cleavage by the CRISPR system, such as CRISPR-Cas9. The PAM is required for a Cas nuclease to cut and is generally found 3-4 nucleotides downstream from the cut site.

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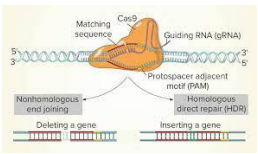
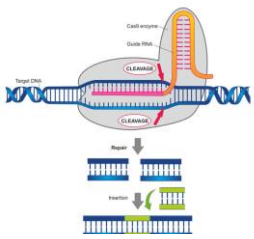


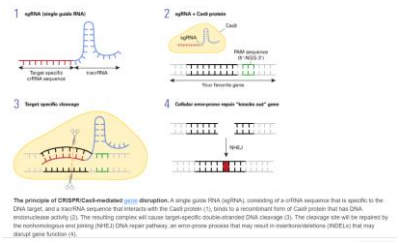
93

CRISP Cas9 Repair and insert



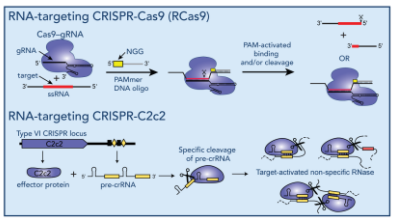
94

CRISPR/CAS9, knock out



95

Targeting RNA



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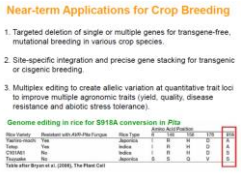
CRISPR-Cas9, applications



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CRISPR-Cas9

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GMO old and new, youtube

https://www.youtube.com/watch?v=JtkhHIG3nx4
https://www.youtube.com/watch?v=4YKFw2KZA5o

Ethics:
https://www.youtube.com/watch?v=PAhtgPDTVag

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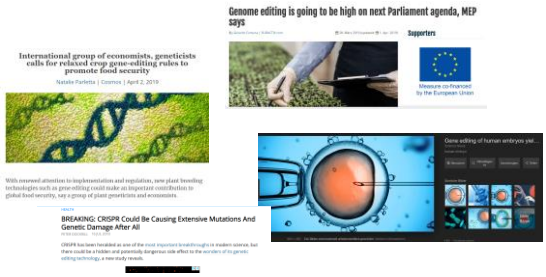

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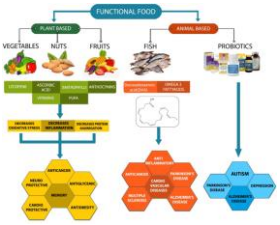
Novel food, functional food, pro, pre, syn, postbiotics



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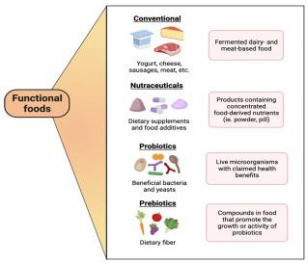
Functional foods



A functional food is a food claimed to have an additional function (often one related to health promotion or disease prevention) by adding new ingredients or more of existing ingredients.

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Fig. 10.3 Continuum between nutraceuticals and pharmaceuticals; https://www.ey.kearney.com/consumer-retail/article/7a/nutraceuticals-the-front-line-of-the-battle-for-consumer-health

Fig. 10.1 Functional foods and dietary supplements; https://www.pmdocksgroup.com/dietary-supplements-vs-functional-foods-safety-and-labeling

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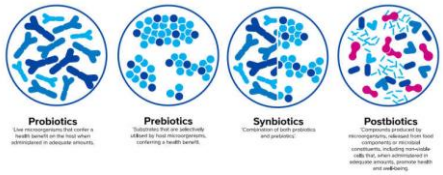
Probiotic, Prebiotic, Synbiotic, Postbiotic



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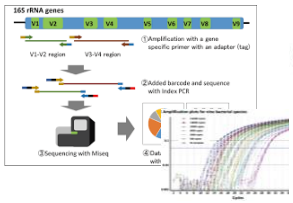
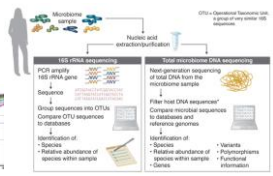
Bacteria, Cultivation : 16S $_{R}$RNA identIficatioN





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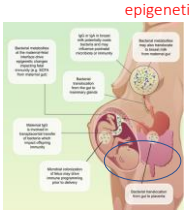
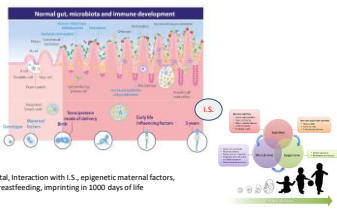
Development of microbiota, I.s., and epigenetic system, imprinting





Development prenatal, Interaction with I.S., epigenetic maternal factors, Diversity:delivery, breastfeeding, imprinting in 1000 days of life

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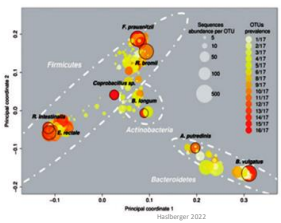
Microbiota: the role of the distribution of groups (and their functions ?)



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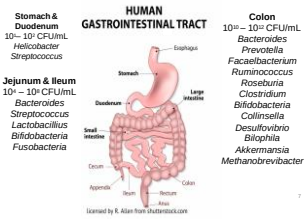
Gut microbiota



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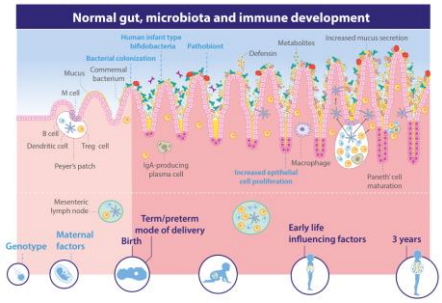

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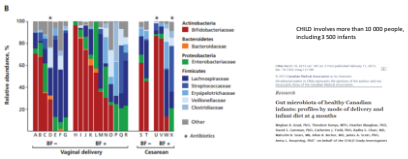
Ways of delivery and microbiota: a long lasting difference

Infants born by elective cesarean delivery had particularly low bacterial richness and diversity. formula-fed infants had increased richness of species, with overrepresentation of *Clostridium difficile*.



CHILD involves more than 10 000 people, including 3 500 infants

Research

Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months

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Core Microbiota

- *Bacteroidetes* (22,9 %)
- *Firmicutes* (64 %)
  (32 % of *C. Cluster IV*, 36 % of *C. Cluster XIVa* and 5 % of *Lactobacilli)*
  (Mariat et al., 2009)
- *Actinobacteria* (1- 4 %)
- Verrumicrobiales (1- 4 %)
- Archaeal domain (1- 2,5 %)
- Eukaryotic microorganisms (< 0,1 %)
  (Gerritsen et al., 2011)

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Microbiota Functions

- Protective functions
- Structural functions
- Metabolic functions
- Fermenting dietary fiber into
- short-chain fatty acids
- Synthesizing vitamins

- *Bacteroidetes* (22,9 %)
- *Firmicutes* (64 %)
  (32 % of *C. Cluster IV*, 36 % of *C. Cluster XIVa* and 5 % of *Lactobacilli)*
- *Actinobacteria* (1- 4 %)
- Verrumicrobiales (1- 4 %)
- Archaeal domain (1- 2,5 %)
- Eukaryotic microorganisms (< 0,1 %)
  (Gerritsen et al., 2011)

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Variation in microbiota structure is high

Despite high variation, GI microbiota depend on :

1. Individuum
2. Area and lifestyle
3. Diet
4. Interventions

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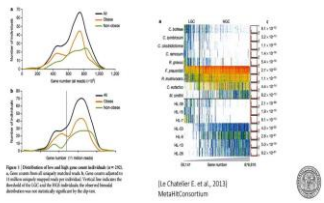
GI microbiota: Diversity of groups and functions important for health



[Le Chatelier E. et al., 2013] MetaHITconsortium

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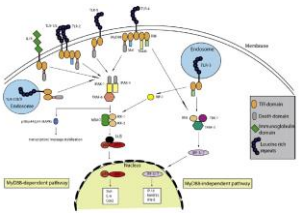
Cooperation between microbiota and the I.S.: PAMPS , TLRs, adaptor molecules



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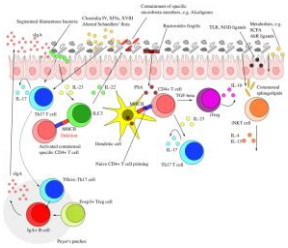
Interaction micobiota Immune system, IS



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Toll-like and NOD-like receptors

Pattern recognition receptors (PRRs)

- PRRs recognize pathogen-associated molecular patterns (PAMPs) such as flagellin, bacterial DNA and RNA Lipopolysaccharide,
- PRRs fall into three families
  - Toll-like receptors (TLRs)
  - NOD-like receptors (NLRs)
  - Retinoicacid-inducible gene I (RIG-I)-like receptors (RLRs)

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Tight junctions. Leaky gut

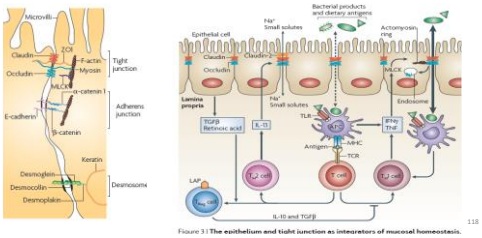

Figure 3 | **The epithelium and tight junction as integrators of mucosal homeostasis.**

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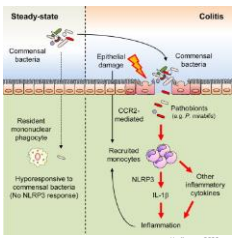
118

Damage of gut wall: Microbiota induce NRLP3 inflammasome and inflammation



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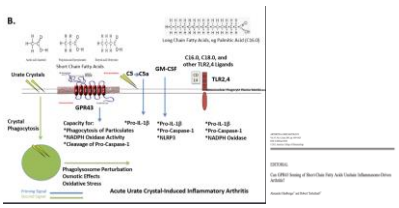
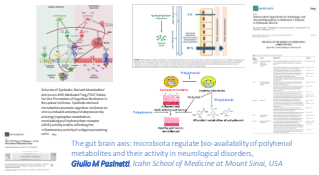
TLR2, TLR4 ligands (endotoxins, long chain fatty acid) trigger inflammation, GPR43 interfers?



120

120

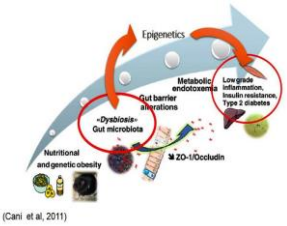
Microbial metabolites regulate treg-th17 balance, cognitive resilience, microbiota and metabolites



The gut brain axis: microbiota regulate bio-availability of polyphenol metabolites and their activity in neurological disorders,
Giulio M Pasinetti, *Icahn School of Medicine at Mount Sinai, USA*

121

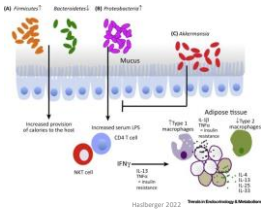
Bacterial cell wall components and Inflammation: dysbiosis, LPS and gut permeability; obesity as a model



(Cani et al, 2011)

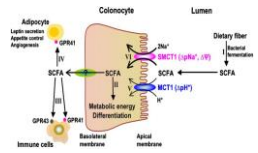
122

Obesity: Firmicutes: Bacteroidetes; Akkermansia and the cell wall



123

Microbiota metabolites: SCFAs bind to G-Protein-Receptors GPR 41/43 (FFARs)



Anti-inflammatory;
Inhibition of NFκB

(Huster et al., 2013; Flint et al., 2009, Nature Rev)

124

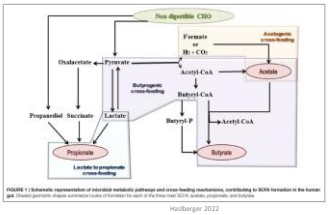
Microbiota and fermentation products e.g. SCFAs

Clostridial cluster IV (Rumminococaceae)	***Clostridial cluster XIVa (Lachnospiraceae)***
Faecalibacterium prausnitzli ***Butyricoccus*** ***Clostridium Leptum***	***Eubacterium hallii*** ***Anaerostipes coli*** ***Roseburia spp.*** ***E. rectale spp.***
Resistent starch	***Non starch Polysaccharides***

(Louis and Flint, 2009, FEMS)

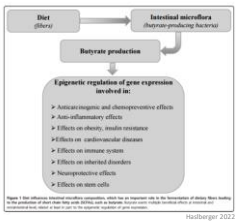
125

Pathways and cross feeding for SCFAs/ Butyrate



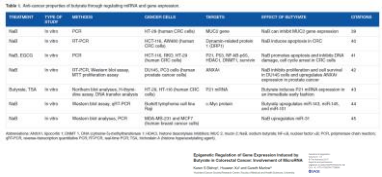
126

Butyrate and epigenetics



127

Butyrate: apoptosis, autophagy, mi- RNAs regulating inflammation, vitro



128

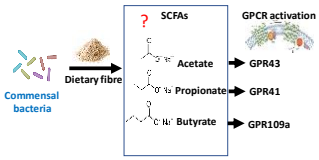
Butyrate: where and how much ?

For good reason it is not possible with current technologies to perform direct measurements of the variation in the butyrate concentration in the portal vein of human subjects, but short-chain fatty acid levels in portal blood from sudden-death victims, subjects undergoing emergency surgery or planned surgery have indicated a higher gut production and absolute and relative concentration of butyrate in non-fasted as compared with fasted human subjects

129

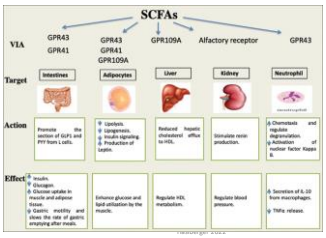
Mechanism of action of fibre: Short-chain fatty acids (SCFAs)?

- SCFAs are major metabolites produced by the microbiota



130

GPR receptors



131

GPRs and therapy, still many unclear

GPR41 AND GPR43 EXPRESSION IS TISSUE-SPECIFIC

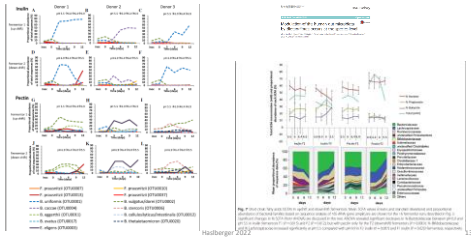
GPR41 AND GPR43 AS POTENTIAL THERAPEUTIC TARGETS FOR OBESITY, COLITIS, ASTHMA, AND ARTHRITIS

REPORTS ON GPR41 AND GPR43 KNOCKOUT MICE PHENOTYPES ARE IN CONSISTENT

Zhiwei Ang and Jeak Ling Ding Front. Imm. 2016*

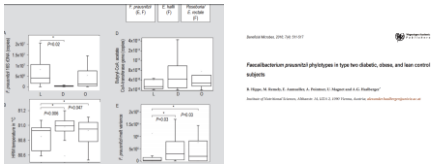
132

Microbiota and SCFA responses vary very individually



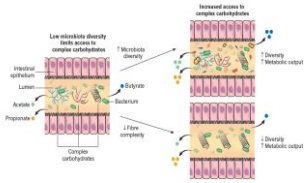
133

SCFAs producers, phylotypes differ in obese, diabetes



134

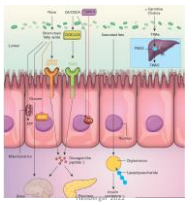
Diet dictates the production of SCFAs, diversity of the microbiota, many types of complex carbs



135

Endotoxins, saturated fats/ chylomicrons trigger inflammation, insulin resistance; SCFAs may trigger GLP1 activation

GLP1: incretin improves DMII and obesity



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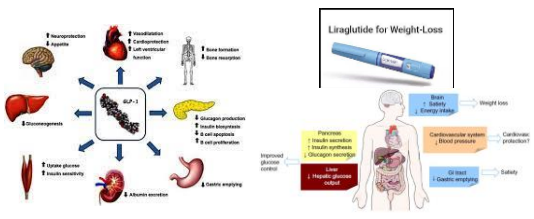
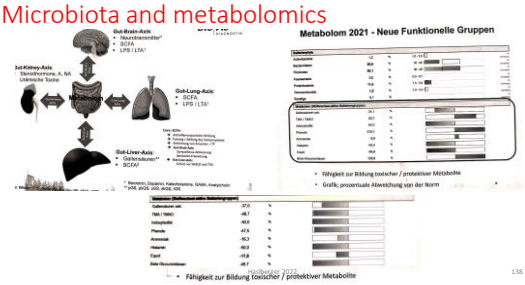
GLP1, analogs



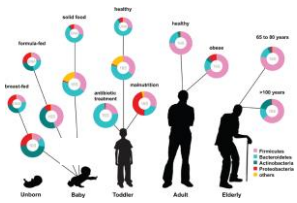
137

Microbiota and metabolomics



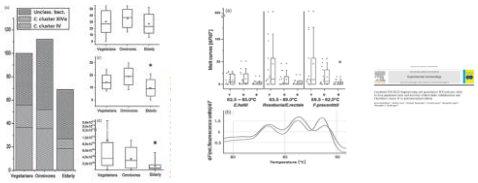
138

Aging and Microbiota



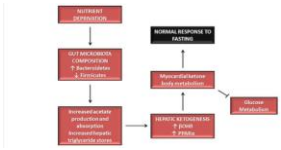
139

SCFAs in elderly:
Decrease in SCFAs producers and „Butyrate Gen producing gene“ in elderly

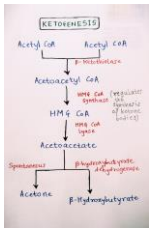


140

Microbiota regulate not only SCFAs but also Ketone bodies in caloric restriction, Beta hydroxy Butyrate , BHB



Crawford PA et al, 2009). Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. *Proceedings of the National Academy of*

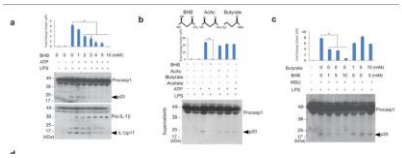


141

Ketone body β-hydroxybutyrate blocks the NLRP3 inflammasome-mediated inflammatory disease(caspase subunit)

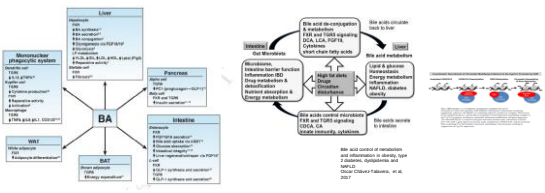
Ketogeneic diet may improve inflammation via epigenetics, but can also lead to an overload of LPS thru high SFA and low vegtable intake

Yun-Hee Youm et al. Nat med. 2015



142

Microbiota modulated Bile acids are epigenetically active and via FXR regulate inflammation



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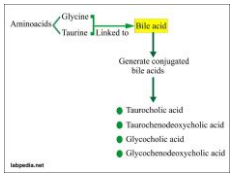
Bile acids

Primary bile acids are synthesized by the liver. Secondary bile acids result from bacterial actions in the colon. In humans, taurocholic acid and glycocholic acid (derivatives of cholic acid) and taurochenodeoxycholic acid and glycochenodeoxycholic acid (derivatives of chenodeoxycholic acid) are the major bile salts in bile and are roughly equal in concentration



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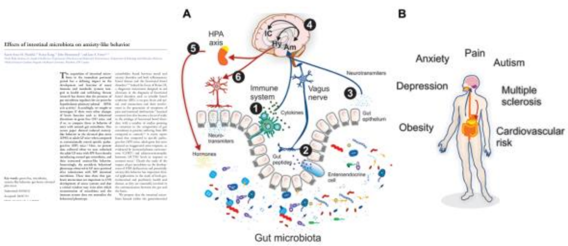
Gut Brain / immune- Axis



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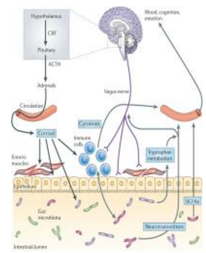
Gut-Microbiota-Brain Communication



- Bidirectional communication
  - Central nervous system (brain and spinal cord)
  - Autonomic nervous system (sympathetic and parasympathetic)
  - Enteric nervous system (intrinsic nervous system of GI tract)
  - Hypothalamic pituitary adrenal axis (HPA)
  - Microbiome (collection of microorganisms and their genomes in the gut)

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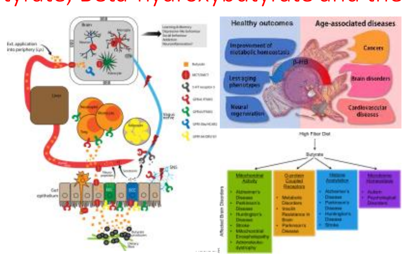
Butyrate, Beta-hydroxybutyrate and the brain



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Vagus Nerve

- Major nerve of the parasympathetic division of the autonomic nervous system
- Important pathway for bidirectional communication between the gut microbes and the brain
- Preclinical/animal studies demonstrate that probiotic effects on brain are dependent on vagal afferent signals
  - *Lactobacillus rhamnosus* directly activates vagal neurons
  - Induces region-dependent alterations in GABA receptor expression in the brain and reduced stress-induced corticosterone and anxiety- and depression-like symptoms via vagus nerve signaling in mice
- Vagotomized mice do not exhibit this effect

Bravo, Javier A., et al. Proceedings of the National Academy of Sciences 108.38 (2011): 16050-16055. 12

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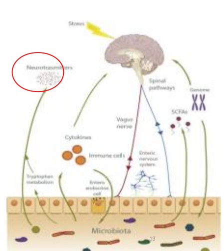
Neurotransmitter and the gut

- Acetylcholine
- Noradrenaline
- Adrenaline
- Gamma-amino butyric acid (GABA)
- Serotonin



Burokas et al., Advances in Applied Microbiology, 91 (2015): 1-62 13

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Bacteria & Neurotransmitters

Neurotransmitter	Genus
GABA	*Lactobacillus, Bifidobacterium*
Norepinephrine	*Escherichia, Bacillus, Saccharomyces*
Acetylcholine	*Lactobacillus*
Serotonin	*Candida, Streptococcus, Escherichia, Enterococcus*

Lyte, Mark. Bioessays 33.8 (2011): 574-581. 15

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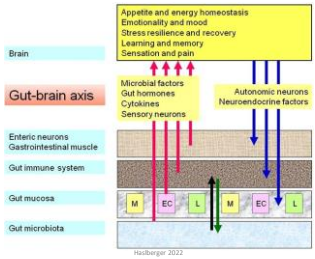
Serotonin

- Biogenic amine that functions as a neurotransmitter
  - Tryptophan is precursor
  - Involved in GI secretion
  - Gut motility
  - Pain perception
  - Maintenance of mood and cognition
- 95% of serotonin is contained in the gut in the mucosa and nerve terminals of the enteric nervous system
- Alterations in serotonin transmission may underlie pathological symptoms
  - Selective serotonin reuptake inhibitors are known to modulate psychiatric and GI disorders (e.g., IBS)

O'Mahony, S. M., et al. Behavioural brain research 277 (2015): 32-48.

151

Functions of gut brain axis, apPetite



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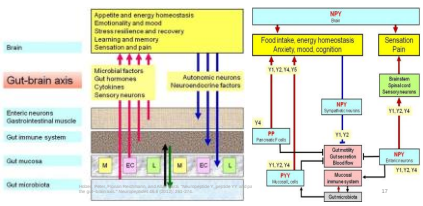
Gut Hormones and Neuropeptides



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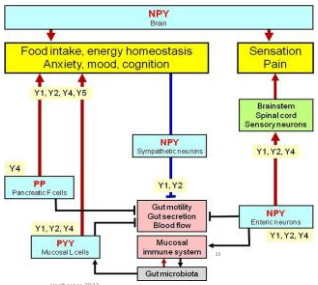
Neuro-peptide Y and the gut



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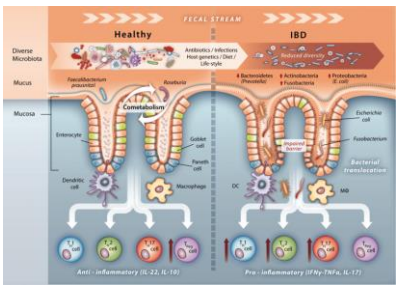
IBD



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Covid, long covid, microbiota and epigenetics

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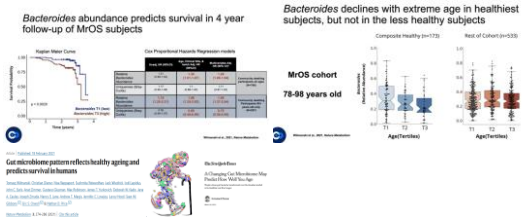
Bacteroidetes decide upon healthy aging ?



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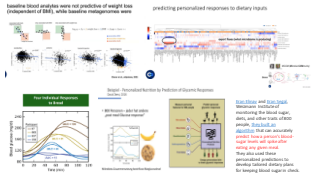
Microbiota predict personal responses to diets



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Probiotic

- Positive effects on health already 100 years ago suggested by Nobel Prize winner Elie Metchnikoff [Metchnikoff, 2004]
- Definition: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [FAO/WHO, 2002]
- Over 8000 research articles published since 2002 → several probiotic products on the market [Hill et al., 2014]
- Cell components of probiotics able to induce effects in host [Dotan and Rachmilewitz, 2005] but requirement for survivable cells remains a crucial factor for efficacy [Ma et al., 2004]

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Antimicrobial substances

- Probiotics produce various antimicrobial acting substances
- Examples: lactic acid, hydrogen peroxide, microcines, deconjugated bile acids [Oelschlaeger, 2010], bacteriocins [Maqueda et al., 2008]
- Antibiotics also produced by probiotics → reuterin:
  - Broad-spectrum antibiotic
  - Active against yeast, gram-positive and gram-negative bacteria, fungi, viruses, protozoa
  - Produced by strain ATCC55730 from L. reuteri [Cleusix et al., 2007]

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Table 6.2 Mechanism of probiotic and host interaction (Guarner et al. 2017)

Immunological benefits	• Stimulation of IgA production • Modulation of cytokine production • Induction of tolerance to food antigens
Nonimmunological benefits	• Production of bacteriocins to inhibit pathogens • Digestion of food and competition for nutrients with pathogens • Alteration of local pH to create an unfavorable local environment for pathogens • Stimulation of epithelium mucin production • Enhance intestinal barrier function • Competition for adhesion with pathogens • Modification of pathogen-derived toxins • Scavenge of superoxide radicals

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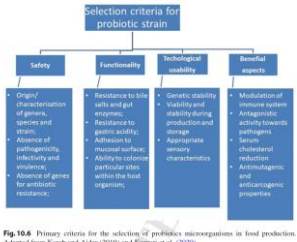

Fig. 10.6 Primary criteria for the selection of probiotics microorganisms in food production. Adapted from Kareb and Aider (2019) and Kumar et al. (2020)

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Species

- Lactobacilli:
  - Present in GIT, oral cavity and vagina of humans [Walter, 2008]
  - Widespread use in production and fermentation of foods → ability to convert hexose sugars to lactic acid → preservation [Fijan, 2014]
  - Excellent for use as probiotics: high tolerance to acid and bile, capability to adhere to intestinal surfaces [Tulumoglu et al., 2013]
- Bifidobacteria:
  - First colonizers of the human gut together with lactobacilli [Turroni et al., 2012]
  - Well known for resistance against bile salts [Fijan, 2014]

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Species

- Bacillus species:
  - Either spore-forming aerobic or facultative aerobic, gram positive bacteria
  - B. subtilis, B. cereus, B. coagulans are members with probiotic characteristics [Fijan, 2014]
- Escherichia coli Nissle 1917:
  - Able to colonize the gut and compete with resident and pathogenic bacteria through multiple fitness factors [Behnsen et al., 2013]
  - Stimulation of epithelial defensin production → restoration of disturbed gut barrier
  - „Sealing effect" on tight junctions of enterocytes [Sonnenborn and Schulze, 2009]

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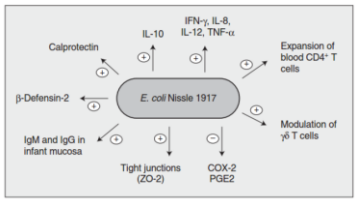

Figure 2. Various ways of immune modulation by E. coli Nissle 1917 (summary of data from in vitro and in vivo experiments) [Behnsen et al., 2013]

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Treatment of antibiotic associated diarrhea with probiotics - meta-analyses

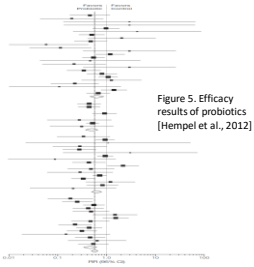

Figure 5. Efficacy results of probiotics [Hempel et al., 2012]

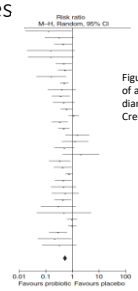

Figure 6. Developement of antibiotic associated diarrhea [Videlock and Cremonini, 2012]

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Treatment of acute diarrhea with probiotics – meta-analyses

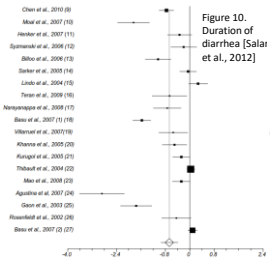

Figure 10. Duration of diarrhea [Salari et al., 2012]

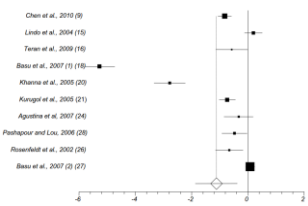

Figure 11. Duration of hospitalization [Salari et al., 2012]

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Probiotic new ways

Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study

Commensal Obligate Anaerobic Bacteria and Health: Production, Storage, and Delivery Strategies

Probiotika: Sind tote Bakterien wirksamer als lebende?

Das Prinzip von Probiotika lernt jeder – egal ob als Joghurt oder Supplement: Dem Körper werden mit der Nahrung Bakterien zugeführt, die sich im Darm vermehren und gesundheitsfördernd sein sollen. Soweit die Theorie. Doch eine aktuelle Studie wirft Fragen auf.

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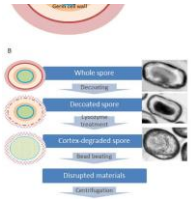
Spores



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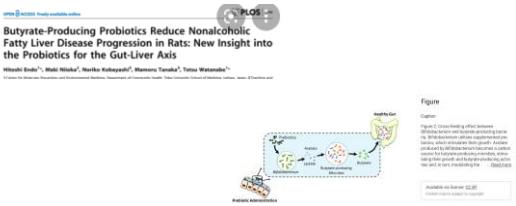
Butyrate production or cross feeding



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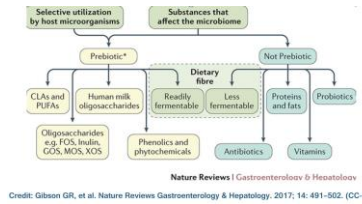
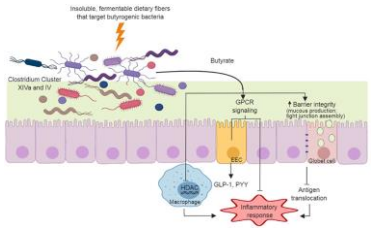
Prebiotics what is it?



171

Fibers and SCFA



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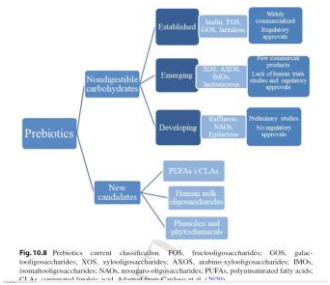

Fig. 10.8 Prebiotics current classification. FOS, fructooligosaccharides; GOS, galactooligosaccharides; XOS, xylooligosaccharides; AXOS, arabino-xylooligosaccharides; IMOs, isomaltooligosaccharides; NAOs, neosugaro-oligosaccharides; PUFAs, polyunsaturated fatty acids; CLAs, conjugated linoleic acid. Adapted from Carbone et al. (2020)

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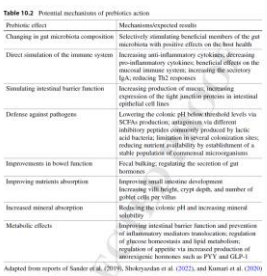

Table 10.2 Potential mechanisms of prebiotics action

Prebiotic effect	Mechanism/expected results
Changing in gut microbiota composition	Selectively stimulating beneficial members of the gut microbiota with positive effects on the host health
Direct simulation of the immune system	Increasing anti-inflammatory cytokines, decreasing pro-inflammatory cytokines; beneficial effects on the mucosal immune system; increasing the secretory IgA; reducing Th2 responses
Simulating intestinal barrier function	Increasing production of mucus; increasing expression of the tight junction proteins in intestinal epithelial cell lines
Defense against pathogens	Lowering the colonic pH below threshold levels via SCFAs production; antagonism via different inhibitory peptides commonly produced by lactic acid bacteria; limitation in several colonization sites; reducing nutrient availability by establishment of a stable population of commensal microorganisms
Improvements in bowel function	Fecal bulking; regulating the secretion of gut hormones
Improving nutrients absorption	Improving small intestine development Increasing villi height, crypt depth, and number of goblet cells per villus
Increased mineral absorption	Reducing the colonic pH and increasing mineral solubility
Metabolic effects	Improving intestinal barrier function and prevention of inflammatory mediators translocation; regulation of glucose homeostasis and lipid metabolism; regulation of appetite via increased production of anorexigenic hormones such as PYY and GLP-1

Adapted from reports of Sander et al. (2019), Shokryazdan et al. (2022), and Kumari et al. (2020)

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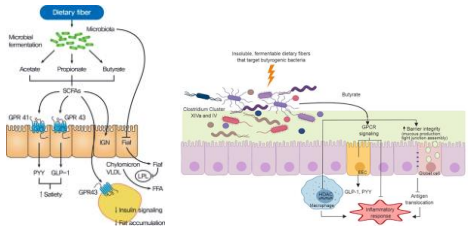
Fibers and obesity, butyrogenic



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Different responses of SCFAS

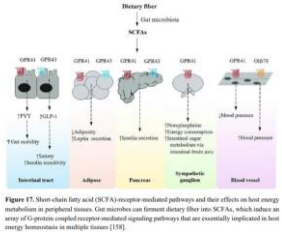

Figure 17. Short-chain fatty acid (SCFA)-receptor-mediated pathways and their effects on host energy metabolism in peripheral tissues. Gut microbes can ferment dietary fiber into SCFAs, which induce an array of G-protein coupled receptor-mediated signaling pathways that are essentially implicated in host energy homeostasis in multiple tissues [158].

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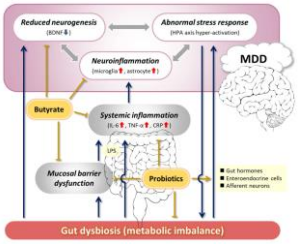
Prebiotics, neuroinflammation, Gut brain axis



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Review article

Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials

Richard T. Liu, P.H., Rachel F.L. Walsh, Ana E. Sheehan

4. Discussion

The current review provided the most comprehensive meta-analysis to date of the effects of probiotics on depression and anxiety. We also conducted the first quantitative syntheses of data on prebiotics for depression and anxiety. Although the current review did not find an ameliorative effect for prebiotics on depression or anxiety, respectively, these findings should be regarded as preliminary, given the relatively small number of eligible studies included in the analyses. We did find general support, however, for an effect of probiotics on depression and anxiety, with small pooled effects in both cases. Although Lactobacillus received the most interest among probiotic trials, when considered alone, it did not have an effect on depression, with a significant difference in effect size existing between Lactobacillus-only trials and others. Lactobacillus did not appear to have an effect on anxiety, regardless of whether considered alone or in combination with prebiotics or other probiotics.

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Postbiotic molecules, responses

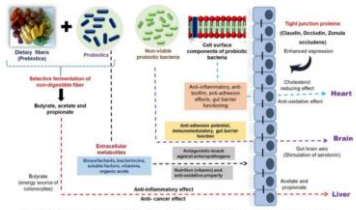

Figure 18. Schematic representation of various health benefits of postbiotic molecules [165]

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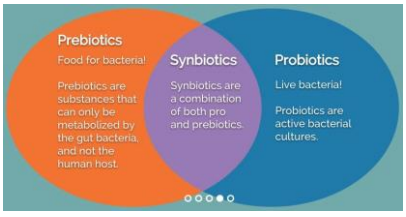
Synbiotics



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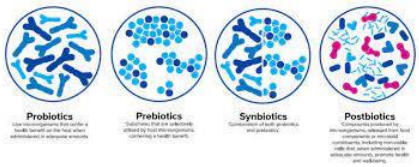

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Postbiotics

- **Bacteriocins** *(protective compounds that make life hard for the bad guys)**
- **Enzymes** *(help to digest food, get rid of toxins and assist other metabolic processes)**
- **Vitamins** *(like the B's and vitamin K)**
- **Amino acids** *(building blocks of protein)**
- **Neurotransmitters** *(carry messages between the nerves and brain and can even affect appetite)**
- **Immune-signaling compounds** *(they support the body's immune cells)**
- **Short-chain fatty acids** *(created from fiber, they keep the intestinal lining strong and healthy)**
- **Nitric oxide** *(crucial for cardiovascular health)**
- **Organic acids** *(such as Fulvic and Humic acid. They combine with minerals, making them easier to absorb and help maintain the correct pH in the GI tract)**

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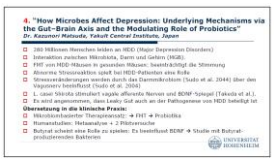

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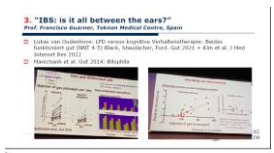

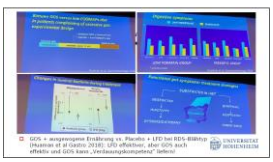

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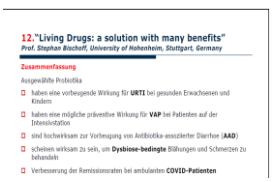

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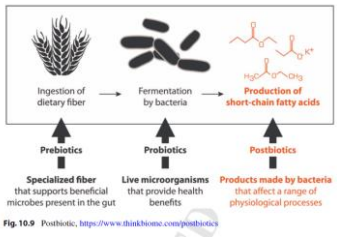

Fig. 10.9 Postbiotic, https://www.thinkbiome.com/postbiotics

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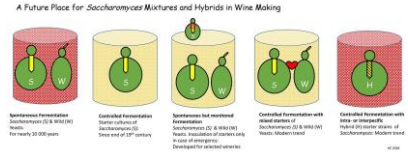
Fermentation spontaneous stater cultures



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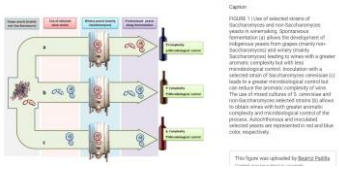
Fermentation, wine



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Bread

- Bread is one of the oldest prepared foods. Evidence from 30,000 years ago in Europe revealed starch residue on rocks used for pounding plants.
- Bread is a staple food prepared from a dough of flour and water, usually by baking.
- Bread is served in various forms with any meal of the day.
- Nutritionally, bread is known as an ample source for the grains category of nutrition.
- maximizes CO_2 production, which leavens bread.
- other microbes used to make special breads (e.g., sourdough bread).
- can be spoiled by *Bacillus* species that produce ropiness.

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Idli

- Idli is a traditional breakfast in South Indian households, especially in Andhra Pradesh, Karnataka, Tamil Nadu and Telangana where it is a popular breakfast dish that is consumed in numerous households.
- The cakes are made by steaming a batter consisting of fermented black lentils (de-husked) and rice.
- In idli made with a 1:1 ratio of black gram to rice, batter volume increased about 47 percent 12 to 15 hours after incubation at 30°C.
- Using a 1:2 ratio of black gram to rice, batter volume increased 113 percent and acidity rose to 2.2 percent in 20 hours at 29°C.

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Sauerkraut

- Sauerkraut is finely cut cabbage that has been fermented by various lactic acid bacteria.
- It has a long shelf life and a distinctive sour flavour, both of which result from the lactic acid that forms when the bacteria ferment the sugars in the cabbage.
- Fermentation by lactobacilli is introduced naturally, as these air-borne bacteria culture on raw cabbage leaves where they grow.
- Sauerkraut is made by a process of pickling called lactic acid fermentation that is analogous to how traditional (not heat-treated) pickled cucumbers and kimchi are made.

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Kimchi

- Kimchi, is a traditional side dish made from salted and fermented vegetables, most commonly napa cabbage and Korean radishes, with a variety of seasonings including chili powder, scallions, garlic, ginger, and *jeotgal*.
- Kimchi is a traditional Korean dish consisting of pickled vegetables, which is mainly served as a side dish with every meal, but also can be served as a main dish.
- Kimchi has been a staple in Korean culture, but historical versions were not a spicy dish.

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Natto

- Nattō is a traditional Japanese food made from soybeans fermented with *Bacillus subtilis var. natto*.
- Nattō is made from soybeans, typically nattō soybeans. Smaller beans are preferred, as the fermentation process will be able to reach the center of the bean more easily.
- Some eat it as a breakfast food.
- It is served with soy sauce, karashi mustard and Japanese bunching onion.
- Nattō may be an acquired taste because of its powerful smell, strong flavor, and slimy texture.

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The best functional food: fasting ?

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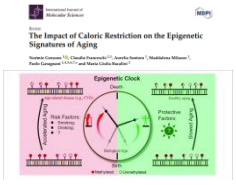
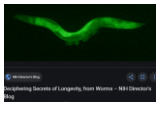
Caloric restriction improves healthy aging, role for epigenetic regulation as seen in epigenetic clock





Also true for mi RNA- marker

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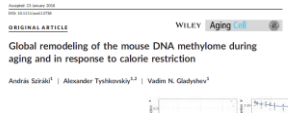
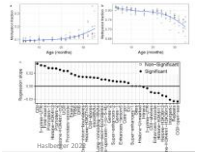
Caloric restriction and aging change epigenetic CpG -methylation structure





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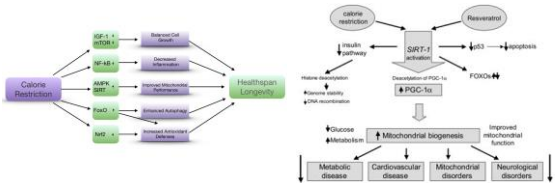
CR, Fasting pathways



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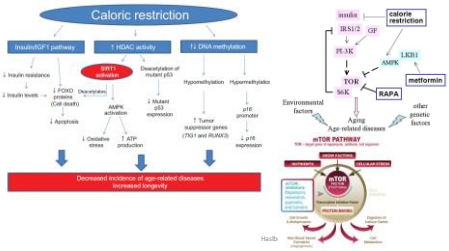
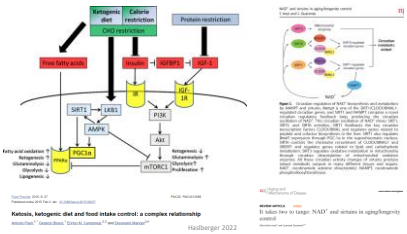
Fasting pathways: Sirt, mTOR pathways



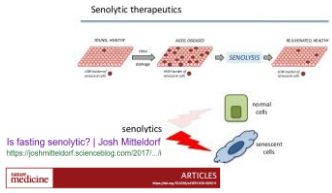
200

Caloric restriction, ketogenic diet involve SIRTs (+NAD, clock genes) + mTOR pathways (Metformin).



201

Caloric restriction: Rejuvenetion by senolysis? role for autophagy ?



83rd ICREA Colloquium, 2018

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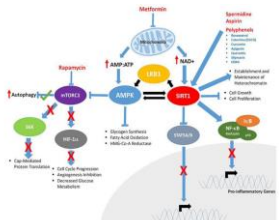
Senolytics, even Cart Tcells



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Fasting and Microbiota

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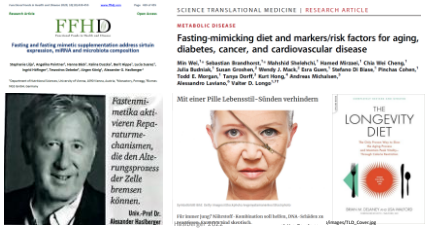
Caloric restriction- longevity
fasting mimetics and senolytics ?



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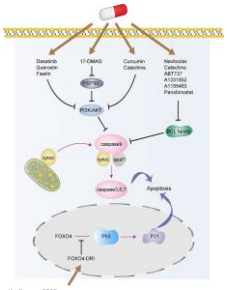
Senolytics
Quecetin,Curcumin,
Catechins, Fisetin,…
+ Dasatinib
markets



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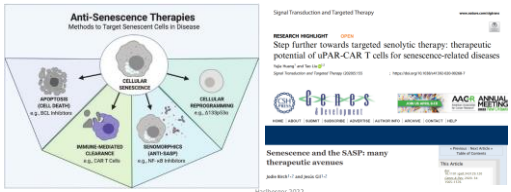
Anti senescence strategies



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CR- mimetics

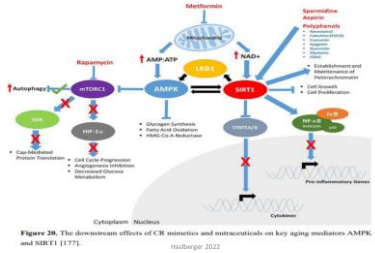

Figure 20. The downstream effects of CR mimetics and nutraceuticals on key aging mediators AMPK and SIRT1 [177].

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STUDY SENOLYTICS, SENESCENCE MARKERS IN BRDU TREATED PRE-ADIPOCYTES, ADIPOCYTES, 3T3

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case study: comparing Fasting and a Fasting mimetic sirt-food shot: Microbiota, epigenetics

Buchinger Fasting < 120 kcal/day
n: 22 in Pernegg Monastery

Feces , blood spots, before and After the end, first sold feces

Active (N. 131) Placebo (n: 30)
Intervention 3 months

Feces, Blood spots before, after 1,3 month

Illuminia sequencing, Line 1 methylation bisulfite qPCR, HR-MCA, RNA, MiRNA RT QPCRi

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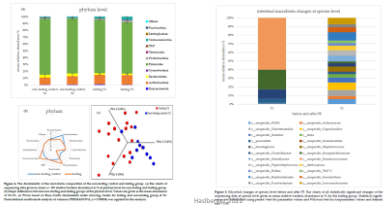
Buchinger fasting resulted in a rise in the distribution of Proteobacteria, increased microbiota diversity and a significant increase in Christensenella



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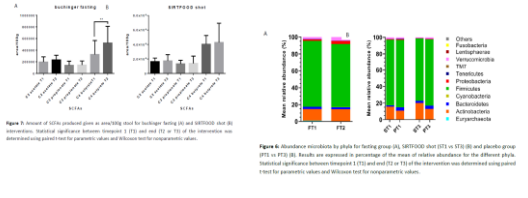
3M sirt inducing drink increased *Actinobacteria.* Firmicutes/*Bacteroidetes* ratio decreased and correlated with BMI. Only Fasting increased Butyrate significantly



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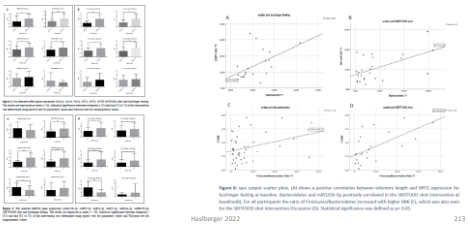
positive correlation of the abundance of butyrate-producing *Bacteroidetes* with Mir125, siRT-1 expression, telomere length



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Conclusions

In conclusion fasting and to some extend fasting mimetics result in beneficial modulation of microbiota (e.g diversity, SCFA, BHP) and metabolism (e.g SIRTS, mtDNA, telomer length)

Microbiota structure seems to interfere with the expression of Sirtuins and metabolism relevant miRNAs

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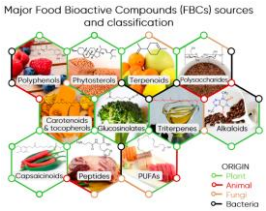
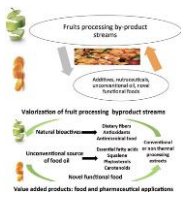
Types and classification of bioactive compounds from food





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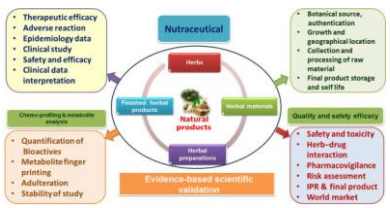
Nutraceuticals



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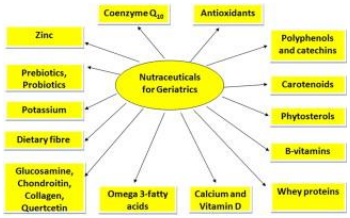
Nutraceuticals for aging



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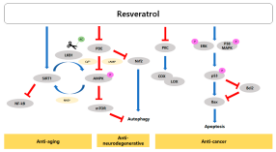
Examples, Resveratrol



Previous studies have demonstrated that resveratrol is well-absorbed following oral administration, with ~75% of the dose absorbed. Following absorption, resveratrol undergoes rapid and extensive metabolism leading to low bioavailability

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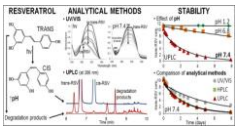
Resveratrol metabolisation, stability



Um 500 mg Resveratrol zu sich zu nehmen, müssten Sie beispielsweise 1528 kg dunkle Schokolade essen oder 400 kg dunkle Trauben oder 3600 kg Erdnüsse oder 1250 kg Äpfel (3).

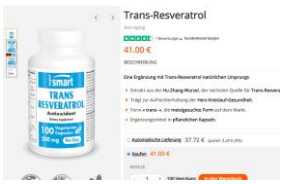
Rotwein 0-15 mg/L

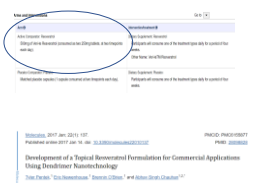
trans-Resveratrol oder cis-Resveratrol

Beide Resveratrol-Formen – trans- und cis-Resveratrol – kommen in der Natur vor. In Nahrungsergänzungsmitteln ist meist das als aktiver geltende trans-Resveratrol enthalten.

219

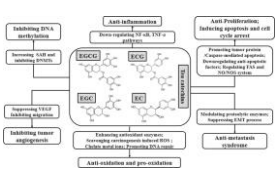
220





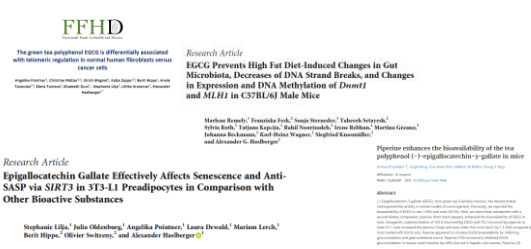
221

Green tea extract, EGCG, Catechines



222

EGCG II


FFHD

The green tea polyphenol EGCG is differentially associated with telomeric regulation in normal human fibroblasts versus cancer cells

Research Article

EGCG Prevents High Fat Diet-Induced Changes in Gut Microbiota, Decreases of DNA Strand Breaks, and Changes in Expression and DNA Methylation of *Dnmt1* and *MLH1* in C57BL/6J Male Mice

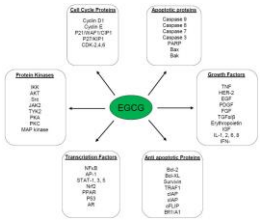
Pipeline enhances the bioavailability of the tea polyphenol (-)-epigallocatechin-3-gallate in mice

Research Article

Epigallocatechin Gallate Effectively Affects Senescence and Anti-SASP via *SIRT3* in 3T3-L1 Preadipocytes in Comparison with Other Bioactive Substances

223

EGCG



224

EGCG auto oxydation, bioavailability

The absolute bioavailability of EGCG after oral administration in rats is found to be 0.1% . Low bioavailability of EGCG seems to be related to its poor membrane permeability and transporter-mediated intestinal efflux

studies conclude that the intake of EGCG with some specific nutrients such as fish oil (omega-3 fatty acids) , vitamins as ascorbic acid which reduce the oxidation of EGCG and minerals as selenium or chrome improves the EGCG bioavailability, enhancing its antioxidant activity.

Epigallocatechin-3-gallate (EGCG) from green tea has anti-cancer effect. The cytotoxic actions of EGCG are associated with its auto-oxidation, leading to the production of hydrogen peroxide and formation of numerous EGCG auto-oxidation products (EAOPs), the structures and bioactivities of them remain largely unclear. In the present study, we compared several fundamental properties of EGCG and EAOPs, which were prepared using 5mg/mL EGCG dissolved in 200mM phosphate buffered saline (pH 8.0 at 37°C) and normal oxygen partial pressure for different periods of time. Despite the complete disappearance of EGCG after the 4-h auto-oxidation, 4-h EAOPs gained an enhanced capacity to deplete cysteine thiol groups, and retained the cytotoxic effects of EGCG as well as the capacity to produce hydrogen peroxide and inhibit thioredoxin reductase, a putative target for cancer prevention and treatment.

225

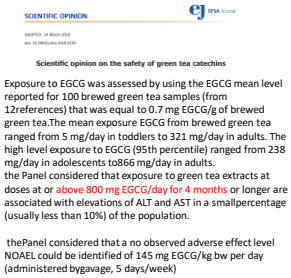
A single cup (8 ounces or 250 ml) of brewed green tea typically contains about 50–100 mg of EGCG. Dosages used in scientific studies are often much higher, but exact amounts have been inconsistent (11Trusted Source, 16Trusted Source).

Daily intakes equal to or above 800 mg of EGCG per day increases the blood levels of transaminases, an indicator of liver damage


efsa

Epigallocatechin gallate (EGCG) in combination with caffeine, and contribution to the maintenance or achievement of a normal body weight

On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of epigallocatechin gallate (EGCG) in combination with caffeine, and contribution to the maintenance or achievement of a normal body weight


SCIENTIFIC OPINION

efsa Journal

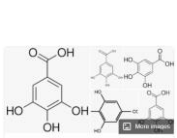
Scientific opinion on the safety of green tea catechins

Exposure to EGCG was assessed by using the EGCG mean level reported for 100 brewed green tea samples (from 12references) that was equal to 0.7 mg EGCG/g of brewed green tea.The mean exposure EGCG from brewed green tea ranged from 5 mg/day in toddlers to 321 mg/day in adults. The high level exposure to EGCG (95th percentile) ranged from 238 mg/day in adolescents to866 mg/day in adults.
the Panel considered that exposure to green tea extracts at doses at or above 800 mg EGCG/day for 4 months or longer are associated with elevations of ALT and AST in a smallpercentage (usually less than 10%) of the population.

thePanel considered that a no observed adverse effect level NOAEL could be identified of 145 mg EGCG/kg bw per day (administered bygavage, 5 days/week)

226

Gallic acid



Gallic acid, a common dietary phenolic protects against high fat diet induced DNA damage

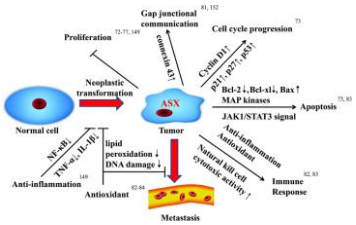
227

Astaxanthin

Astaxanthin is produced naturally in the freshwater microalgae Haematococcus pluvialis and the yeast fungus Xanthophyllomyces dendrorhous (also known as Phaffia rhodozyma).When the algae are stressed by lack of nutrients, increased salinity, or excessive sunshine, they create astaxanthin. Animals who feed on the algae, such as salmon, red trout, red sea bream, flamingos, and crustaceans (shrimp, krill, crab, lobster, and crayfish), subsequently reflect the red-orange astaxanthin pigmentation.

228

Asthaxantin II



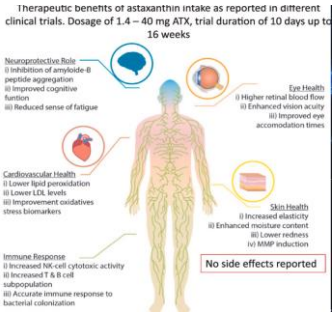
229

Asthaxantin III

Biological Activities
Antioxidant activity
Protection from UV rays
Anti-skin cancer
Anti-inflammatory
Anti-gastric activity
Anti-hepatoprotective
Anti-diabetes
Cardiovascular prevention
Immune response
Neuroprotection

Astaxanthin is a natural C40 carotenoid with numerous reported biological functions, most of them associated with its antioxidant and anti-inflammatory activity, standing out from other antioxidants as it has shown the highest oxygen radical absorbance capacity (ORAC), 100-500 times higher than α-tocopherol and a 10 times higher free radical inhibitory activity than related antioxidants (α-tocopherol, α-carotene, β -carotene, lutein and lycopene).

230

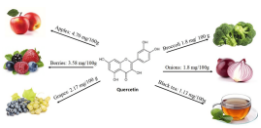


European Food Safety Authority has set an Acceptable Daily Intake of 0.2 mg per kg body weight, as of 2015.[9] As a food color additive, astaxanthin and astaxanthin dimethyldisuccinate are restricted for use in Salmonid fish feed only.[10]

231

Quercetin, flavonoid

biological systems. These studies showed that the 3'- and 4'-hydroxyl groups of quercetin, (catechol hydroxyl groups) were important targets for methylation, sulfation and glucuronidation. Methylation of a catechol hydroxyl group of quercetin proved to decrease the pH-dependent radical scavenging capacity of the compound, both by increasing its pK_a for deprotonation and by decreasing its electron-donating properties. Methylation of a catechol



232

Quercetin, RDS, health claim

233

Potential Clinical Benefits of Quercetin in the Early Stage of COVID-19: Results of a Second, Pilot, Randomized, Controlled and Open-Label Clinical Trial

Quercetin in the Prevention and Treatment of Coronavirus Infections: A Focus on SARS-CoV-2

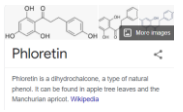
Scientific Opinion on the substantiation of health claims related to quercetin and protection of DNA, proteins and lipids from oxidative damage (ID 1647), "cardiovascular system" (ID 1844), "mental state and performance" (ID 1845), and "liver, kidneys" (ID 1846) pursuant to Article 13(1) of Regulation (EC) No 1924/2006

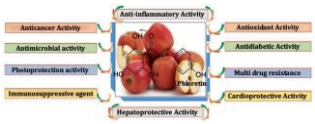
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

European Food Safety Authority (EFSA), Parma, Italy

234

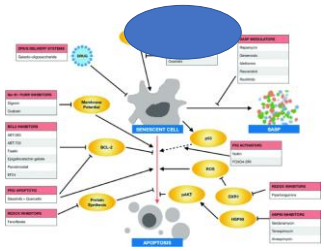
Phloretin, adipocytes, apoptose, senolytic

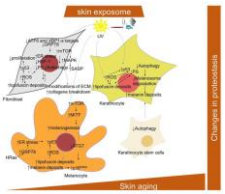




235

Senolytic





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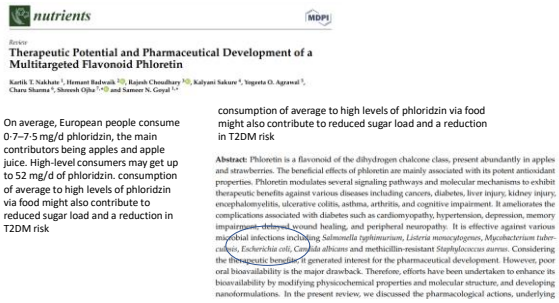
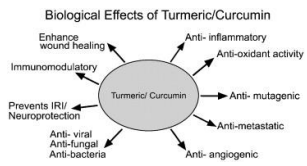


237

Fisetin

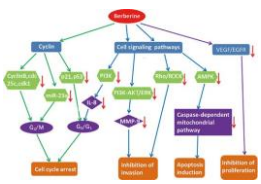
238

Curcumin



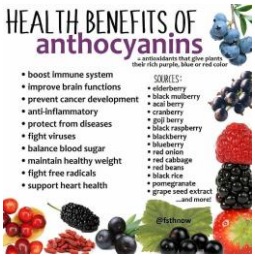
239

Berberin, Berberitze



240

Anthocyans

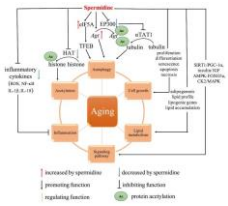


241

Spermidin

242

Spermidine mechanisms



Molecular and cellular mechanisms of spermidine in age-related diseases. Spermidine is an inducer of autophagy, which is the main mechanism of anti-aging. First, spermidine **triggers autophagy by modulating the expressions of *Atg* genes**. Second, it regulates **transcription factor eIF 5A to promote the synthesis of transcription factor TFEB.** Third, spermidine **inhibits EP300, which directly promotes the acetylation of Atg genes** and **indirectly stimulates deacetylation of tubulin due to inhibition of αTAT1**. Besides, spermidine exerts potent anti-inflammatory roles by **suppressing of multiple inflammatory cytokines, such as ROS, NF-κB, IL-1β and IL-18**. Moreover, it is involved in regulation **of cell proliferation, differentiation, senescence, apoptosis and necrosis**, ultimately promoting cell growth and inhibiting cell death.
As an anti-aging agent, spermidine suppresses histone acetylation. Moreover, spermidine regulates lipid metabolism. On the one hand, it promotes the differentiation of preadipocytes into mature adipocytes. On the other hand, it alters lipid profile, modulates lipogenic gene expressions, and represses lipid accumulation. Furthermore, spermidine can delay aging through specific signaling pathways, such as SIRT1/PGC-1α, insuli n/ IGF, AMPK-FOXO3a, and CK2/MAPK signaling pathways.

243

Shiitake

High Vitamin D Mushroom Powder has been Approved as a Novel Food Ingredient by EFSA

opinion on pea and rice protein fermented by Shiitake mushroom (Lentinula edodes) mycelia as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is a mixture of fermented pea and rice protein concentrates (65% and 35%, respectively). The NF is proposed to be used as a food ingredient in specific food categories. The target population is the general population. Th

The Panel concludes that the NF, pea and rice protein fermented by Shiitake (Lentinula edodes) mycelia, is safe under the proposed conditions of use.

244

Ganoderma

Application for consultation to determine the status of a novel food, pursuant to Article 4(2) of the Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods.

Recipient Member State: SPANISH AGENCY FOR FOOD SAFETY AND NUTRITION (AESAN)

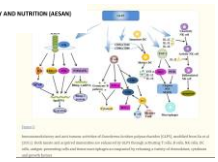
***Ganoderma lucidum* dehydrated mycelium powder**

Also, after consulting other Member States, none of these has confirmed a history of consumption of ***Ganoderma lucidum* dehydrated mycelium powder** prior to May 15, 1997.

Conclusion:

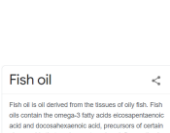
***Ganoderma lucidum* dehydrated mycelium powder** is considered as **novel food** since it does not present history of safe and significant consumption in the European Union before May 15, 1997 and it is not possible to establish an equivalence relationship between the reproductive and vegetative part of the fungus in terms of nutritional composition, metabolites and potentially active substances, falling within the scope of Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods.

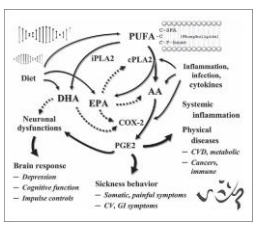
Doses ????



245

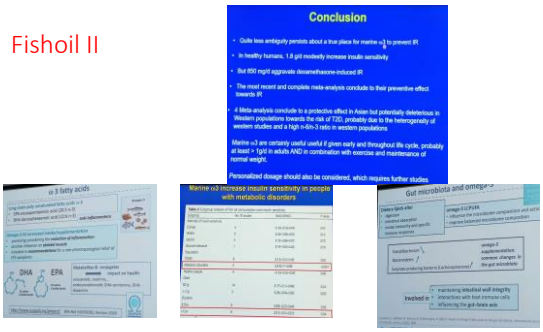
Fishoil, EPA, DHA





246

Fishoil II



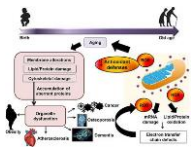
247

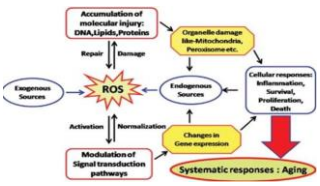
Discussed activities of nutraceuticals along the hallmarks of aging, age related complex diseases

Anti oxydative	Epigenetic active
inflammation	neuroinflammation
Telomers	Mitochondria
Autophagy	Apoptose
Senolytic	DNa repair
Immune senescence	Nuro infl
Anti bacterial	Anti viral
AGING	

248

ROS and antioxydative activities

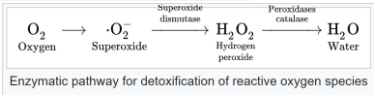




249

Antioxydants

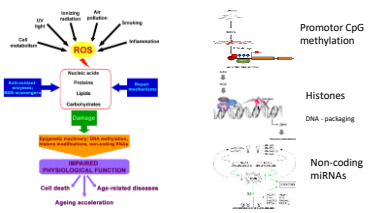
Antioxidant	Solubility	Concentration in human serum (μM)	Concentration in liver tissue (μmol/kg)
Ascorbic acid (vitamin C)	Water	50–60[23]	260 (human)[24]
Glutathione	Water	4[25]	6,400 (human)[24]
Lipoic acid	Water	0.1–0.7[26]	4–5 (rat)[27]
Uric acid	Water	200–400[28]	1,600 (human)[24]
Carotenes	Lipid	β-carotene: 0.5–1[29][30] retinol (vitamin A): 1–3[30][31]	5 (human, total carotenoids)[31]
α-Tocopherol (vitamin E)	Lipid	10–40[32]	50 (human)[24]
Ubiquinol (coenzyme Q)	Lipid	5[33]	200 (human)[24]



Enzymatic pathway for detoxification of reactive oxygen species

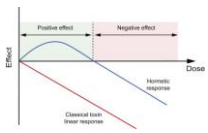
250

Ros, stress impairs all mechanisms of the epigenetic machinery **- > aging**



251

Stress Mitormesis



ECG and EGCG are considered antioxidants, which means they counteract or prevent oxidative stress in the body caused by aggressive free radicals of oxygen," said senior co-author Professor Michael Ristow, a researcher in the Department of Health Sciences and Technology at ETH Zurich and the Department of Human Nutrition at the Friedrich Schiller University Jena, and his colleagues. "Until now, it was assumed that these catechins neutralize free radicals and thus prevent damage to cells or DNA."
"One source of oxygen free radicals is metabolism; for example, when the mitochondria — the powerhouses of the cell — are working to produce energy."
"We took a closer look at how catechins act in the nematode worm *Caenorhabditis elegans* and came to a different, seemingly paradoxical conclusion: rather than suppressing oxidative stress, green tea catechins promote it."
In their experiments, the researchers found that applying the green tea catechins EGCG and ECG at a low dose extends the lifespan of *Caenorhabditis elegans*.
The long-term effects also included reduced fat content in the nematodes after 5 days of catechin treatment.
"ECG and EGCG initially increase oxidative stress in the short term, but that this has the subsequent effect of increasing the defensive capabilities of the cells and the organism," they explained.

252

Health, novel foods, functional foods, areas

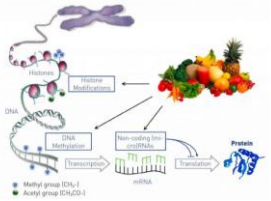


Figure 4. Polyphenols address all epigenetic mechanisms.

253

Antioxydants, mithormesis


Antioxidants

Abstract

Antioxidants have the ability to scavenge free radicals in the human body and have been suggested to contribute to the protective effect of plant based foods on diseases such as cardiovascular disease (CVD), cancer, and type 2 diabetes. However, evidence from supplementation studies using various antioxidants, including vitamin C, vitamin E, carotenoids, zinc, or selenium, does not support the hypothesis that antioxidants decrease risk of these diseases. Intervention studies highlight a lack of information on the safety of sustained intakes of moderate to high doses of micronutrient supplements and suggest that long-term harm cannot be ruled out, particularly in smokers.

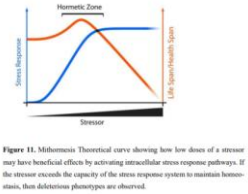
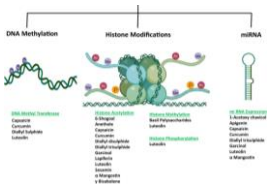


Figure 11. Mithormesis Theoretical curve showing how low doses of a stressor may have beneficial effects by activating intracellular stress response pathways. If the stressor exceeds the capacity of the stress response system to maintain homeostasis, then deleterious phenotypes are observed.

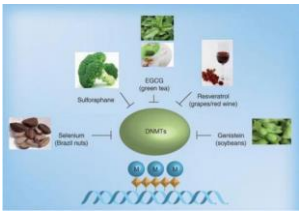
254

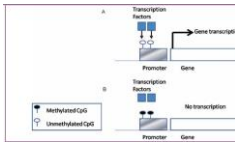
Bioactive plant ingredients and epigenetics



255

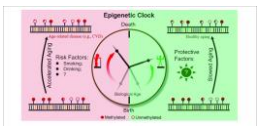
DNA, CpG methylation

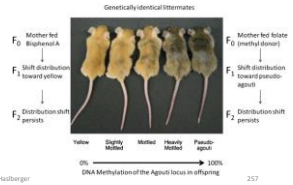




256

Best marker for aging: The epigenetic clock (Horvath) evaluates the biological age, accelerated or decelerated, healthy aging (CpG methylation of 100s of genes)





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Effects on histones, chromation

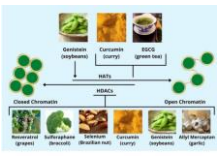

Figure 6. Effects of epigenetic active plant ingredients on histones, chromatin, and gene expression

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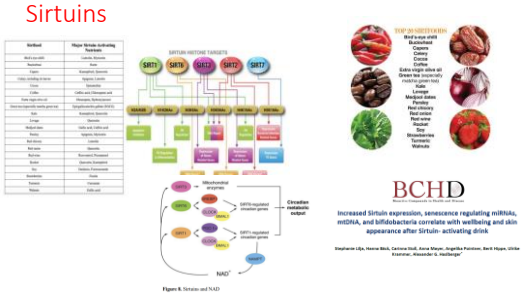
Sirtuins



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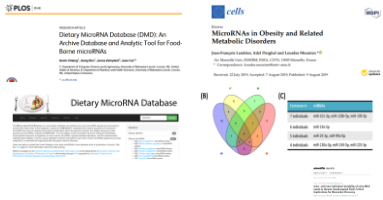
Epigenetic miRNAs: food borne, marker for mechanisms, phenotypes, disorders



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MI RNAS

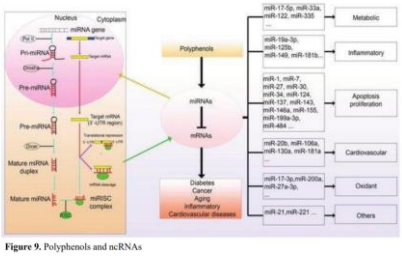

Figure 9. Polyphenols and ncRNAs

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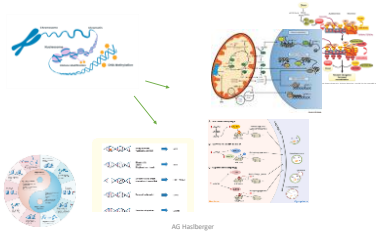
Epigenetics regulates aging mechanisms involved in telomere attrition, mitochondrial functions, autophagy, I.S./inflammation, senescence and DNA-repair



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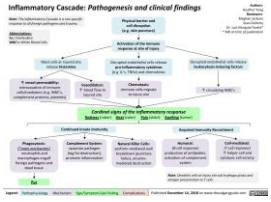
Polyphenols and Inflammation mechanisms



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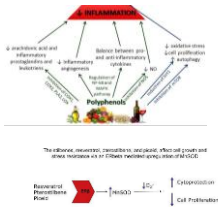
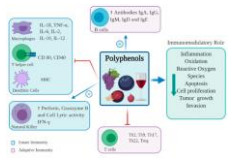
Inflammation: interactions novel foods facts or hypothesis ?





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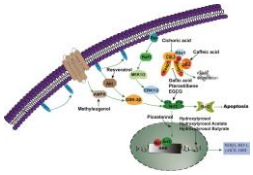
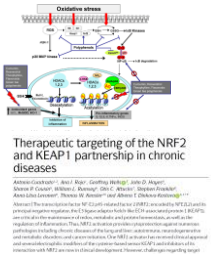
Polyphenols and NRF2





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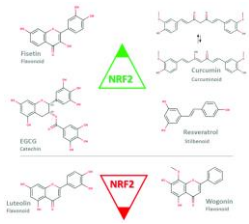
NRF2 agonists, antiagonists



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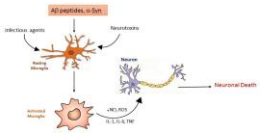
Neuro- inflammation



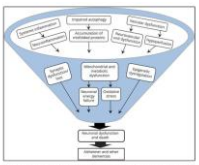


Figure 1. Microglial cells respond differently depending upon type and intensity of activation signals. [17].

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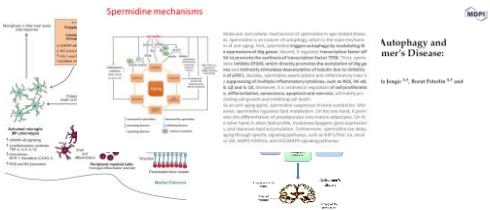
Polyphenols and microglia



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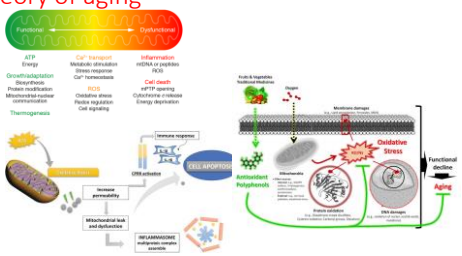
Polyphenols and mitochondria, the oldest theory of aging



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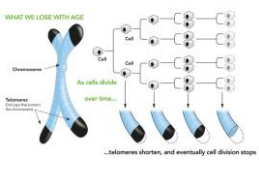
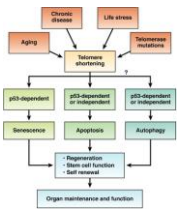
Telomers





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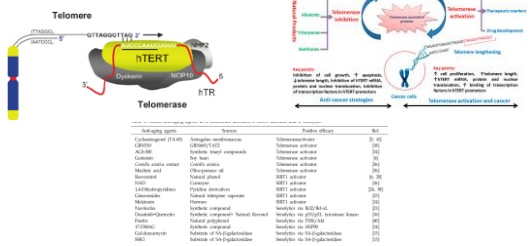
Telomers, telomerase



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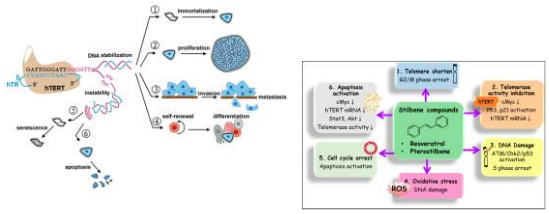
Polyphenols in the regulation of telomerase, hTERT



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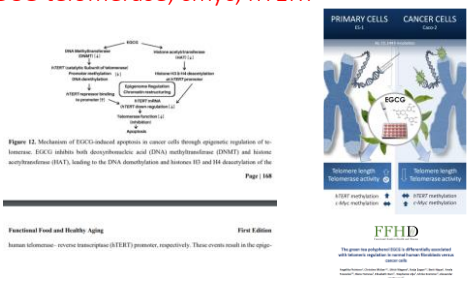
EGCG telomerase, cmyc, hTERT



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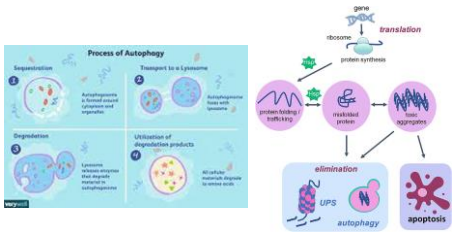
Autophagy, apoptosis



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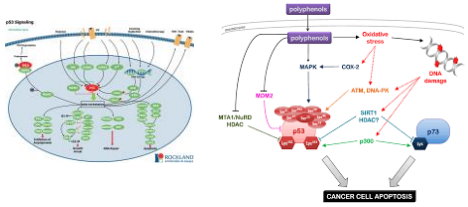
Apoptosis, p53 and polyphenols



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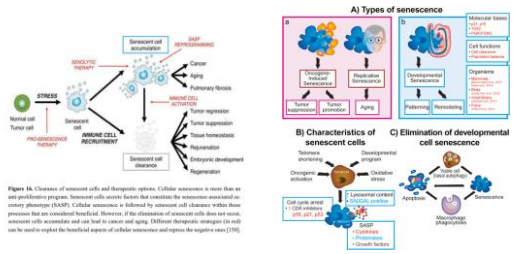
Senescence and polyphenols



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Polyphenols and senescence

Review

Natural Polyphenols Targeting Senescence: A Novel Prevention and Therapy Strategy for Cancer

Yan Bian, Juntong Wei, Changsheng Zhao and Guorong Li *

Received: 3 December 2019; Accepted: 17 January 2020; Published: 20 January 2020

Abstract: Cancer is one of the most serious diseases endangering human health. In view of the side effects caused by chemotherapy and radiotherapy, it is necessary to develop low-toxic anti-cancer compounds. Polyphenols are natural compounds with anti-cancer properties and their application is a considerable choice. Pro-senescence therapy is a recently proposed anti-cancer strategy and has been shown to effectively inhibit cancer. It is of great significance to clarify the mechanisms of polyphenols on tumor suppression by inducing senescence. In this review, we delineated the characteristics of

Figure 2. Potential functions of resveratrol in anti-tumor therapy. Resveratrol inhibit tumor microenvironment for cancer prevention, reduce drug/radiation resistance and induce cancer cell senescence for cancer therapy. SASP, senescence-associated secretory phenotype; DLC1, deleted in liver cancer1; ROS, reactive oxygen species.

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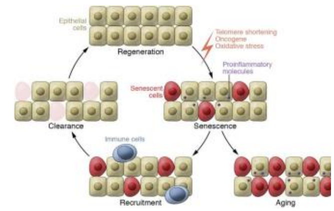
Senolytics between rejuvention of tissues and cancer prevention

Table 1. Polyphenols and polyphenol derivatives as cancer cell senescence inducers and their effect

Classification	Compounds	Concentration	Pathways
Resveratrol and its derivatives	Resveratrol	25/50 (μM)	p53/CXCR2
		50 (μM)	BRCA1/DDR
		30 (μM)	ROS/DDR
		100 (μM)	ROS/DLC1/SASP
		6/20 (μM) 100 (μM)	Histone H2B Pokemon
		25/50 (μM)	SIRT1
		50 (μM)	Rictor/RhoA-GTPase
	Pterostilbene	2.5/5/50 (μM)	hTERT DDR
	Pauciflorol B	10 (μM)	p16/Rb
	3,3',4,4'-tetrahydroxy-trans-stilbene	10/50/100 (μM)	ROS DDR
Flavonoids	Quercetin	50/100/200 (μM)	RAS/MAPK/ERK PI3K/AKT
	Beta-naphthoflavone	10 (μM)	PI3K/AKT/cyclinD1/D3 MAPK/ERK
	Baicalin	10/20/40 (μM)	DEPP/RAS/Raf/MEK/ERK DEPP/p16/Rb
	IdB 1016	63.2/126.5 (μg/mL)	HER-2/neu p53
	Diosmin	5/10 (μM)	ROS DDR
	Apigenin	Above 25 (μM)	ROS/RNS p16/cyclin D1/p-Rb p21/cyclin E/p-Rb
	Coumestrol	50 (μM)	CK2/ROS/p53/p21
	Rotenone	0.4 (μM)	Ca^{2+}/ROS
	Epigallocatechin gallate	10 (μM)	DDR
	Oroxin A	5/10/15/20 (μM)	p38/ER stress
	Cristacarpin	1 (μM)	p38/ER stress/ROS/p21
	Flavokawain B	3 (μg/mL)	ATF4/DDIT3/TRIB3/AKT/mTOR



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CR mimetics,

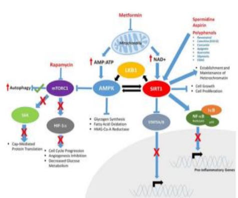

Figure 20. The downstream effects of CR mimetics and nutraceuticals on key aging mediators AMPK and SIRT1 [177].

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STUDY SENOLYTICS, SENESCENCE MARKERS IN BRDU TREATED PRE-ADIPOCYTES, ADIPOCYTES, 3T3

Hindawi

Research Article

Epigallocatechin Gallate Effectively Affects Senescence and Anti-SASP via SIRT3 in 3T3-L1 Preadipocytes in Comparison with Other Bioactive Substances

Stephanie Lilja, Julia Oldenburg, Angelika Pointner, Laura Dewald, Mariam Lerch, Berit Hippe, Olivier Switzeny, and Alexander Haslberger

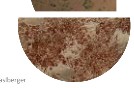
Stem Cells. Author manuscript; available in PMC 2015 Aug 18.
Published in final edited form as:
Stem Cells. 2008 Dec; 26(12): 3218–3227.
Published online 2008 Sep 18. doi: 10.1634/stemcells.2008-0299

Bromodeoxyuridine Induces Senescence

- EGCG
- Anthocyanins Biogenes
- Ayurvedic Spermidine Biogenes
- BLUEZONES Resveratrol

Phloretin, BHB, Butyrate (Merck)

B-Gal, senescence



Adipocytes, fat droplets

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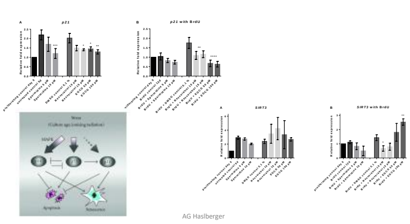
Egcg Effectively reduce Senescence (p21) and SASP
EGCG, spermidine, resveratrol, anthocyans stimulate SIRT3



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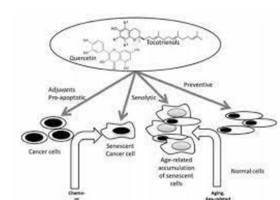
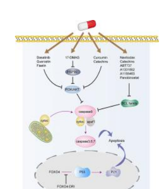
Quecetin, senolytics and millio $ markets





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Immuno senescence and nutraceuticals

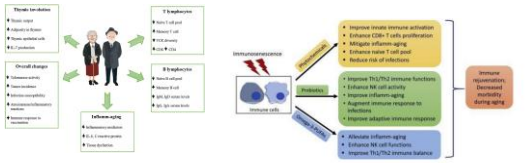

Figure 13. Nutraceuticals-Based Immunotherapeutic Concepts and Opportunities for the Mitigation of Cellular Senescence and Auine

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Quercetin, Fisetin, Piperlongumine, and Curcumin; and in the latter one, Resveratrol, Kaempferol, Apigenin, and Epigallocatechin gallate (EGCG) (16, 21).

The senescence of the immune system (immunosenescence) represents a challenge, especially when associated with the presence of age-related chronic inflammation (inflammaging) and affecting the metabolic programming of immune cells (immunometabolism). These aspects are linked to poorer health outcomes and therefore present an opportunity for host-directed interventions aimed at both eliminating senescent cells and curbing the underlying inflammation. Senotherapeutics are a class of drugs and natural products that delay, prevent, or reverse the senescence process – senolytics; or inhibit senescence-associated secretory phenotype – senomorphics. Natural senotherapeutics from food sources – nutritional senotherapeutics – may constitute an interesting way to achieve better age-associated outcomes through personalized nutrition. In this sense, the authors present herein a framework of nutritional senotherapeutics as an intervention targeting immunosenescence and immunometabolism, identifying research gaps in this area, and gathering information on concluded and ongoing clinical trials on this subject. Also, we present future directions and ideation for future clinical possibilities in this field.

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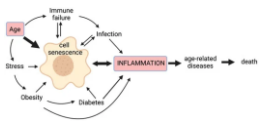
Immunosenescence and inflammation



Similarly, levels of the anti-inflammatory cytokine IL-10 [114] negatively correlate with age [75], and high IL-10 was not associated with any detrimental outcomes in physical performance [81]. In fact, IL-10 was included in an anti-inflammation cluster together with HDL cholesterol and is associated with good prognosis in acute coronary syndrome patients (~67 years old) [78]. Counterintuitively, however, a number of factors classified as anti-inflammatory appear to correlate with poor physiological function when they increase with age. For instance, soluble TNF receptor I (sTNFR-I) and it act as inhibitors of TNF-α pro-inflammatory signalling by preventing its interaction with membrane TNFR. Hence they would be anticipated to be anti-inflammatory, yet they have a strong positive correlation with increasing age [75] and are associated with a lower physical performance [77]. An index that combined IL-6 and sTNFR-I was the best predictor of 10-year mortality in a longitudinal study of two large cohorts of older adults [83], suggesting that sTNFR-I acts with IL-6 to promote inflammation. The interleukin-1 receptor antagonist (IL-1RA) blocks IL-1 signalling and has been described as anti-inflammatory. However, levels of IL-1RA positively correlate with age [75,80], and it clusters with IL-6 and CRP [76,81]. In fact, IL-1RA is a significant predictor of mortality in older adults independent of inflammatory exposure or genotype [83,93]. Two other soluble interleukin receptors, IL-2 soluble receptor (IL-2sR) and IL-6sR, have been included in the same cluster as TNF-α and sTNFRs and are associated with poor physical performance [77]. However, in another study, IL-6sR was not associated with negative performance [81] and was not correlated with age in men [75],

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thymic function through the use of growth hormone (GH) in combination with metformin and DHEA (dehydroepiandrosterone to counteract the diabetogenic action of GH) was shown in the TRIMM trial to improve overall health and reduce biological ageing, as assessed through methylation clocks [427]. The engraftment of young thymic epithelial cells into the thymus of middle-aged mice improved thymic T cell production [428], demonstrating the importance of the tissue microenvironment as well as immune cells themselves in retaining immune function. Other strategies using transplantation of induced thymic epithelial cells showed that a novel complete and functional thymus could be generated in vivo [429], which could ameliorate immune function in older patients. Thymus-targeted expression of IL-7 also rejuvenated the thymus, increased naïve T cell production, and improved immune responses to viral infection in old mice [430]. Similar results have been found when overexpressing FOXN1 expression in the thymus of middle-aged and old mice [431,432]. Stimulating haematopoietic stem cells to proliferate by depleting aged immune cells also appears promising, since rejuvenation of the B cell lineage has been achieved by complete B cell depletion (e.g., treatment with the monoclonal anti-B cell antibody, rituximab), which triggers the expansion of progenitors; such B lineage rejuvenation was sufficient to enable treated individuals to mount a response to hepatitis B vaccination, but it did not fully restore immune system function [433,434]. The rejuvenation is thought to be a consequence of the elimination of old long-lived B cells [433], as similarly suggested in a genetic model of age-associated B cell removal [336]. Induced pluripotent stem cells derived from old HSCs were also able to restore haematopoiesis to levels seen in young mice and also avoided skewing towards myeloid lineage [435], suggesting that the epigenetic reprogramming to return to the iPSC state was sufficient to remove age-related epigenetic information. Multiple other strategies to counteract immunosenescence, such as the use of growth factor IL-7 and checkpoint inhibitors, have been investigated. Approaches may combine immunostimulatory and immunosuppressive strategies and rejuvenation of immune tissues, and have been reviewed elsewhere [436,437].

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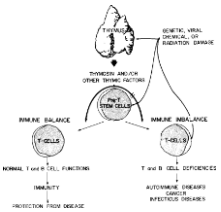
Zinc, vitamin B6, and vitamin C are perhaps the most critical. Supplementation with these nutrients has been shown to improve thymic hormone function and cell-mediated immunity. Zinc may be the critical mineral involved in thymus gland function and thymus hormone action.



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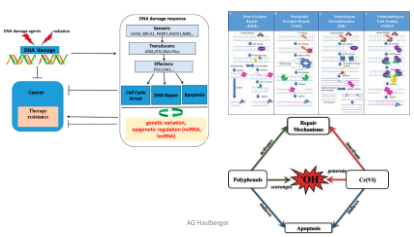
Aging DNA-damage response, DNA-repair, Epigenetics, Polyphenols



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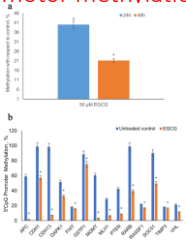
MGMT and MLH1 DNA repair enymes and promotor methylation, EGCG



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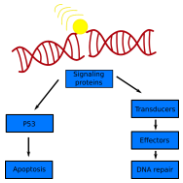
Epigenetics regulates DNA repair



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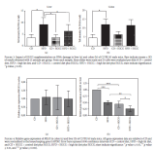
Mouse study: EGCG reduced high fat diet induced strandbreaks, DNmt1, comet assay



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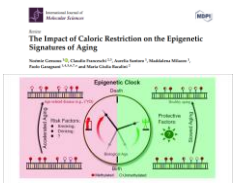
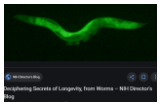
Caloric restriction improves healthy aging, role for epigenetic regulation as seen in epigenetic clock





Also true for mi RNA- marker

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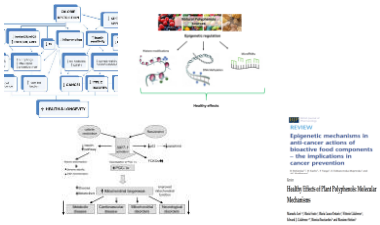
Caloric restriction **as well as special polyphenols** improve healthy aging including similar epigenetic mechanisms, especially Sirt enzymes



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Aging, ageotypes and prevention

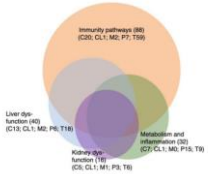

Figure 21. Ageotypes, personalized aging [136]

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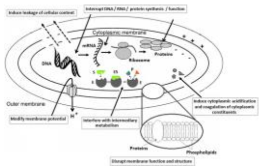
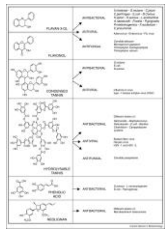
Anti bacterial polyphenols





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Polyphenols in food preservation, processing

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Antiviral nutraceuticals

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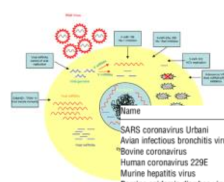
RNA and Corona viruses



Name	Abbrev.	Accession	Length	Base composition
SARS coronavirus Urbani	SARS	AY278741	29,727	(0.28, 0.20, 0.21, 0.31)
Avian infectious bronchitis virus	AIBV	NC_001451.1	27,608	(0.29, 0.16, 0.22, 0.33)
Bovine coronavirus	BCoV	NC_003045.1	31,028	(0.27, 0.15, 0.22, 0.36)
Human coronavirus 229E	HCoV	NC_002645.1	27,317	(0.27, 0.17, 0.22, 0.35)
Murine hepatitis virus	MHV	NC_001846	31,357	(0.26, 0.18, 0.24, 0.32)
Porcine epidemic diarrhea virus	PEDV	NC_003436.1	28,033	(0.25, 0.19, 0.23, 0.33)
Transmissible gastroenteritis virus	TGV	NC_002306.2	28,586	(0.29, 0.17, 0.21, 0.33)
Rubella virus	RUV	NC_001545.1	9,755	(0.15, 0.39, 0.31, 0.15)
Equine arteritis virus	EAV	NC_002532.2	12,704	(0.21, 0.26, 0.26, 0.27)
Rabies virus	RV	NC_001542.1	11,932	(0.29, 0.22, 0.23, 0.26)
Human immunodeficiency virus 1	HIV-1	NC_001802.1	9,181	(0.36, 0.18, 0.24, 0.22)

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S. No.	Molecule	Target	Type of Study/ Techniques Used	Results	Study, Year, Reference
1	Luteolin	SARS-CoV S2 protein	• Frontal-affinity chromatography-mass spectrometry • HIV-luc/SARS pseudotype virus assay • MTT assay with wild-type SARS-CoV	• Luteolin-inhibited SARS-CoV infection in a dose-dependent manner. • EC_{50} was 10.6 μM, CC_{50} was 0.155 mM, LD_{50} in mice was 232.2 mg/kg	Yi et al. 2004
2	Quercetin	SARS-CoV S2 protein	HIV-luc/SARS pseudotype virus assay	EC_{50} of 83.4 μM and CC_{50} of 3.32 mM	Yi et al. 2004
3	GCG (gallocatechin gallate)	SARS-CoV 3CLPro	• Expression of recombinant 3CLPro in Pichia pastoris and its inhibition. • Molecular docking	• 91% inhibition by 200 μM. • IC_{50} of 47 μM. • Binding energy of −14 kcal/mol	Nguyen et al. 2012
4	Quercetin	SARS-CoV 3CLPro	• Expression of recombinant 3CLPro in Pichia pastoris and its inhibition. • Molecular docking	• 80% inhibition at 200 μM. • IC_{50} of 23.8 μM • Binding energy −10.2 kcal/mol	Nguyen et al. 2012
5	EGCG	SARS-CoV 3CLPro	• Expression of recombinant 3CLPro in Pichia pastoris and its inhibition. • Molecular docking	• 85% inhibition at 200 μM. • IC_{50} of 73 μM • Binding energy −11.7 kcal/mol	Nguyen et al. 2012
6	Resveratrol	MERS-CoV NP	• MTT assay using vero-E6 cell line • Nucleocapsid protein staining	• Found to be effective in the 125–250 μM range on viral titre as well as viral RNA amount. • Inhibits caspase 3 cleavage.	Lin et al. 2017
7	Hesperetin	SARS-CoV 3CLPro	Cell free and cell-based cleavage assays	IC_{50} of 60 μM in cell free assay, IC_{50} of 8.3 μM in cell-based assay and a CC_{50} of 2718 μM	Lin et al. 2005
8	Quercetin	ACE2 and FURIN	• Gene silencing • Expression studies • Transgenic mouse models	• Quercetin affected ACE2 expression. • In addition, it was found to alter the expression of 98 of 332 (30%) genes encoding human proteins that serve as target for the SARS-CoV-2.	Glinsky, 2020

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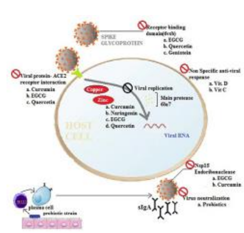
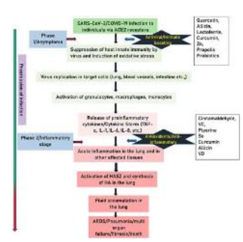
Covid , SARS-2





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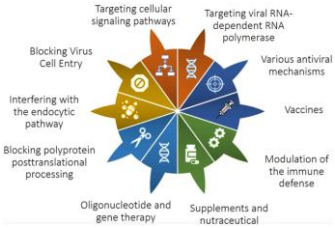
Strategies



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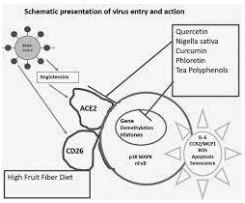
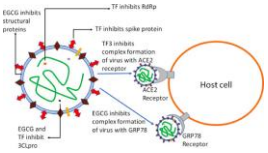
Nutraceuticals, epigenetics and inhibition of RNA viruses





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Novel Protein Sources

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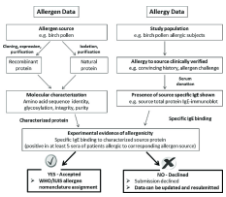
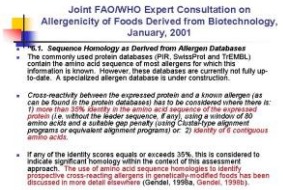
Main problem allergy





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Algae

- Algae have been used as human food for thousands of years in all parts of the world.
- The most commonly consumed macro algae include the

1. Red algae Porphyra(nori, kim, laver)
2. Asparagopsis taxiformis(limu)
3. Chondrus crispus(Irish moss)
4. Kelps Laminaria(kombu)
5. Palmaria palmata(dulse)
6. Macrocystis
7. Undaria(wakame)
8. Green algae Caulerpa racemosa
9. Codium

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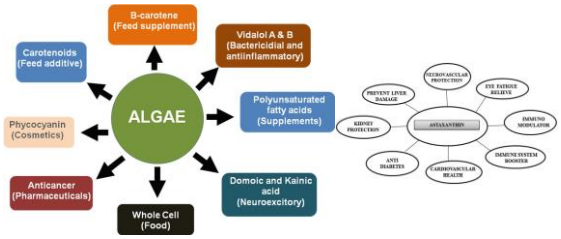

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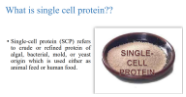
Single cell proteins



- The term single cell protein was introduced in the 1960s to describe protein-rich foods manufactured from yeasts that served as dietary supplements for livestock and humans.
- The production and utilization of microbial biomass as a source of food proteins gained particular interest as an alternative source for proteins of agricultural origin due to its high content of protein.
- Algae as a source of SCP is a term which refers to either microscopic single-cell true algae or prokaryotic cyanobacteria, and their growth is based on use of carbon dioxide and light energy.
- Quorn is produced from a multi-cellular, filamentous fungus, the term single cell protein is inaccurate and **mycoprotein** is the preferred name.
- **Mycoprotein** is a form of single-cell protein, also known as **fungal protein** "Protein derived from fungi, especially as produced for human consumption.

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efsa

Safety of astaxanthin for its use as a novel food in food supplements

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Jacqueline Castenmiller, Stefaan de Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Karl-Heinz Engel, Thomas Frenzel, Marina Heinonen, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Yolanda Sanz, Josef Rudolf Schlatter, Henk van Loveren, Reinhard Ackert, Wolfgang Gelbmann, Hans Steinkellner and Helle Katrine Knutsen

Abstract

Following a request from the European Commission, the Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the safety of astaxanthin when used as a novel food in food supplements at maximum levels of 8 mg/day, taking into account the overall cumulative intake of astaxanthin from all food sources. In 2014, the NDA Panel assessed the safety of the novel astaxanthin-rich ingredient derived from microalgae *Haematococcus pluvialis* in the context of an application submitted under Regulation (EC) No 258/1997. In that opinion, the NDA Panel considered that the acceptable daily intake (ADI) for astaxanthin was 0.034 mg/kg body weight (bw) set by the EFSA FEEDAP Panel in 2014. In 2019, the FEEDAP Panel adopted an opinion which concerned the renewal of the authorisation of dimethyldisuccinate-astaxanthin and a new use of the additive for crustaceans and other fish than salmonids. In that assessment, the FEEDAP Panel derived a new ADI of 0.2 mg astaxanthin/kg bw which replaced the ADI of 0.034 mg/kg bw established in 2014. By taking into account an updated exposure assessment for astaxanthin from the background diet (fish and crustaceans) in combination with 8 mg from food supplements, the NDA Panel concludes that (i) such combined exposure to astaxanthin is safe for adults, (ii) 14 to < 18 years old adolescents reach the ADI, and (iii) the ADI is exceeded by 28% in children aged 10 to < 14 years and up to 524% in infants aged 4–6 months.

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Microalgae as a novel food

Potential and legal framework

Tomke F. Prüser, Peggy G. Braun, Claudia Wiacek

Abstract

Microalgae such as *Chlorella* and spirulina have high dietary potential, because they contain a large number of nutrients which seem to make them predestined for use in human nutrition. They are characterized by fast growth and enable low-resource production of important nutrients, such as n-3 fatty acids.

Alongside a few approved species of microalgae, there are several thousand microalgae that are not used in human nutrition despite their interesting nutrient profile. The reasons for this are explored in this outline paper and can be traced back to Europe's legal framework for consumer protection. As a result of the Regulation on novel foods, foods are only approved for use on the European market after a time-consuming investigation process, in order to protect consumers from unsafe foodstuffs.

Keywords: microalgae, novel food, Novel Food Regulation, n-3 fatty acids, vitamin B_{12}

Microalgae

The name "algae" is a collective term for a large polyphyletic group of living things including both plants and bacteria. What they almost all have in common is that they contain chlorophyll and are thus also able to produce energy from light, carbon dioxide and water through oxygenic photosynthesis [1]. They differ from mosses and ferns in that algae are not specialised for life on land [2]. Even this very general definition is incomplete and excludes whole taxa of algae which have lost the ability to photosynthesise over the course of their development [1, 2].

The algae group is divided into microalgae and macroalgae, whereby macroalgae are multi-

Molke

Microalgae in the food industry

Microalgae such as *Chlorella* and spirulina have considerable dietary potential due to their spectrum of nutrients. They thus became a focus of research as early as 1950 and the first microalgae were cultivated and marketed on a commercial scale from 1960 [8]. Since then interest in microalgae has steadily increased due to their adaptability and the number of different constituents which can be obtained from them [8, 11]. Whereas initially microalgae were sold mainly as nutritional supplements in the form of powders, capsules, and tablets, today they are also incorporated into various products like pasta, smoothies, soft drinks, chocolate, and ice cream [12, 13]. In 2018 the global market volume for microalgae products was already USD 9.4 billion and with a projected annual growth rate of over 7% market volume could reach USD 14.99 billion by 2024 [14]. There are large production plants in countries such as Israel, United States, Australia and China. In Germany too there are at least 13 plants producing microalgae [15].

Spirulina is promoted mainly for its protein and vitamin B12 content. Tablets of dried spirulina have a vitamin B12 content of 120–240 µg/100 g, although 83% is in the form of non-bioavailable pseudovitamin B12 [17]. The protein content in spirulina is around 50–60% of the dry mass with a biological value of 50–70 [18]. However, even microalgae not approved up to now have major potential. For instance, not only does the dry mass of Phaeodactylum tricornutum contain 1.7-5.0% of eicosapentaenoic acid (EPA) [29], it also contains the carotenoid fucoxanthin

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Plant-based proteins

- Made from soy, peas, lentils, wheat, or other proteins mixed with ingredients such as oils
  - Binding agents such as methylcellulose may be added
- May be called "meat analogues", "veggie burgers"
- Some products have been formulated to "bleed" like meat
  - Impossible™ burger uses genetically engineered soy leghemoglobin
  - Beyond Meat® uses beet juice

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Plant-based proteins- 2

Regulation: FDA regulates
- Daily regulation not required
- Food processors must have risk-based preventive food safety system in place
- Discussion in many states and federal level on what can be called a "burger", "sausage", "meat" or similar terms

EU Novel food ?

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Plant-based proteins- 3

- Food safety considerations: consumers with allergies to wheat, soy, etc should check label
  - Cook to 165F, use same good practices as with meat
- Marketplace status: Available in many restaurants and grocery stores

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Plant-based proteins- nutrition comparison

- Slightly different than meat

Table 1. Nutritional comparison of a regular Whopper® to an Impossible™ Whopper® (patty only)

	Regular Whopper®	Impossible™ Whopper®
Calories (Kcal)	240	210
Fat (g)	18	12
Saturated Fat (g)	8	7
Trans Fat (g)	1.5	0
Cholesterol (mg)	80	0
Sodium (mg)	230	330
Carbohydrates (g)	0	9
Fiber (g)	0	2
Sugar (g)	0	1
Protein (g	20	17

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Further nutrition considerations

- Noted nutrients likely lacking in most beef replacements and meat replacements include:
  - Monounsaturated fatty acids
  - Vitamins B_3 (niacin), B_{12}*
  - Zinc
  - Choline
  - Selenium

*Lack of B_{12} represents a well-known and potentially serious limitation of plant based diets

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Cultured meat is coming

- NOT currently available for many consumers
  - Not currently produced on large scale
- Grown in laboratories from animal cells in culture medium
  - Grown on an edible non-meat scaffold that holds cells in position
- May be called "cultured protein", "clean meat", "lab-grown meat", "in vitro meat", others

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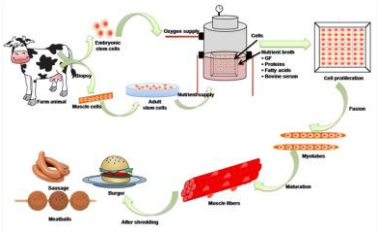
Cultured meat, production



There are three stages in the production of cultured meat.

1. Selection of starter cells,
2. Treatment of growth medium,
3. Scaffolding,

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Cells, media, scaffolds (Gerüst)

- To collect cells that have rapid rate of proliferation.
- Stem cells does not develop toward a specific kind of cells. So cells such as myosatellite and myoblast cells are often used.
- Because the cells will helps in producing a structural cells.

- Cells are then treated by applying a solution that promotes tissue growth known as growth medium.
- Medium should contain necessary nutrients and appropriate quantities of growth factor.
- Then they are placed in a bioreactor which is able to supply the cells with energetic requirements.
-
- To cultured 3 dimensional meat, the cells are grown on scaffold.
- The idea scaffold is edible so meat does not have to be removed and periodically moves to stretch the developing muscle.
- Scaffold must maintain flexibility in order to not detach from developing myotubes.
- Scaffold d must allow vasucularization (creation of blood vessel) in order to develop normal muscle tissue.

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3D printing ?

- Additive manufacturing:

An Israeli company Meatech proposes to use 3 dimensional printing techniques to improve the texture of cultured meat.

- Sacffold based production technique can be only appropriately used in boneless or ground meats.
- End result of this process would be meat for hamburger and sausages.

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Proteins from arthropods, insects

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Insekten als Lebensmittel

- in über 200 Ländern als Lebensmittel verzehrt
- v.a. in Asien, Afrika, Lateinamerika

- in Kenia und Thailand
-> Massenzüchtungen

- in westlichen Ländern Säugetiere als Hauptproteinquelle -> kaum Insektenverzehr

[Garino et al., 2019]

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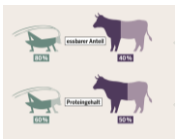
gesundheitliche Vorteile von Insekten

- vergleichbare Nährstoffgehalte wie Fleisch und Fisch

- **hohe Gehalte an:**
  - essentielle Aminosäure
  - mehrfach ungesättigten Fettsäuren
  - Ballaststoffen
  - Mineralstoffen: Kupfer, Eisen, Magnesium, Mangan, Phosphor, Selen und Zink



[1]

[FAO, 2013]

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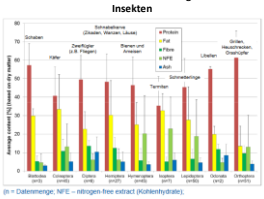
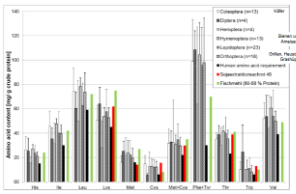
Durchschnittliche Zusammensetzung verzehrter Insekten



Aminosäurespektren verzehrter Insekten



[BfR, 2016]

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ökologische und ökonomische Vorteile

- geringer Futter und Wasserverbrauch
  -> effizientere Futterverwerter
  -> 2kg Futter ≙ 1kg Insektenmasse
  -> 8kg Futter ≙ 1kg Rindermasse
- weniger Landverbrauch
- geringer Treibhausemissionen

[1]

- Zucht auch mit geringen Ressourcenaufwand möglich
  -> auch für Schwellen- und Entwicklungsländer

[FAO, 2013]

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Risiko allergenes Potential

- direkte Allergie bei Mehlwürmern und Seidenraupe
- Kreuzreaktivität bei Hausstaubmilben- und Meeresfruchtallergikern zu Tropomyosin und Argininkinasen der Insekten
  -> bei Mehlwürmern, Grillen, Grashüpfer, Motte, Termiten, Schabe

Vorkommen:

- 7,6% allergische Reaktionen
- davon 18% anaphylaktischer Schock

Symptome:

Hautreaktionen (Rötung, Urticaria), GI-Probleme (Bauchschmerzen, Diarrhoe), respiratorische Störungen (Asthma, Dyspnoe)

[De Gier & Verhoeckx, 2018]

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Risikoanalyse-System allergenes Potential

- Verhinderung einer Übertragung von allergenen Material auf andere Lebensmittel
  -> Schutz von Allergikern

- Stellung eines Novel Food- Antrags
  -> Beweis, dass kein allergenes Protein in Lebensmittel enthalten
  -> Vergleich der AS-Sequenz mit Sequenz von allergenen Proteinen

[Garino et al., 2019]

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Risikoanalyse-System Allergene (Mehlwürmer)

1. **Stufe: Gefahrenidentifikation**
   -> allergische Reaktionen durch Hautkontakt, Inhalation oder Verdauung
   -> IgE-Körper Produktion
2. **Stufe: Gefahrencharakterisierung**:
   -> Bestimmung Grenzwert-Dosis für allergische Reaktion (durch klinische Studie)
   -> Effektive Dosis (5%, 10%, 50%)
3. **Stufe: Aufnahme Beurteilung**:
   -> Menge von konsumierten Produkt
   -> Konzentration Allergen in Produkt
   -> Wahrscheinlichkeit, dass allergenes Produkt aufgenommen wird
   -> Charakterisierung und Prävalenz von klinischen Subgruppen
4. **Stufe: Risiko Charakterisierung**
   -> Charakterisierung des Risikos bei verschiedenen Leveln von Allergenen
   -> Entwicklung eines sicheren Grenzwertes für allergene LM

[Garino et al., 2019]

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Risiko: biologische und chemische Gefahren

Biologische Gefahren

- pathogene Bakterien
- Mykotoxin-produzierende Pilze
- Parasiten
- Viren
- Antibiotika resistente Gene

Chemische Gefahren

- Schwermetalle
- toxisch-chemische Verbindungen

Abhängig von:

- Spezifische Produktionsmethoden
- Substratverwendung
- Phase der Ernte
- Insektenspezies
- Verarbeitungsmethoden

[Garino et al., 2019]

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gesetzliche Regelungen

- EU Regulation 2015/2283: Insektenbasierte Lebensmittel gehören zu Novel Food
- EU Regulation 2017/893: Liste mit 7 erlaubten Insektenspezies
  - Hermetia illucens (Soldatenfliege)
  - Musca domestica (Stubenfliege)
  - Tenebrio molitor (Mehlkäfer)
  - Alphitobius diaperinus (Getreideschimmelkäfer)
  - Acheta domesticus (Hausgrille)
  - Gryllodes sigillatus (Kurzflügelgrille)
  - Gryllus assimilis (Steppengrille)

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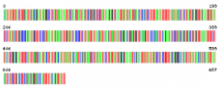
Nachweismethode Insekten

- Für Gen-Identifikation C01-Gen verwendet

-> Cytochrom C Oxidase 1-Gen in Mitochondrien aller Tierarten



- C01-Gensequenz bei allen Spezies unterschiedlich
- je näher verwandt, desto ähnlicher

- Gensequenzen erlaubter Insektenspezies in Datenbank „Barcode of Life Data System (BOLD)" gespeichert

⇒ Nachweis durch Vergleich Gensequenz von Probe mit Datenbank

[Garino et al., 2019]

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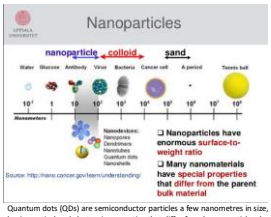
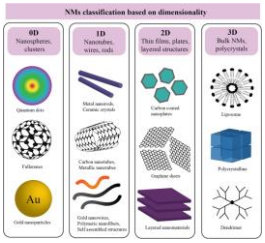
Nano technology and nutrition



Quantum dots (QDs) are semiconductor particles a few nanometres in size, having optical and electronic properties that differ from larger particles due to quantum mechanics



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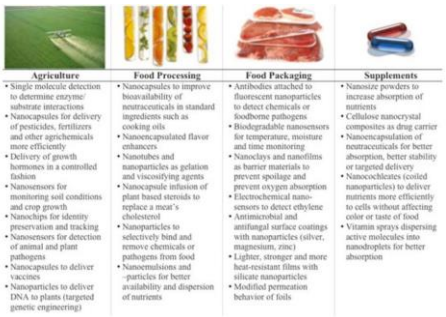

Agriculture	Food Processing	Food Packaging	Supplements
• Single molecule detection to determine enzyme/ substrate interactions • Nanocapsules for delivery of pesticides, fertilizers and other agrichemicals more efficiently • Delivery of growth hormones in a controlled fashion • Nanosensors for monitoring soil conditions and crop growth • Nanochips for identity preservation and tracking • Nanosensors for detection of animal and plant pathogens • Nanocapsules to deliver vaccines • Nanoparticles to deliver DNA to plants (targeted genetic engineering)	• Nanocapsules to improve bioavailability of neutraceuticals in standard ingredients such as cooking oils • Nanoencapsulated flavor enhancers • Nanotubes and nanoparticles as gelation and viscosifying agents • Nanocapsule infusion of plant based steroids to replace a meat's cholesterol • Nanoparticles to selectively bind and remove chemicals or pathogens from food • Nanoemulsions and –particles for better availability and dispersion of nutrients	• Antibodies attached to fluorescent nanoparticles to detect chemicals or foodborne pathogens • Biodegradable nanosensors for temperature, moisture and time monitoring • Nanoclays and nanofilms as barrier materials to prevent spoilage and prevent oxygen absorption • Electrochemical nano-sensors to detect ethylene • Antimicrobial and antifungal surface coatings with nanoparticles (silver, magnesium, zinc) • Lighter, stronger and more heat-resistant films with silicate nanoparticles • Modified permeation behavior of foils	• Nanosize powders to increase absorption of nutrients • Cellulose nanocrystal composites as drug carrier • Nanoencapsulation of neutraceuticals for better absorption, better stability or targeted delivery • Nanocochleates (coiled nanoparticles) to deliver nutrients more efficiently to cells without affecting color or taste of food • Vitamin sprays dispersing active molecules into nanodroplets for better absorption

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Methods Nano

Nanotechnique	Characteristic feature	Examples	Reference
Edible coatings	To preserve the quality of fresh foods during extended storage	Gelatin-based edible coatings containing cellulose nanocrystal	Fakhouri et al., 2014
		Chitosan/nanosilica coatings	Shi et al., 2013
		Chitosan film with nano-SiO_2	Yu et al., 2012
		Alginate/lysozyme nanolaminate coatings	Medeiros et al., 2014
Hydrogels	Can be easily placed into capsules, protects drugs from extreme environments, and to deliver them in response to environmental stimuli such as pH and temperature	Protein hydrogels	Qui and Park, 2001
Polymeric micelles	Solubilize water-insoluble compounds in the hydrophobic interior, high solubility, low toxicity	PEO-b-PCL [poly(ethylene glycol)block-poly(caprolactone)] polymeric micelles	Ma et al., 2008
		Methoxy poly(ethylene glycol) palmitate polymeric micelles	Sahu et al., 2008
Nanoemulsions	(i) Greater stability to droplet aggregation and gravitational separation; (ii) Higher optical clarity; and, (iii) increased oral bioavailability	β-Carotene-based nanoemulsion	Kong et al., 2011
		β-Carotene-based nanoemulsion	Yuan et al., 2008
Liposomes	Since liposome surrounds an aqueous solution inside a hydrophobic membrane, it can be used delivery vehicles for hydrophobic molecules (contained within the bilayer) or hydrophilic molecules (contained in the aqueous interior)	Cationic lipid incorporated liposomes modified with an acid-labile polymer hyper-branched poly(glycidol) (HPG)	Yoshizaki et al., 2014
Inorganic NPs	They display good encapsulation capability and their rigid surfaces allow controlled functionalization	Mesoporous silica nanoparticles	Tang et al., 2012

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Nanoparticles: Delivery, stability, release

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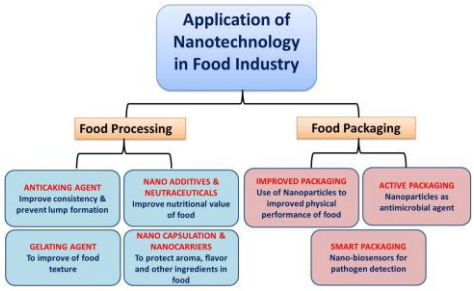

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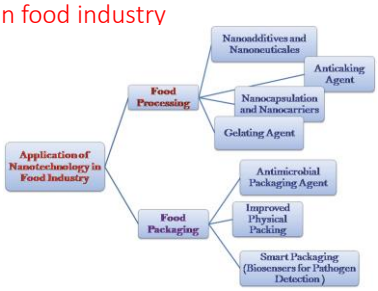
Nano in food industry



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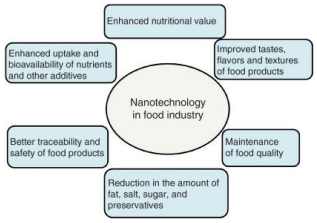
Nano and nutrition



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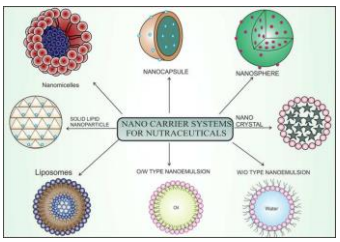
Nano carriers



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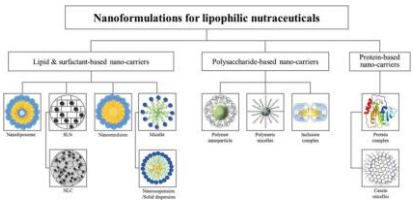
Nano and nutraceuticals



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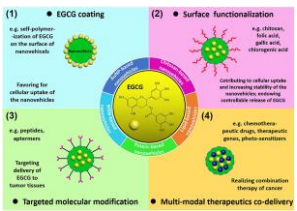
Nutraceuticals delivery



Gold nanoparticles , GNPs

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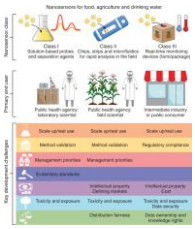
Nano sensors



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Nanosilver as an example

WHAT IS NANO SILVER?

- Nano Silver particles typically measures 25nm.
- Nano Silver is high efficacious and got the power to produce an intended effect
- Nano Silver is in suspension form with deionized water
- 80% of Silver is in the form of metallic silver nano-particles and remaining silver is in ionized form.

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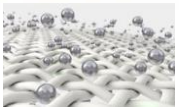
WHY SILVER ON NANOSCALE?

- Silver has superior antibacterial and antibiotic characteristics but there has been limitation on the application in real life because of darkening at high temperature and high cost
- Nanotechnology solved all these two problems at the same time by Silver Nanoparticles



- Silver Nanotechnology Commercial Inventory (SNCI) provides products ranging from cleaning sprays, skin creams, ATM buttons and sport clothing
- Nano Silver technology is applied in wide range of health care products ranging from bandages, burn care treatments and catheters and almost any product where infection control is critical
- Has marked antibacterial and antifungal properties
- Emits ultra-red rays, which are known to help in improving blood circulation and metabolism

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- Electrostatic and a natural safe deodorizer against unpleasant odor
- Powerful germ killing agent and have been incorporated into a number of consumer products such as clothing, kitchenware, toys and cosmetics
- Researchers are incorporating 'smart packaging' that would be able to tell if the packaged food becomes contaminated, as well as respond to changes in environmental conditions and self-repair holes and tears

HOW GOVERNMENTS AND BUSINESSES USE SILVER NANOPARTICLES AROUND THE WORLD

- Water Treatment , Purifications and Waste Water
- Health Care Industry
- Electronics & Communications Industry
- Textile & Clothing Industry
- Painting Industry
- Agriculture
- Cosmetic Industry
- Soft Drinks Industry
- Shoes Industry
- Aerospace Industry
- Consumer Products
- Packaging Industry
- Automotive Industry
- Military And Government Services

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"If the government added it to our drinking water instead of Fluoride we would all be far healthier!"

- In the United States, Environmental Protection Agency (EPA) recommended silver be present in drinking water at 100 ppb.
- The system injected with 10 parts per billion of ionic silver into the drinking water distribution grid. Within 24 hours of installing the system, the fecal coliform count (a count of microorganisms from human or animal waste, frequently accompanied by disease-causing pathogens) was brought to zero, and it remained at zero for the next eighteen days.

NANOSILVER BASED ANTIMICROBIAL FABRICS

- Nanosilver-based antimicrobial clothing and fabric products includes shoes, socks, sportswear, dish rags, facial towels, sleeping bags etc.
- nylon fused with nanosilver ions is woven into a variety of fabrics.
- Many nanosilver-based fabric products are available in the market. e.g.,
- Silver based fiber is designed which is impregnated into the shoes to prevent foot furgus and bacteria from growing. This is extremely important to diabetics as they are prone to serious foot infections such as gangrene
- Nanosilver fiber is incorporated into sleeping bags and cold-weather jackets to prevent growth of bacteria and fungi
- A wide variety of sports apparel are weaved with nanosilver fibres to provide sustained antibacterial and antifungal action during prolonged sporting events such as cycle races or cross country skiing.

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FIGHT AGAINST VIRUS

- Use of nano silver-titanium dioxide coating (NSTDC) spray is utilized to help fend off the spread of the flu virus in different areas of the country from where people can easily get colds and the flu through common surfaces
- NSTDC disinfectant spray has been certified as effective at killing wide ranges of bacteria, mold and viruses, including the H1N1 virus
- It is demonstrated that only very smallest silver particles no larger than 10 nm have the ability to inactivate the virus.
- Silver nanoparticles are able to bond to the deadly viral microorganisms and prevented the virus from bonding with the host cell to cause infection

NANOSILVER TECHNOLOGY IN DAILY LIFE

- The Nano Silver Technology has influenced electronic products in the market including nano-silver lined refrigerators, air conditioners and washing machines.
- Nano Silver Technology unites microparticles of ceramic and silver for a unique combination of benefits that create smooth, silky hair and generate negative ions that eliminate frizz and close the cuticle, creating a smooth, glossy shine
- Other current applications for silver nanoparticle impregnated materials include: Toys, Baby pacifiers, Clothing, Food storage containers, Face masks, HEPA filters, Laundry detergent

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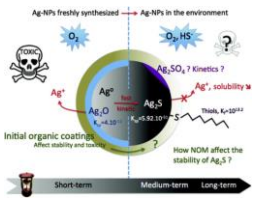
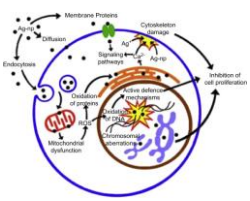
Silber-Ion , Silbersulfid, Silbersulfat , Tox





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Novel foods

What are novel foods?
Novel foods are all foods that have not been used for human consumption to a significant degree within the European Union before 15 May 1997, irrespective of the dates of accession of Member States to the Union, and fall into at least one of the following 10 food categories:
1.with a new or intentionally modified molecular structure (e.g. tagatose, salatrim)
2.consist of or are isolated from microorganisms, fungi or algae (e.g. algae oil from the microalgae *Ulkenia sp.*)
3.consist of or are isolated from materials of mineral origin (e.g. clinoptilolite (zeolite))
4.consist of or are isolated from plants and parts of plants (e.g. noni juice (*Morinda citrifolia*), chia seeds*(Salvia hispanica)*)
5.consist of or have been isolated from animals or their parts (e.g. insects, oil from Antarctic krill*(Euphasia superba)* , peptides from the fish *Sardinops sagax*)
6.cell and tissue cultures from animals, plants, microorganisms, fungi or algae (e.g. extract from cell cultures of *Echinacea angustifolia*, in vitro meat)
7.food resulting from a production process not used for food production within the Union before 15 May 1997 resulting in a change in composition or structure (e.g. high pressure pasteurised fruit preparations, UV-treated mushrooms*(Agaricus bisporus)*, UV-treated baker's yeast*(Saccharomyces cerevisiae)*, UV-treated milk)
8.consist of engineered nanomaterials (according to Article 3, Para. 2, lit f)
9.vitamins, minerals and other substances (e.g. iron (II) ammonium phosphate, vitamin K2 (menaquinone), chromium picolinate)
10.used exclusively in food supplements (not permitted in food categories other than food supplements) (e.g. maqui berry (*Aristotelia chilensis*), rose root*(Rhodiola rosea*)

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Novel foods

The Commission considers foods and food ingredients that have not been used for human consumption to a significant degree in the EU before 15 May 1997 novel foods and novel food ingredients.

Applies to foods and food ingredients which satisfy the decription and fall into one of the following categories:
Foods and food ingredients
- which present a new or modified primary molecular structure;
- which consist of micro-organisms, fungi or algae;
- which consist of or are isolated from plants and ingredients isolated from animals;
- whose nutritional value, metabolism or level of undesirable substances has been significantly changed by the production process.

They:
Must be safe for consumers.
Must be properly labelled to not mislead consumers.
Can not be nutritionally disadvantageous.

What the Novel Food Regulation does not cover
The Regulation does not cover :
- Food additives
- Flavourings for use in foods
- Extraction solvents used in the production of foods
- GMOs for food and feed

If foods and/or food ingredients were used exclusively in food supplements, new uses in other foods require authorisation under the Novel Food Regulation e.g. food fortification require authorisation.

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Do novel foods have to be safe?
Novel foods must be subject to a uniform safety assessment before they can be placed on the market in the EU. Novel foods must not pose a risk to the consumer and must not be misleading. Furthermore, they must not differ from the conventional foods and food ingredients they are intended to replace in such a way that their normal consumption would result in nutritional deficiencies for the consumer.

What is not covered by the Novel Food Regulation?
Food additives, food flavourings, food enzymes, genetically modified food and extraction solvents for the production of food are not novel foods, as they are subject to their own legal regulations (according to Article 2, para. 2).
Clarification of Novel Food Status

The food business operator is responsible for verifying whether the food to be placed on the market is a novel food. To clarify the Novel Food status, it is recommended to consult the Union list (Implementing Regulation (EU) 2017/2470 as amended consolidated version) as well as the Novel Food Catalogue of the European Commission. The Novel Food Catalogue of the European Commission provides information on the Novel Food status of foods and ingredients. Since 01 January 2018 there is the Union list, a positive list in which all approved Novel Foods are listed. If a Novel Food is already listed in the Union list, it can be placed on the market under compliance with the conditions of use and specifications. Another aid for clarifying the Novel Food status are the German Substance Lists, which are intended to provide an overview of the use of plants and fungi in foodstuffs.
For determining the criterion "significant consumption before 15 May 1997", the guideline "human consumption to a significant degree" published by the European Commission is used.
In case of existing uncertainty as to whether the food is an unauthorised novel food, the food business operator may consult the competent authority of the Member State in which the potentially novel food is to be placed on the market first (= Consultation procedure according to Article 4 of Novel Food Regulation (EU) 2015/2283).

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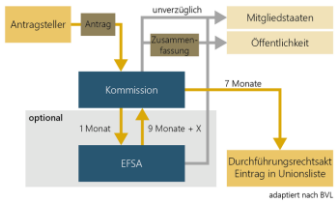
Authorisation process



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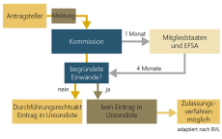
Notification of a traditional food from third countries
There is facilitated market access into the EU for traditional foods from third countries. However a safe history of use of at least 25 years outside the EU has to be proven. But this only applies to plants, animals, micro-organisms, fungi, algae and cell and tissue cultures.
If there are no objections to the notification of the traditional food, it is entered on the Union list by means of an implementing act. In case of safety concerns, an authorisation procedure with shorter deadlines is possible (Article 16). EFSA has also published guidance on the notification of traditional foods from third countries. The procedure for notification of a traditional food is regulated in the Implementing Regulation (EU) 2017/2468. Currently ongoing applications for authorisation of a novel food as well as a traditional food from third countries can be viewed online at the European Commission.



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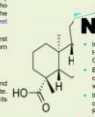
Stevia

Historical Background:

Stevia has been used over centuries by the Guarani people of Brazil and Paraguay, who called it *ka'a he'e* ("sweet herb"), to sweeten the local yerba mate tea, as medicine, and as a "sweet treat"

In 1899, botanist Moisés Santiago Bertoni first described the plant as growing in eastern Paraguay, and observed its sweet taste.

In 1931, chemists M Bridel and R Lavielle isolated the glycosides stevioside and rebaudioside that give the leaves their sweet taste. The exact structures of the aglycone steviol and its glycosides were published in 1955.



Novelty

- In 1991, the FDA refused to approve stevia as a sweetener as an additive in foods. However, in 2008, after the purification process was developed and patented by Coca-Cola, the FDA approved the stevia extracts as GRAS.
- Based on the JECFA (Joint Expert Committee on Food Additives) declaration, safe consumption of steviol glycosides for humans is determined to be 4 mg per kg body weight per day.
- It was also agreed by the European Commission in 2011 for use in food in European countries. Steviol glycosides have also been accepted in the US as GRAS (Generally Recognized as Safe).
- Stevia leaf and raw extracts are not treated as GRAS and their import into the US is not allowed for usage as sweeteners. Nutrition, Center for Food Safety and Applied (9 February2019). "Additional Information about High-Intensity Sweeteners Permitted for Use in Food in the United States". FDA.
- **Note:**
Stevioside was found to be nontoxic in acute toxicity studies.

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Authorisations of novel foods and novel food ingredients by Commission Decisions

" the placing on the market of…as a novel food ingredient"

2013
- zeaxanthin
- an extension of use of Chia (Salvia hispanica) seed

2012
- bovine lactoferrin
- dihydrocapsiate
- Gamma-Cyclodextrin
- novel chewing gum base

2011
- novel chewing gum base
- yeast beta-glucans
- Phosphatidylserine from soya phospholipids
- fermented black bean extract
- phosphated maize starch
- Chromium Picolinate
- chitin-glucan from Aspergillus niger
- mycelial extract from Lentinula edodes (Shiitake mushroom)
- Chromium Picolinate ingredient
- a fish (Sardinops sagax) peptide product
- a chitin-glucan from Aspergillus niger
- a mycelial extract from Lentinula edodes (Shiitake mushroom)

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2010
- ferrous ammonium phosphate
- Ferric Sodium EDTA
- puree and concentrate of the fruits of Morinda citrifolia (Noni)

2009
- Chia seed (Salvia hispanica)
- a leaf extract from Lucerne (Medicago sativa) *sinimailanen*
- the uses of algal oil from the micro-algae Schizochytrium sp.
- the uses of algal oil from the micro-algae Ulkenia sp.
- a lipid extract from Antarctic Krill Euphausia superba
- lycopene
- lycopene from *Blakeslea trispora*
- lycopene oleoresin from tomatoes
- lycopene as a novel food ingredient
- Ice Structuring Protein type III HPLC 12
- Vitamin K2 (menaquinone) from *Bacillus subtilis natto*

2008
- leaves of Morinda citrifolia (Noni)
- arachidonic acid-rich oil from Mortierella alpina (belong to soil fungi)
- Baobab dried fruit pulp (a tree native to Africa, Australia, Madagaskar, Arabian Peninsula)
- allanblackia seed oil (flowering plant in the Clusiaceae family, African)
- refined echium oil (a genus of 60 species of flowering plant in the family Boraginaceae. Native to North Africa, mainland Europe and the Macaronesian islands)
- alpha-cyclodextrin
- rice drinks with added phytosterols/phytostanols (Teriaka Ltd)

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2006-2007
- oil enriched with phytosterols/phytostanols
- diacylglycerol oil of plant origin
- lycopene from Blakeslea trispora
- rapeseed oil high in unsaponifiable matter
- maize-germ oil high in unsaponifiable matter"
- foods and food ingredients derived from genetically modified maize line MON 863
- rye bread with added phytosterols/phytostanols (Fazer, Pharmaconsult)
- foods and food ingredients produced from genetically modified Roundup Ready maize line GA21

2004-2005
- isomaltulose
- foods and food ingredients derived from genetically modified maize line NK 603
- milk based beverages with added phytosterols/phytostanols
- sweet corn from genetically modified maize line Bt11
- yellow fat spreads, milk based fruit drinks, yoghurt type products and cheese type products with added phytosterols/phytostanols (Teriaka Ltd)
- milk type products and yoghurt type products with added phytosterol esters
- yellow fat spreads, milk type products, yoghurt type products, and spicy sauces with added phytosterols/phytostanols (Pharmaconsult Oy Ltd. (formerly MultiBene Health Oy Ltd))
- yellow fat spreads, salad dressings, milk type products, fermented milk type products, soya drinks and cheese type products with added phytosterols/phytostanols

2000-2003
- Salatrim
- oil rich in DHA
- 'noni juice'
- coagulated potato proteins and hydrolysates thereof
- dextran preparation produced by Leuconostoc mesenteroides.
- pasteurised fruit-based preparations produced using high-pressure pasteurisation.
- trehalose
- "yellow fat spreads with added phytocolesterol esters"
- "phospholipides from egg yolk"

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Refusals of authorisation of novel foods and novel food ingredients by Commission Decisions

2000-2005
- Betaine
- "Nangai nuts" *Canarium indicum* L (dried seed kernels)
  http://old.eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:004:0035:0035:EN:PDF
- *Stevia rebaudiana Bertoni* plants and dried leaves
  http://old.eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:061:0014:0014:EN:PDF

Note! Usage of steviol glycosides from leaf extracts as sweeteners has been accepted (since 2.12.2011, EU food additive legislation)

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Examples of open status

- Fresh dried Noni powder
- Juices and nectars with added phytosterols
- Glucosamine hydrochloride from *Aspergillus niger*
- Conjugated Linoleic Acid (CLA)
- Policosanol
- Cis-9-cetyl myristoleate
- CLA
- Lactoferrin
- D-Ribose
- Astaxanthin
- Methyl Vinyl Ether-Maleic Anhydride Copolymer
- Glicine propionyl L-Carnitine hydrochloride
- *Arracacia xanthorrhiza* (with objections, is undergoing EFSA consultation)
- Isomaltooligosaccharide
- Bee venom
- L-Theanine
- Pectine-derived Acidic Oligosaccharide
- Taxifolin
- Selenitetriglycerides
- Rooster combs extract
- Modified apple drink
- *Tetrasemilis chuii* (microalga)
- *Nannochloropsis gaditana* (micro-alga)
- Coriander seed oil
- Cranberry extract powder

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- Hydroxypropyl methyl cellulose (HPMC)
- (6S)-Methyltetrahydrofolic acid, Glucosamine salt
- *Clostridium butyricum* as an ingredient to food supplements
- Oil from the crustacea *Calanus finmarchicus*
- UV treated yeast
- Citicoline
- Tolerase
- Rapeseed protein
- UV treated bread
- Methyl Cellulose
- Resveratrol
- UV treated milk
- Chia oil (applied 2012)
- Pyrroloquinoline Quinone Disodium Salt)
- Milk based products with *Bacteroides xylanisolvens*
- Astaxanthine
- UV-treated bread with vitamin D

Applications 2014
- Peels of Sporopollen
- Lacto-N-neotetraose
- Phospholipids-rich krill oil
- Cycloastragenole
- EstroG-100
- Novel use of Glavonoid
- Nattokinase (fermented soy bean extract)
- *Hovenia dulcis* (applications not accepted by any country)

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Novel Food Catalogue
http://ec.europa.eu/food/food/biotechnology/novelfood/nfnetweb/mod_search/index.cfm

- lists products of plant and animal origin and other substances subject to the Novel Food Regulation, after EU countries and the Commission agree in the Novel Food Working Group.
- non-exhaustive, and serves as orientation on whether a product will need authorisation under the Novel Food Regulation.
- EU countries may restrict the marketing of a product through specific legislation. For information, businesses should address their national authorities.
- In some cases, it shows EU countries' history of use of food supplements and ingredients used exclusively in food supplements.
- If foods and/or food ingredients were used exclusively in food supplements, new uses in other foods require authorisation under the Novel Food Regulation.

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Novel foods and novel foods ingredients – Authorisations
http://ec.europa.eu/food/food/biotechnology/novelfood/authorisations_en.htm

Foods that may be placed on the market in the EU (Regulation (EC) No. 258/97 Article 4.2 first indent)

2012
- DHA and EPA rich oil from the microalgae *Schizochytrium*
- Synthetic Vitamin K 2
- Krill oil (extension of uses)

2011
- Arachidonic acid-rich oil from the fungus Mortierella alpina
- Magnolia bark extract
- Zinc L-pidolate
- Wheat bran

2010
- Guar gum
- Sucromalt

2005
- D-Tagatose

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Noni

By 2003
only 1 traditional food product was authorized: noni juice by Morinda Inc (2003)
Other noni products require separate authorization

In 2004
application for the use of noni leaves as herbal tea submitted by Morinda Inc

In 2008
authorization of the use noni leaves as herbal tea

By 2009
substantial equivalence of products from 48 companies to those of Morinda Inc demonstrated, resulted in authorization (33 from Europe, 7 from Polynesia, 3 from USA, 3 from Central America and Caribbean, 1 from New Zealand, 1 from Switzerland)

source: Hermann M. Food Policy 34 (2009) 499-507

Now also noni puré and concentrate authorized

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Points to consider (from Hermann M. The impact of the European Novel Food Regulation on trade and food innovation based on traditional plant foods from developing countries. Food Policy 34 (2009) 499-507.)

- Market access outside EU (many of the novel foods available in Canada, USA, Switzerland and Japan), re-directing of the marketing due to restrictions in Europe?
- Importance of traditional exotic foods to the economics of poor countries and to the diet diversification among EU consumers? The regulation is critized being a non-tariff trade barrier for food that is "exotic" from the EU perspective.
- Would separate categories be needed for exotic traditional foods and "true" novel, innovative foods with no long-term consumption outside the EU?
  - Now extensive data is required of composition, nutritional aspects, intake, toxicology and allergenic potential, also for products that are generally regarded as safe (GRAS) outside the EU
  - Are the requirements even stricter than those required for accepted traditional European foods?
  - Are the scientific requirements proportionate to the potential risks they pose?
  - Currently, the history of safe use outside the EU is not considered - should traditional knowledge be admitted for food safety assessment?
  - Lack of peer-reviewed publications, lack of data from certified laboratories – should be taken into account in project design, product development and trade promotion
  - Traditional knowledge from the local people should be used.
- Would the potato be authorized nowadays (glycoalcaloids)? Wheat (gluten)?

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European Federation of Associations of Health Food Products Manufacturers (EHPM) Briefing Paper 2014
http://www.federsalus.it/drive/File/documenti_news/1397809900EHPM%20Position%20paper%20novel%20food%20aprile%202014.pdf

criticism of the revision proposal of European Commission

- Lack of categories:
  According to the proposal, categories are not used to define novelty of food. This would deny countless products from the market access without no true safety concern
  New proposal: categories are needed and they will need to be fine-tuned. The extent to which the novel food system can be applied to plant extracts should be clarified.
- Definition:
  "used to significant degree…" What is significant? Companies are only required to keep accounts dating back a period of 7 years. Evidence of sales through pharmacies is given more importance than other channels.
  New proposal: guidance from the commission should be revised to take all channels of distribution into account.
- Lack of timeframe for decision:
  No timeframe for a Standing Committee to make the decision based on a proposal from the Commission decision.
  New proposal: timeframe of 5-9 months should be set for a Standing Committee decision.
- Borderline, novel vs medicinal food
  Member states are able to classify certain products containing a novel food as medicinal based on the functionality of the novel food. Therefore, the product can stay out of the market in certain member states despite authorisation.
  New proposal: This internal trade barrier should be addressed and guarded against.

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Health claim regulation

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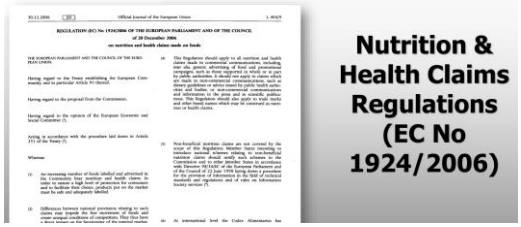

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- Depends on nature of claim & evidence it's based on…
- 3 mechanisms in Regulation:
  - Article 13(1) claims based on "generally accepted scientific evidence"
  - Article 13(5) claims based on new or emerging science or which use proprietary data (i.e. company specific)
  - Article 14 claims based on "generally accepted scientific evidence" or new/emerging science

Article 13(5) claims

- Based on newly developed scientific evidence and/or involve proprietary data
  "Sugar beet fibre contributes to an increase in faecal bulk."
- EFSA has 5 months to evaluate & give opinion (but can be extended by 1 month)
- After 30 day consultation period, EC has 2 months to authorise

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Article 14(1)(a) & (b) claims

- Can be based on GAS or newly developed scientific evidence
  "Plant stanol esters have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease." 14(1)(a)
  "Calcium is needed for normal growth and development of bone in children." 14(1)(b)

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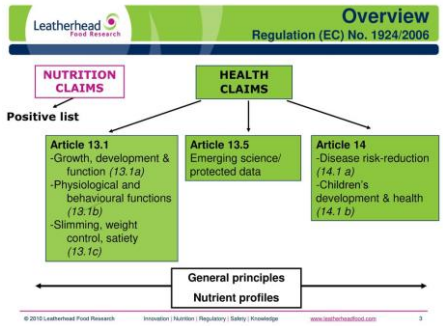

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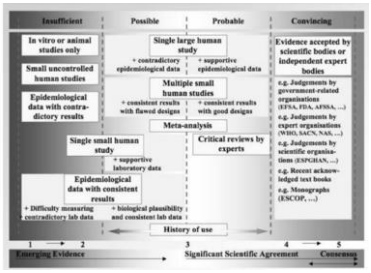

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Examples types of authorised claims	Examples of nutrient, substance, food or food category	Examples of nature of claim	Main conditions of use	Comments
Article 13.1 'GAS'	Barley grain fibre	Increases faecal bulk	Food to meet 'high fibre' as defined in NHC Annex	229 claims cover only 68 distinct nutrients; most relating to vitamins & minerals
Article 13.5 'new' science	Carbohydrates	Help muscle recovery after exercise	Food to provide 4 g/kg BW within 4-6 hours … etc.	5 claims, 4 of which have been granted data protection
Article 14(1)a 'reduction of disease risk'	Plant sterols/stanols	Lowers blood cholesterol, a risk factor in CHD (coronary heart disease)	Daily food to supply 1.5-3 g plant sterols/stanols, for 2-3 weeks etc. www.	14 claims, 7 of which relate to cholesterol/CHD; 3 to improved bone density & reduced risk of osteoporotic fractures; 3 to reduced risk of dental caries, & 1 folic acid) to reduced risk of foetal neural tube defects.
Article 14(1)b 'children's development/health'	Docosahexaenoic acid (DHA)	Contributes to normal visual development in infants to 12 months	Daily food to supply 100 mg of DHA or in follow-on formulae, minimum 0.3% of the total fatty acids as DHA	11 claims, 4 of which relate to essential fatty acids, 6 to essential vitamins/ minerals, & 1 to protein

Table 1. Summary of authorised claims on EU foods (derived from the EU register on nutrition and health claims)

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Examples types of non-authorised claims	Examples of nutrient, substance, food or food category	Examples of nature of claim	Reasons for non-authorisation	Comments
Article 13.1 'GAS'	Bifidobacterium animalis ssp. lactis BB-12®	Probiotic. Contributes to beneficial gut bacteria, aiding digestion.	Claim not substantiated	1,729 'GAS' claims rejected
Article 13.5 'new' science	'NPU' food supplements containing hops	'NPU' imitate female breasts enhancement process by 8-PN (8-prenylnaringenin)	Claim not substantiated	32 'new' science claims rejected
Article 14(1)a 'reduction of disease risk'	Glucosamine HCl	Reduces articular cartilage destruction & risk of osteoarthritis.	Claim not substantiated	20 'reduction of disease risk' claims rejected
Article 14(1)b 'children's development/health'	Beta-palmitate	Contributes to increased calcium absorption	Claim not substantiated	26 claims on 'children's development or health' rejected

Table 2. Summary of non-authorised claims on EU foods (derived from the EU register on nutrition and health claims)

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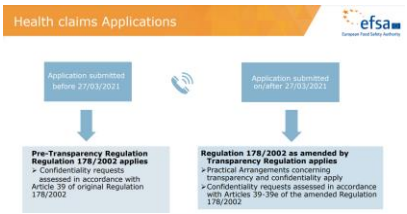

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Personalisation

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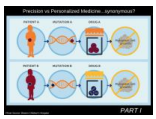
Discussion:
Prevention, intervention: personal precision medicine, personal precision nutrition



Objectives aging:
- longevity,?
- healthy life span ?
- age related complex diseases?

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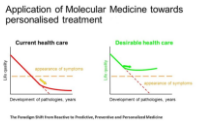
Discussion
Analysis of molecular markers of different aging mechanisms and functional foods adressing the personal hazard may contribute to a personal, preventive health care, disease prevention, healthy aging



Objectives aging:
- longevity,
- healthy life span
- age related complex diseases?

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Discussion: Epigenetic and Salutogenesis the bridge between scientific reductionism of markers and mechanisms and the need address the entire person ?

Markers and mechanims, omics

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1.2 Was sind „neuartige Lebensmittel"?

„Neuartige Lebensmittel" (englisch *„Novel Foods"*) sind nicht nur *„gentechnische hergestellte Lebensmittel"*, sondern auch Lebensmittel, die unter Verwendung von ***nicht traditionellen Rohmaterialien*** und dem Einsatz ***neuartiger Technologien*** produziert oder zubereitet worden sind, welche bis zum 27.05.1997 ***nicht in nennenswertem Umfang*** in der EG verzehrt wurden.

Mögliche Beispiele sind:

- Produkte mit neuen Strukturen:
  - **Phytosterolester, Fettersatzstoffe, Süßungsmittel**
- Lebensmittel aus nicht traditionellen Rohstoffen:
  - **Single Cell Proteine, Algen**
- Produkte aus fremden Kulturkreisen:
  - **Geröstete Heuschrecken, exotisches Obst und Gemüse**
- Neue technische Verfahren:
  - **Hochdrucksterilisierung**

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Fettersatzstoffe

2.5.2 Fettersatzstoffe

- Einteilung, Anforderungen
- **Salatrim**
  - Spezifikation
  - Sicherheitsbewertung
- **Olestra**
  - Spezifikation
  - Sicherheitsbewertung

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LM aus nicht traditionellen Rohstoffen

2.6 Lebensmittel aus nicht traditionellen Rohstoffen

- Lycopin aus Pilz Blakeslea Trispora
- Synthetisches Lycopin
- Lycopin-Oleoresin aus Tomaten
  - Sicherheitsbewertungen durch die EFSA
  - Anwendung
    Herstellmethoden von Nährstoffen / Probleme bei der lebensmittelrechtlichen Abgrenzung als Novel Food

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Zb Lycopin

Lycopin - Herstellung

- **Mit Hilfe spezieller, lebensmittelrechtlich zugelassener Lösungsmittel wird Lycopin aus Tomaten (*Lycopersicon esculentum* L.) extrahiert. Ein Kilogramm Tomaten enthält etwa 20 mg Lycopin.**
- **Häufiger als der isolierte Farbstoff wird Tomaten-Extrakt eingesetzt. Er gilt, wenn nicht der enthaltene Anteil Lycopin gezielt erhöht wurde, als färbendes Lebensmittel. Wenngleich Tomaten-Extrakt keine E-Nummer trägt, ist er doch in der Zutatenliste aufgeführt.**
- **Lycopin kann auch chemisch-synthetisch hergestellt werden. Gemäß einer Stellungnahme des Wissenschaftlichen Lebensmittelausschusses der EU vom Dezember 1999 darf synthetisches Lycopin jedoch nicht als Zusatzstoff eingesetzt werden (SCF/CS/ADD/COL/160 Final). Dies wird damit begründet, dass das synthetische Präparat anders als das durch Extraktion gewonnene zusammengesetzt sei und entsprechende toxikologische Untersuchungen bisher fehlten.**

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LM ethnic

2.7 Produkte aus fremden Kulturkreisen

- Noni-Saft (Fruchtsaft aus *Morinda citrifolia*)
  - Taxonomie, traditionelle Verwendung
  - Herstellung und Verwendungszweck
  - Wirkungsbehauptungen
  - Sicherheitsbewertung von Noni-Saft
- Nangai-Nüsse (Canarium indicum L.) aus südpazifischen Anbau
  - Taxonomie, traditionelle Verwendung
  - Sicherheitsbewertung von Nangai-Nüssen
  - Entscheidung der Kommission vom 19. Dezember 2000 zum Verbot des Inverkehrbringens von „Nangainüssen" als neuartiges Lebensmittel

Sicherheitsbewertung

- In seiner Stellungnahme vom 4. Dezember 2002 war der SCF zu der Auffassung gelangt, dass Tahitian Noni®-Saft in den beobachteten Verzehrmengen akzeptabel ist.
- Die **EFSA** hat am 6. September 2006 erneut die Sicherheit von Noni-Saft bewertet. Sie war von der Europäischen Kommission um eine wissenschaftliche Stellungnahme gebeten worden. Sie sollte bewerten, ob die berichteten Fälle von akuter Hepatitis einen Einfluss auf die Sicherheit von Noni-Saft haben würden.
- Noni-Saft ist seit 2003 als neuartiges Lebensmittel zugelassen, vermarktet zu werden. Das NDA-Gremium der **EFSA** kam zu dem Ergebnis, dass es keine schlüssigen Beweise für einen kausalen Zusammenhang zwischen den in den berichteten Fällen beobachteten akuten Hepatitis und dem Verzehr von Noni-Saft gibt.
- Unter Berücksichtigung der verfügbaren Informationen ist es unwahrscheinlich, dass der Verzehr von Noni-Saft in den festgestellten Verzehrmengen unerwünschte Nebenwirkungen auf die menschliche Leber auslösen könnte.

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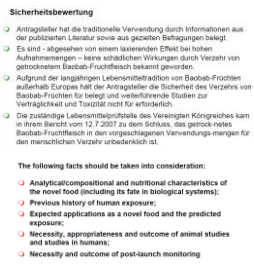
2.7 Baobab-Fruchtfleisch

Taxonomie, traditionelle Verwendung

- Der Baobab, *Adansonia digitata* Linné (1753), wird zur Familie der Bombacaceae (Wollbaumgewächse) gezählt. Häufig wird er auch als Affenbrotbaum bezeichnet, da die Früchte gern von Affen gefressen werden.
- Früchte und auch andere Teile (Samen, Wurzeln, Blätter, Blüten und Rinde) des Affenbrotbaumes werden von der afrikanischen Bevölkerung traditionell vielseitig verwendet.



Sicherheitsbewertung

- Antragsteller hat die traditionelle Verwendung durch Informationen aus der publizierten Literatur sowie aus gezielten Befragungen belegt.
- Es sind - abgesehen von einem laxierenden Effekt bei hohen Aufnahmemengen – keine schädlichen Wirkungen durch Verzehr von getrocknetem Baobab-Fruchtfleisch bekannt geworden.
- Aufgrund der langjährigen Lebensmitteltradition von Baobab-Früchten außerhalb Europas hält der Antragsteller die Sicherheit des Verzehrs von Baobab-Früchten für belegt und weiterführende Studien zur Verträglichkeit und Toxizität nicht für erforderlich.
- Die zuständige Lebensmittelprüfstelle des Vereinigten Königreiches kam in ihrem Bericht vom 12.7.2007 zu dem Schluss, das getrock-netes Baobab-Fruchtfleisch in den vorgeschlagenen Verwendungs-mengen für den menschlichen Verzehr unbedenklich ist.

The following facts should be taken into consideration:

- **Analytical/compositional and nutritional characteristics of the novel food (including its fate in biological systems);**
- **Previous history of human exposure;**
- **Expected applications as a novel food and the predicted exposure;**
- **Necessity, appropriateness and outcome of animal studies and studies in humans;**
- **Necessity and outcome of post-launch monitoring**

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Functional foods,additives
health claim regulation
Personalisation

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- Lebensmittel vs. Arzneimittel?
  - Bei Pflanzen-Extrakten große kulturelle Unterschiede in den Mitgliedstaaten der EU
    Länder mit langer Tradition, aber sehr unterschiedlichen Handhabungen (pos. vs neg. Liste, LM vs. AZM)
- Neuartig (Novel Food) oder nicht?
  - Vielfältige und nicht konsistente Interpretation sowohl auf Mitgliedstaaten als auch EU-Ebene
  - **Folge** → große Unsicherheiten, eingeschränkte Entwicklungsmöglichkeiten

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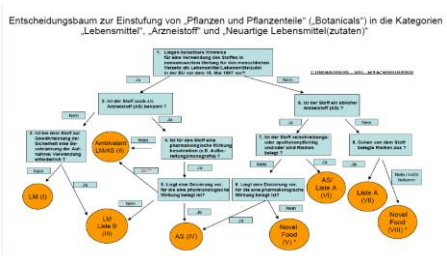
Food improvement



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Safe level

Setting the "safe level"
As part of its safety evaluations of food additives EFSA seeks to establish, when possible (e.g. when sufficient information is available), an Acceptable Daily *Intake* (*ADI*) for each substance.

The ADI is the amount of a substance that people can consume on a daily basis during their whole life without any appreciable health risk. ADIs are usually expressed in mg per kg of body weight per day (mg/kg bw/day). The ADI can apply to a specific additive or a group of additives with similar properties. When re-evaluating previously authorised additives, EFSA may either confirm or amend an existing ADI following review of all available evidence.
When there are insufficient data for establishing an ADI, a *margin of safety* may be calculated to determine whether estimated *exposure* might be of potential concern.
In other cases, for example, for substances that are already present in the body or regular components of the diet or that did not indicate adverse effects in animal studies, there is no need to set an ADI.

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Green tea is produced from the leaves of *Camellia sinensis* (L.) Kuntze, without fermentation, which prevents the oxidation of polyphenolic components. Most of the polyphenols in green tea are catechins.
The Panel considered the possible association between the consumption of (-)-epigallocatechin-3-gallate (EGCG), the most relevant catechin in green tea, and hepatotoxicity. This scientific opinion is based on published scientific literature, including interventional studies, monographs and reports by national and international authorities and data received following a public 'Call for data'.
The mean daily intake of EGCG resulting from the consumption of green tea infusions ranges from 90 to 300 mg/day while exposure by high-level consumers is estimated to be up to 866 mg EGCG/day, in the adult population in the EU. Food supplements containing green tea catechins provide a daily dose of EGCG in the range of 5–1,000 mg/day, for adult population. The Panel concluded that catechins from green tea infusion, prepared in a traditional way, and reconstituted drinks with an equivalent composition to traditional green tea infusions, are in general considered to be safe according to the presumption of safety approach provided the intake corresponds to reported intakes in European Member States. However, rare cases of liver injury have been reported after consumption of green tea infusions, most probably due to an idiosyncratic reaction. Based on the available data on the potential adverse effects of green tea catechins on the liver, the Panel concluded that there is evidence from interventional clinical trials that intake of doses equal or above 800 mg EGCG/day taken as a food supplement has been shown to induce a statistically significant increase of serum transaminases in treated subjects compared to control.

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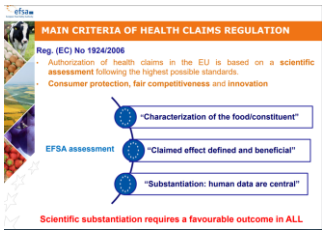
Health claim regulation



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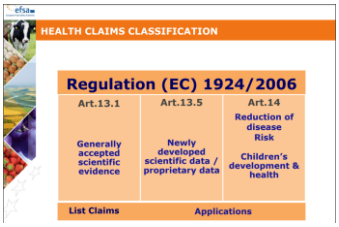
Health claim classification



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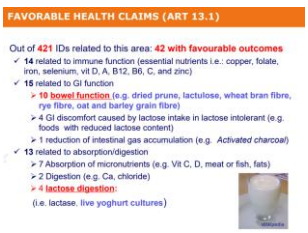
Examples 13.1



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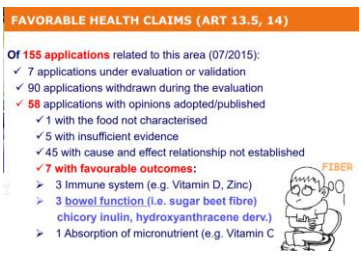
Examples 13.5



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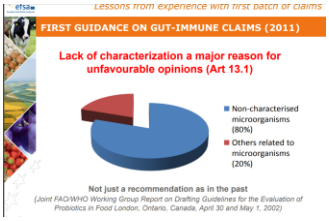
Problems of gut immune claims (eg probiotoics



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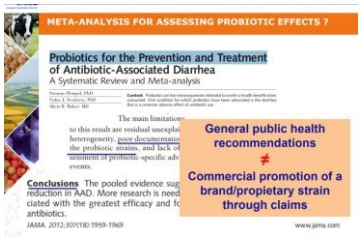

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New developmements

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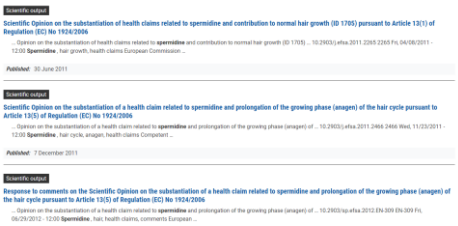
Spermidine



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Basis: dietary reference values
1992 US USDA-Pyramide, an individualised approach, age, lifestyle (work)

John Neustadt
Integrative Medicine 2005

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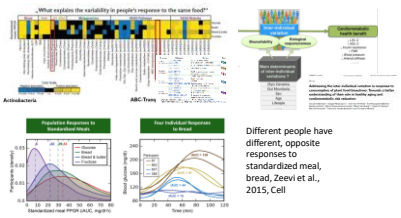
What explains the variability in responses to food ?
E.g. Highly different personal post- prandial glycemic responses



Different people have different, opposite responses to standardized meal, bread, Zeevi et al., 2015, Cell

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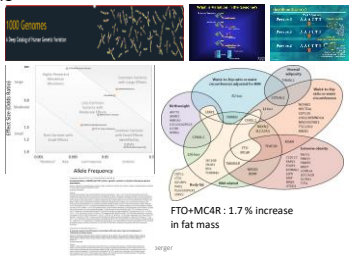
GWAs, SNPs, rare: common variants moderate, large effects; in different areas



FTO+MC4R : 1.7 % increase in fat mass

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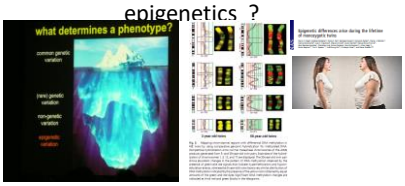
The penetrance of SNPs,
DTC genetic testing for nutritional advice

For diseases controlled by 1000 loci of mean relative risk of only 1.04, a case-control study with 10,000 cases and controls can lead to selection of ~75 loci that explain >50% of the genetic variance. The 5% of people with the highest predicted risk are three to seven times more likely to suffer the disease than the population average, depending on heritability and disease prevalence. Whether an individual with known genetic risk develops the disease depends on known and unknown environmental factors.

Prediction of individual genetic risk to disease from genome-wide association studies

404

Missing heritability: what is missing to understand a phenotype, a person: gene- environment interactions epigenetics ?



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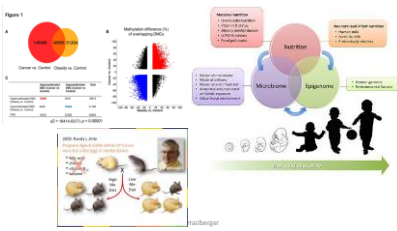
Methylation of CpGs established as marker in nutrition, Agouti mouse: nutrition modulated: interaction nutrition- microbiome-epigenome



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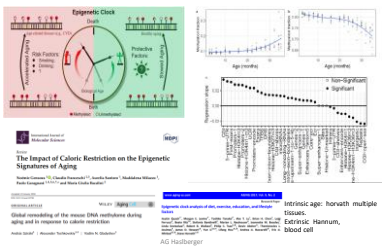
Epigenetic clock, CR, nutrition



Intrinsic age: horvath multiple tissues,
Extrinsic Hannum, blood cell

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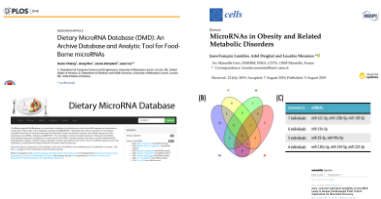
Epigenetic miRNAs: food borne, marker for mechanisms, phenotypes, disorders



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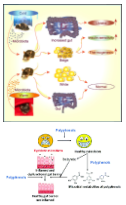
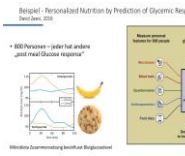
Individual diversity of gut microbiota reflects nutrition and lifestyle, mediates individual different energy extraction, and different metabolisation

Davis SC et al. / Microbiologyopen 2017



409

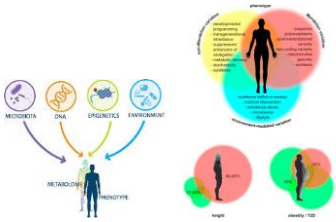
Individual glucose-responses mediated by microbiota structure, personal nutrition predictor, advise



"it's fun to get a selfie of "your inner self", Joel Dore

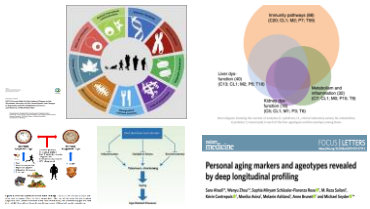
410

Personalisation: obesity: only 30-40 % linked to mendelial variation, role for gene- environment (epigenetic?) interactions



411

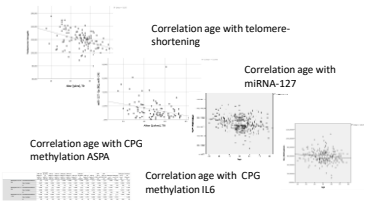
Personal different responses to nutriton affect personal different developments of hallmarks of aging, types of aging, ageotypes



Personal aging markers and ageotypes revealed by deep longitudinal profiling

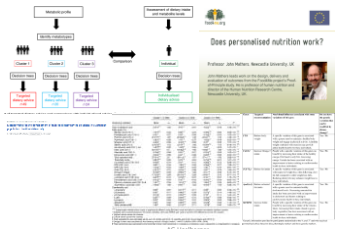
412

Faces of personal aging: correlations age with telomers, miRNAs, CPG-methylation, inflammation



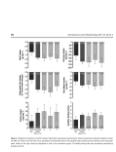
413

Consequences personalised nutrition, EU- Food4me Study,

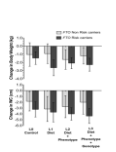


414

EU- Food4me study results prove „personal nutrition does better than on size fits all“,
J. Mathers



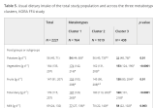
Changs of dietary intake after personalised advice
Healthy eating index



Changes in adiposity markers were greater in participants who were informed that they carried the *FTO* risk allele (level: 3 AT/AA carriers) than in the nonpersonalized group

415

Definition of metabotypes from biochemical, genetic-, microbiota- based information,
(air-)digestion trackers; Consequences

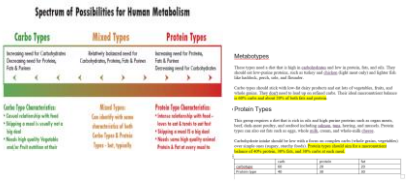


Triacylglycerol (TAG), total cholesterol (TC), HDL cholesterol, and glucose
Metabotyping seems to be a promising tool to simplify the delivery of effective advice.

Metabotype cluster 3 with the highest occurrence of diseases and risk factors, generally advised to reduce the relatively high consumption of meat and to increase the relatively low consumption of vegetables and physical activity compared to clusters 1 and 2.

416

Consequences of metabotyping, example

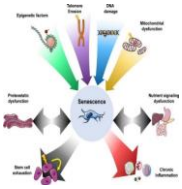


417

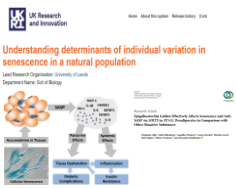
health/aging. Use of supplements, functional foods which address specific mechanisms „ Achilles Fersen Concept“

418

Aging: Increase of senescent cells in tissues, senolysis enables re-juvenation



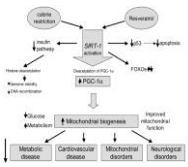
Senescence-Associated Secretory Phenotype

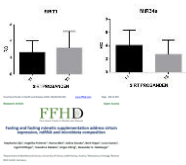


Domhnall McHughand Jesús Gil, JCB 2017

419

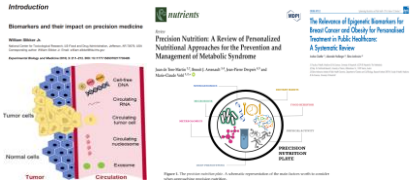
Functional foods, food additives, and precision nutrition
Sirt activation drink mimics certain effects of fasting/CR in the area of healthy aging and balances microbiota.





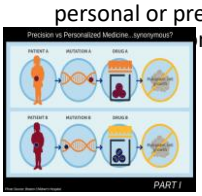
420

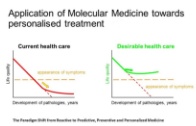
Importance of good biomarkers, the way to precision medicine, especially cfDNA
Precision Nutrition



421

intervention:
personal or precision medicine, synonyme?
personal or precision nutrition, nyme?





422

Precision, personalised nutrition, where we are, where to go

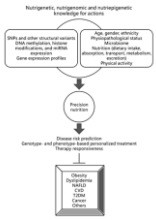
Fig. Achievements already made and challenges faced by personalised nutrition (Prasad et al., 2016)

Personalisierte Ernährung und Einteilung/ Klassifizierung von metabolischen Typen basierend auf genetischen, epigenetischen und mikrobiologischen Analysen

Personalized nutrition and classification of metabolic types based on genetics, epigenetics and gut microbiota

423

Precision-, personalised nutrition, the way we may go



Mobile apps and wearable devices facilitate real-time assessment of dietary intake and provide feedback which can improve glycaemic control and diabetes management.

By integrating these technologies with big data analytics, precision nutrition has the potential to provide personalised nutrition guidance for more effective prevention and management of complex metabolic diseases

(D. D. Wang & Hu, 2018).

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Many open questions remain …

www.alexander-haslberger.at
www.my-personal.health

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425

Vortrag AKE 2021

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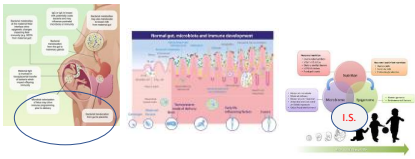
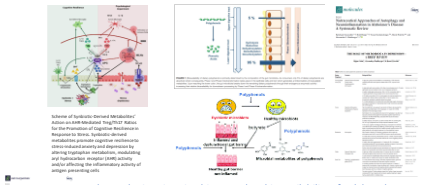
Development of microbiota, I.s., and epigenetic system, imprinting



Development prenatal, Interaction with I.S., epigenetic maternal factors, Diversity:delivery, breastfeeding, imprinting in 1000 days of life

427

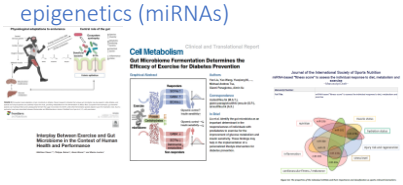
Gut brain axis: Microbial metabolites regulate treg, th17 balance, important for cognitive resilience



The gut brain axis: microbiota regulate bio-availability of polyhenol metabolites and their activity in neurological disorders,
Giulio M Pasinetti, *Icahn School of Medicine at Mount Sinai, USA*

428

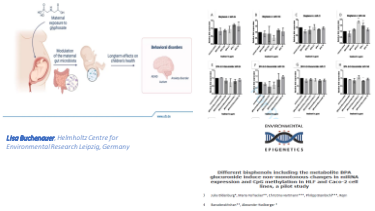
Lifestyle: Exercise modulates microbiota metabolites and epigenetics (miRNAs)



Enhanced amount of SCFA producers by exercise ?
Production of metabolites decides effectivity of exercise against diabetes

429

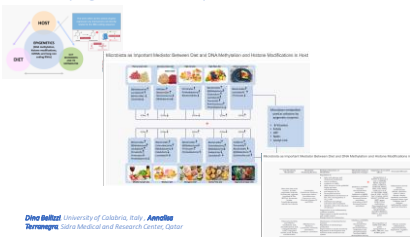
Toxins: Glyphosate, bisphenols, microbiota and epigenEtics



Lisa Buchenauer, *Helmholtz Centre for Environmental Research Leipzig, Germany*

430

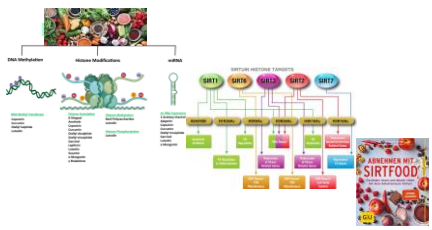
Interactions diet microbiota and Epigenetics, experience



Dina Bellizzi, *University of Calabria, Italy*, ***Annalisa Terranegra***, *Sidra Medical and Research Center, Qatar*

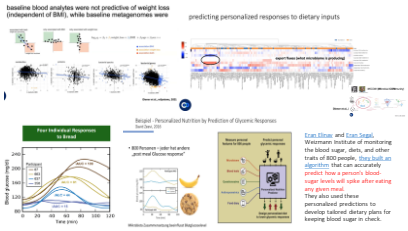
431

Dietary ingredients and microbiota derived metabolites (SCFAS) address all elements of the epigenetic system



432

Microbiota predict personal responses to diets



433

Definition of metabotypes from genetic-, microbiota-metabolomics- based information, Metabotyping, food4me

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Personalisation of Additives for Prevention: Monitoring basic hallmarks of health/aging. Use of mixes of supplements, functional foods which address specific mechanisms „Achilles Fersen Concept"

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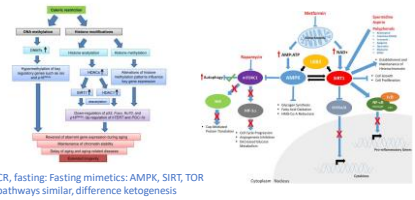
Gut host interactions of central importance case study: comparing caloric restriction : fasting Mimetics



CR, fasting: Fasting mimetics: AMPK, SIRT, TOR pathways similar, difference ketogenesis

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Study design

Buchinger Fasting < 120 kcal/day
n: 22 in Pernegg Monastery

Feces , blood spots, before and After the end, first solid feces

Active (N. 131) Placebo (n: 30)
Intervention 3 months

Feces, Blood spots before, after 1,3 month

Illuminia sequencing, Line 1 methylation bisulfite qPCR, HR-MCA, RNA, MiRNA RT QPCRi

A.G. Hadberger 2021 437

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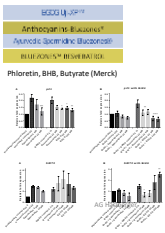
In vitro confirmation that selected compounds are active in preadipocytes, 3T3. Markers for Senescence, autophagy, expression SIRT 3. Best EGCG



Pre, Adipocytes, fat droplets
B-Gal, senescence

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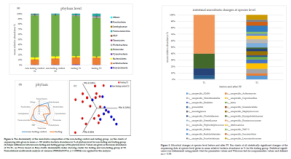
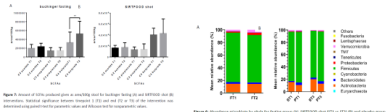
Buchinger fasting resulted in a rise in the distribution of Proteobacteria, increased microbiota diversity and a significant increase in Christensenella



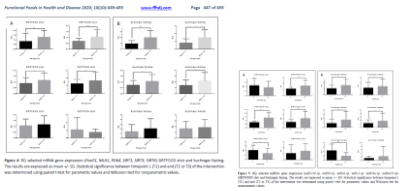
439

3M. sirt inducing drink increased *Actinobacteria (BIF),* Firmicutes/*Bacteroidetes* ratio decreased (nS) and correlated with BMI. Only Fasting increased Butyrate significantly



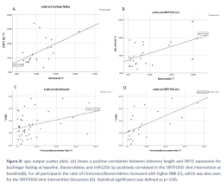
440

Fasting, but also the fasting mimetic could increase expression of *FoxO1, SIRTs, MLH1 and miRNAs* discussed in aspects of autophagy, senescence , DNA repair and longevity.



441

positive correlation of the abundance of butyrate-producing *Bacteroidetes* with Mir125, siRT-1 expression, telomere length



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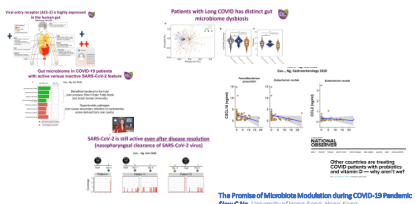
Conclusions

In conclusion fasting and to some extend fasting mimetics result in beneficial modulation of microbiota (e.g diversity, SCFA, BHP) and metabolism (e.g SIRTS, mtDNA, telomer length)

Microbiota structure seems to interfere with the expression of Sirtuins and metabolism relevant miRNAs

443

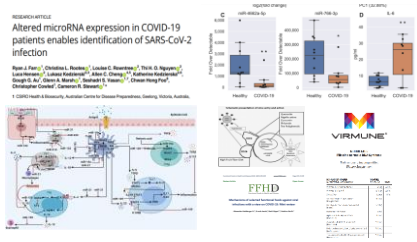
Covid, long covid, microbiota and epigenetics



The Promise of Microbiota Modulation during COVID-19 Pandemic
Siew C Ng, University of Hong Kong, Hong Kong

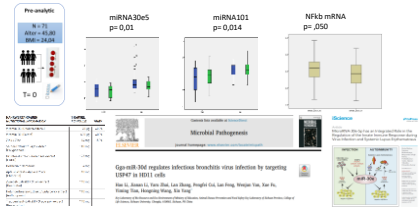
444

3 MIRNAS monitor SARS-COV-2 Infection, MIRNAS Monitor anti-viral immune- Responses



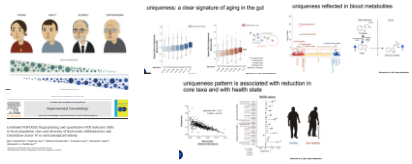
445

Modulation of I.S. -, and viral infection relevant miRNAs and inflammation related NFkb after 2 M



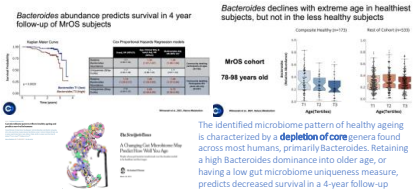
446

Aging, bacterial diversity, uniqueness and health



447

Bacteroidetes decide upon healthy aging ?



The identified microbiome pattern of healthy ageing is characterized by a **depletion of core** genera found across most humans, primarily Bacteroides. Retaining a high Bacteroides dominance into older age, or having a low gut microbiome uniqueness measure, predicts decreased survival in a 4-year follow-up

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Aging and diets are reflected in the epigenetic Clock, CPG-DNA methylation; personal types of aging can be seen in ageotypes

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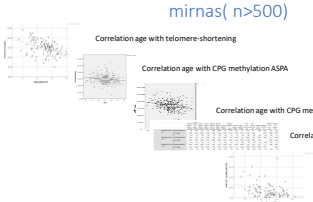
Faces of personal aging: correlations of age with telomers, CPG-methylation, inflammation, mirnas(n>500)



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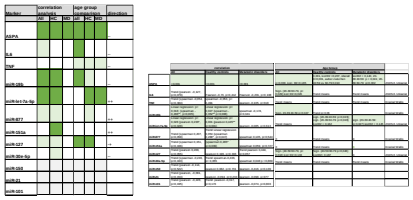
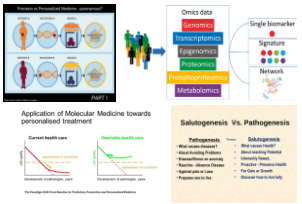
Age dependent epigenetic markers: In the Metabolic disease group (MD) correlations are disrupted, n>300



451

Discussion: personalised (precision) prevention, medicine, nutrition Salutogenesis ?



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