

exposed to the sample for too long. Growth of cyanobacterial cells during sample incubation will decrease the concentrations of all nutrients and increase the number of cells that can emit luminescence. In other words, a biological response would alter the sample.

A variety of other cyanobacterial reporters using *luxAB* for bioavailability determination have been described: *Synechococcus* PCC 7942 for heavy metals [10] and phosphorus [11], and *Synechocystis* PCC 6803 for iron [12] and nitrogen [13]. Other hosts such as the salt water *Synechococcus* PCC 7002 might be explored in the future. In an attempt to overcome the drawbacks of cyanobacterial reporters, with regards to handling and robustness issues, Schreiter and colleagues immobilized the phosphorus reporter [11] in microtitre Plate [14]. The sensor showed a slightly reduced sensitivity on microtitre Plate, but it could be stored fully active for a period of 3 weeks and was very easy to handle and robust. In a similar way, Mbeunkui *et al.* extended this approach to the assessment of nitrogen bioavailability using the nitrogen bioreporter [13] in an immobilized format [15]. In future, using optical fibres to monitor the reporter gene responses at the single-cell level could be possible, as has already been described for other microbial reporters [16].

Conclusion

Cyanobacterial reporters used in ecotoxicology and environmental monitoring provide a means for the biologically relevant assessment of chemical residues and nutrients that cannot be achieved by conventional analyses. Advanced developments will certainly include other species for the design of reporters; for example, from a marine environment. Genomics and proteomics methods will help identify genes that respond to particular analytes. Finally, further optimization of handling issues, in particular relating to assay time, will bring to light further possibilities for cyanobacterial reporter applications.

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Letter

Improved evaluation of potential allergens in GM food

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One of the main issues in the safety assessment of a genetically modified (GM) crop is its potential allergenicity. Genetic modification can affect the allergenicity in two ways: by introducing allergens, or by changing the level or nature of intrinsic allergens. Potentially, allergens can be introduced by the expression of transgenic proteins because proteins are the causative agents of food allergies, contact allergies and inhalant allergies (pollen, fungal

spores) [1–3]. A recent study reviews the current practice of allergological safety assessment for GM crops in the European Union. This investigation covers 11 notifications for placing on the market and sets recommendations for the standardization of the assessment (see Spök, A. *et al.* (2002) Toxicology and Allergology of GMO-Products, Federal Environment Agency, Vienna, Austria).

Notably, several notifications use the decision-tree approach that is currently discussed within the Codex alimentarius committee of the joint Food and Agriculture

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Organisation and World Health Organisation (FAO/WHO) in preparation of Codex guidelines (see <http://www.fao.org/es/SN/food/pdf/allergygm.pdf> and <ftp://ftp.af.o.org.codex/alnorm01/al0134ae.pdf>). It can be anticipated that many of the source organisms that provide candidate proteins for genetic engineering will lack a safe history of food use. The path through the decision tree depends on data and outcomes, such as the allergenicity of the source of the foreign gene, the comparison of the amino acid sequence of the foreign protein to the sequences of known allergens using computer databases, and the stability of the foreign protein to digestive enzymes (most food allergens are stable to digestion). The transfer of genes from commonly allergenic foods is discouraged by a joint FAO/WHO expert consultation unless it is documented that the gene transferred does not encode an allergen. In some cases, further testing with allergy patients' sera, followed by skin-prick tests and food challenges might be recommended.

In the notifications reviewed, no direct testing of potentially allergenic properties was carried out. The absence of allergenic properties was justified solely in an either argumentative way and/or by giving rather indirect evidence (e.g. digestion studies, sequence homology comparisons). Unintended secondary effects possibly caused by the gene insertion, such as possible upregulated expression of other allergens through insertion and expression of the foreign gene, are not considered (see Spök report mentioned above). To evaluate the allergenic potential, the authors of the study recommend comparing the GM crop with the corresponding parental line using allergy patients' sera. Immunization studies and further estimation of IgE (the class of immunoglobulin associated with allergy) levels could be carried out in mice (see Spök report mentioned above). Other immune hypersensitivity reactions caused by food are not associated with IgE [3].

Linear epitopes can be distinguished from conformational epitopes consisting of amino acid residues that occur separated from each other within the primary, one-dimensional protein sequence, but that are within each other's proximity and accessible for antibodies on the surface of the folded, three-dimensional allergenic protein. It might be worth noting that also structural overall similarity with an allergenic protein, that is 35% identity within an 80-amino acid long stretch, is being considered to become part of the assessment of potential allergenicity by Codex alimentarius. In addition to linear- and conformational-peptide epitopes, glycans are major IgE binding sites in allergenic glycoproteins.

With regard to the prediction of linear epitopes within transgenic proteins, discussions within the FAO/WHO currently focus on whether the minimal degree of identity should be six contiguous amino acids or eight contiguous amino acids, as devised by the joint International Life Sciences Institute – International Food Biotechnology Council (ILSI/IFBC) to reduce false positive results [4]. For some IgEs, identical stretches with a minimum length of six amino acids are sufficient to bind. Recently, an alternative approach to reduce false positives among the identical stretches of six contiguous amino acids identified during the

sequence alignment of transgenic proteins with allergenic proteins was described [5]. In the absence of literature data on epitopes, antigenicity prediction by computer helps to select potential antibody binding sites that will need verification of IgE binding by sera binding tests.

Using this alternative approach, 33 transgenic proteins have been screened for identities of at least six contiguous amino acids shared with allergenic proteins. Twenty-two transgenic proteins showed positive results of six- or seven-contiguous amino acids length. The *Bacillus thuringiensis* (Bt) toxin Cry1Ac shares two identical peptides with the same allergen [5]. Exposure to Bt sprays could lead to allergic skin sensitization and induction of IgE and IgG antibodies, or both [6]. Insect resistant GM maize encoding the protein variant Cry9C was subject to a massive product recall in the USA owing to unresolved allergenicity concerns. In cases in which multiple potential epitopes have been identified within a transgenic protein, methods to estimate the ability of the protein to cross-link IgE molecules on mast cell surfaces would enable prediction of allergic reactions owing to mast cell stimulation by the particular protein.

Only a limited number of identical stretches shared by transgenic proteins and allergenic proteins could be identified as (part of) potential linear epitopes. A peptide of the papaya ringspot virus coat protein is predicted to be the antigenic determinant of an allergenic protein. So far, antigenicity prediction algorithms have not been used for the safety assessment of GM food before marketing. Such a prediction might prove helpful if a transgenic protein shares with allergenic proteins identical stretches for which it is unknown if they are part of an epitope. In the cases of the transgenic enzymes acetolactate synthase GH50 and glyphosate oxidoreductase, these potential IgE epitopes are based on published data and would have been missed if the eight-amino-acids threshold were applied [5]. Care should be taken not only to reduce false positives but also to reduce the likelihood of false negatives in further refinement of methods to screen for potential IgE epitopes in transgenic proteins. The positive outcomes of this approach warrant further clinical testing for potential allergenicity. In addition, supplementary methods are needed for the prediction of conformational epitopes and glycan-containing epitopes.

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