Report from the Second Generation Prebiotics Working Group

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1. Justification for the value of the second generation prebiotics group

Despite their considerable use in Europe, Japan, and the U.S., the current generation of prebiotic oligosaccharides was not developed with specific functionality in mind. Generally, they are extracted from plants or synthesised from sucrose or lactose by enzymatic methods (1). The working group argues that a more rational approach is not only feasible but is highly desirable. It was also recognised that a greater variety of oligosaccharides than is currently available is likely to lead to new applications in the future (2). In addition, there are many sources of complex carbohydrates that have not yet been evaluated as candidate prebiotics or prebiotic precursors. Many of these, such as waste biomass, would be economically attractive substrates.

2. State of the science and the next level

The scientific basis for selection of prebiotic oligosaccharides has been mainly empirical, being based largely on limited screening studies (3) and on only a few materials that happen to be available. Advances in carbohydrate chemistry, microbial physiology, and biochemical engineering now offer opportunities for rational development of novel and effective prebiotics. Many of the current prebiotics are poorly substantiated by data, with much historical use of pure culture studies and inadequately designed human volunteer trials. This is now changing but more good quality studies are needed.

The working group feels that the definition of a prebiotic (4) might usefully be revised, and that a new definition might prove necessary for the next generation of products. One way forward might be to add specific qualifiers to the definition, e.g., butyrogenic prebiotic, antiadhesive prebiotic, bifidogenic prebiotic, anticancer prebiotic, immunomodulating prebiotic etc. The working group are concerned with the misuse of the term by certain companies. Some companies use the term to mean an oligosaccharide that is fermented in the colon. The term prebiotic as currently defined should only be used for *selective* fermentation to increase desirable microbial groups. It is recognized that the definition of the term prebiotic may also depend on the definition of the term probiotic.

The group considered that in the future a more outcome-oriented definition of a prebiotic might be appropriate rather than the current mechanism-oriented definition. It is clear that oligosaccharides have other health-promoting effects in addition to the selective fermentation of desirable hind gut bacteria. For example, if the prebiotic selectively enriches for bacteria that don't produce tumor promoters and induces apoptosis of colon cancer cells, then the definition should include these anticancer activities as the outcome. Many of the outcomes considered for probiotics may also be appropriate for the definition of a prebiotic. The concept of synbiotics is important in this context. In the future, it may be possible to not only cause selective fermentation of beneficial bacteria but it may also be possible to select for the health-promoting outcomes of the probiotic based on what prebiotic is used in the synbiotic (5).

One major problem with the area of prebiotic oligosaccharides is our incomplete knowledge of the colonic ecosystem (6). Much diversity remains to be described and the role individual species play should be characterised, especially with regards to their bioactivities in the colon. It is likely that new knowledge about the colonic microflora will lead to the identification of new targets for prebiotic intervention.

3. Where are the gaps in our knowledge?

The single, most-important gap in our current knowledge is our poor understanding of structure-function relationships in prebiotics. Very few studies aim to generate mechanistic data and there are too few data on the metabolism of prebiotics by probiotics. The original paradigm was of cell-associated exo-glycosidases hydrolysing the oligosaccharides prior to uptake of the resulting monosaccharides by the cell (7). It is now known that this is not the only mechanism involved and that at least some species of lactic acid bacteria posses specific transport systems for fructo-oligosaccharides. It is essential that the extent and the significance of the two mechanisms in a wide range of probiotic bacteria is discovered.

In particular, the enzymatic systems underlying the degradation of large polysaccharides and metabolism of the oligosaccharide products needs further study. Little is known about the biochemical and physiological basis for oligosaccharide transport and metabolism, and essentially nothing is known about the genes coding for these pathways.

In addition to developing a greater understanding of the physiology of the probiotic bacteria, we also need a much better understanding of the operation of the entire ecosystem. The nutritional relationships between members of the colonic microflora are not well known. In particular, the degree of cross-feeding resulting from production of saccharolytic enzyme systems by non-probiotic bacteria is poorly understood.

Many structures of many oligosaccharides remain to be investigated as candidate prebiotics. It is notable that there is little or no data on the fermentation of cello-oligosaccharides or chito-oligosaccharides in mixed culture systems. However, cellulose and chitin are the most abundant polysaccharides in nature and oligosaccharides derived from them are common food ingredients.

Many health benefits have been suggested for prebiotics (8). The mechanisms of effect are, in many cases, however unknown. Notable in this regard are important bioactivities such as improved calcium absorption, modulation of blood lipid levels, bile acid binding, inhibition of bacterial toxin binding, stimulation of apoptosis in colon cancer cells, and immunomodulation.

4. Suggested strategies to fill the knowledge gaps

The group recognised that multidisciplinary cooperation will be critical to the development of enhanced prebiotics. Close liaison between carbohydrate chemists, microbiologists, biochemical engineers and nutritionists will be needed to realise the potential enhancements, and fundraising attempts should fully recognise this.

A meta-analysis of the literature should be performed to correlate specific microflora changes and changes in levels of fermentation end points with specific prebiotics.

As there is increasing potential for the manufacture of novel oligosaccharides at reasonable cost, it is suggested that a library of oligosaccharides be built up and the fermentation of such oligosaccharides tested for all known probiotics and candidate probiotics. This will require an international effort to succeed and will be facilitated by ISAPP. Following on from this, we propose that prebiotic testing systems need to be standardised across the world. This is likely to demand the widespread adoption of new molecular methods of microbial characterisation. The group proposes that ISAPP should

produce a defined sequence of experimental tests or criteria that will support a claim for a carbohydrate to be classed as a prebiotic. ISAPP must at the same time be careful not to impose such rigid guidelines that innovation is discouraged or novel classes of prebiotics might be excluded.

The group also felt that the characterisation of the prebiotic properties of candidate oligosaccharides should be more quantitative and that a prebiotic index that provides a qualitative measure of prebiotic activity should be developed.

Development of the second generation of prebiotics will demand new manufacturing technologies. These will initially need to be implemented at the lab scale but they should be designed to be scaleable. If a manufacturing technology is to be utilised for prebiotic manufacture it must be economical, and this should be a primary concern when designing new manufacturing technologies.

Mechanistic studies are needed to elucidate and clarify the health benefits of prebiotics. These should involve the rational use of *in vitro* data and animal and human studies and should be hypothesis driven. Most importantly, the mechanisms of regulation of blood lipids and cholesterol by prebiotics should be elucidated. The role of prebiotics in the promotion of mineral uptake from the colon should also be investigated. Such studies need to be performed with a wider rage of prebiotics than at present. More studies are needed to establish the role of prebiotics in colon cancer prevention and the possible connection to bile acid binding and immunomodulation. More structure/function studies are needed to elucidate the role of prebiotics in bacterial toxin binding to epithelial receptors.

5. Hypothesis based substantiation in humans or animals/livestock

The group recognises that there is a need for more hypothesis-driven human intervention trials to complement investigative trials designed to ascertain the prebiotic activity of an oligosaccharide. The group identified several hypotheses that should be tested:

i) A prebiotic carbohydrate will display a molecular weight optimum, representing a compromise between persistence and selectivity

ii) The presence of specific oligosaccharide transport and metabolic systems by probiotic bacteria will facilitate the isolation and development of carbohydrate compounds with prebiotic activity

iii) A given prebiotic will always stimulate the growth of a specific population of *Bifidobacterium* spp. and of *Lactobacillus* spp. in a human host if other ecological factors are not limiting

iv) Isogenic mutants defective in the ability to utilize a given prebiotic will not be stimulated by prebiotics in mixed culture fermentations or in animal hosts.

v) The health-promoting outcome of a probiotic can be regulated by the prebiotic supplied.

vi) Prebiotics in animal feeds can be used to reduce gas production in livestock.

6. Future directions

In the longer term the group identified several research avenues that should be explored:

i) Targeting synbiotics for particular consumers. The EU Crownalife project is developing functional foods for the elderly, and this approach might be extended to other groups such as infants, companion and livestock animals and, potentially, even individuals.

ii) Cheaper better manufacturing. New technologies for the synthesis and in particular the downstream processing and separation of product oligosaccharides will be critical to success.

iii) Prophylactic application of prebiotics and synbiotics before travelling to reduce incidence of traveller's diarrhoea.

iv) Investigate fully the regulation of induction of antimicrobial activities against gastrointestinal pathogens. The ecological interactions and nutritional factors that regulate the differential expression of the various antimicrobial agents known to be produced by probiotics should be investigated. The role of individual antimicrobial agents in the gut should be studied in detail.

v) The potential for prebiotics to replace antibiotics as growth enhancing agents or to reduce carriage of pathogenic bacteria in pets, poultry and livestock animals should be investigated. The group recognised that this application will demand the ability to manufacture prebiotics at a very economical rate.

vi) The concept of using soluble oligosaccharide receptor analogues as dietary antiadhesive agents should be investigated. In principle, it might be possible to modulate the gut flora in a healthy way by inhibiting the adhesion of pathogens to intestinal cells. The paradigm for this approach is human milk, which contains a wide array of such receptor-active oligosaccharides. New manufacturing technologies will be required to make receptor-active oligosaccharides on an economical scale. Potential negative effects on the adhesion of probiotics and on the biological function of cell surface receptors should also be considered.

vii) The ability of prebiotics to reduce the incidence of colon cancer should receive more attention. We currently have very little knowledge on the utility of specific prebiotics to regulate the production of specific carcinogens and tumour promoters by specific microorganisms in the gut. Further, we have little experimental data correlating such changes with incidence of cancer. There is some recent evidence that fructo-oligosaccharides and pectic oligosaccharides can stimulate apoptosis in colon cancer cell lines. The significance of such observations on normal colonocytes should be studied.

viii) New and emerging sources of prebiotic oligosaccharides should be sought. Particularly attractive sources include:

a) Waste agricultural biomass (residues from plant, animal and microbial processing), containing a range of oligosaccharides and complex polysaccharides that may be broken down into a variety of oligosaccharides.

b) Sucrose, which can be used as substrate for a variety of microbial sucrose-dependant glycosyl transferases

c) Bacterial extracellular polysaccharides, which represent a source of rare residues and glycosyl linkages

d) Lactose from inexpensive whey feedstocks, which can be used as a substrate for transgalactosylating reactions

e) Starch-derived oligosaccharides, particularly those produced by transglycosylase/hydrolase-type enzymes

f) Fungal mycelia, which contain chitin and β-(1,3)(1,6)-glucan.

ix) In principle, the prebiotic concept should be applicable to other complex microbial communities. One important area for the future is the skin microflora. There has been some development activity in Europe of “cosmeceuticals” which selectively stimulate lactic acid bacteria on the skin. The group felt that this should be developed further with particular emphasis on extra-intestinal sites of infection that are currently being studied with respect to probiotic therapy, e.g. vagina, oral cavity.

x) The group recognised that new DNA technologies, especially DNA micro-arrays, will have a huge impact on microbial population analysis on the study of microbial physiology. As our knowledge of the diversity of the colonic microflora and our knowledge of the ecological and nutritional interactions

between these organisms increases, new targets for prebiotic intervention will become apparent, e.g. non-clostridial butyrate producers.

xi) There is potential for the *in situ* manufacture of prebiotics during food processing operations. This might, for example involve the enzymatic modification or acid hydrolysis of various carbohydrates and polysaccharides during food processing.

xii) Analytical methods, which can identify novel prebiotic oligosaccharides in food need to be developed. Ideally these should have good reproducibility and reliability and should not involve expensive, specialist analytical equipment. An example of this kind of analytical approach is fluorophore assisted carbohydrate electrophoresis (FACE).

The group discussed sources of funding and recognised the support of the USDA, the EU, the UK FSA, NIH, Dairy Management Inc. and industry for research into prebiotic oligosaccharides.

7. Publication issues

The working group felt that journal editors should insist on better standards of definition of prebiotic oligosaccharides. Full characterisation should be provided including purity information. Standard chemical nomenclature should be used and oligosaccharides should be described in terms of their residue identification, glycosidic linkage, anomeric configuration, degree of polymerization and molecular weight distribution as a minimum. A standardised set of abbreviations should be used.

The group also recognised that more published studies should be carried out with the prebiotic formulated into the intended foodstuff. A prebiotic activity for an ingredient does not automatically confer that activity on the final food product.

8. References

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