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A wine-induced breakdown

A polysaccharide called rhamnogalacturonan II is a substantial component of some fruits, but humans rely on their gut microbiota to digest it. The microbes and processes responsible for this digestion have been revealed. See Article p. 65-70

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When you sip a glass of wine, you might be feeding some of your gut bacteria their favourite food: a polysaccharide named rhamnogalacturonan II (RGII). This is because humans do not produce the enzymes needed to break down most of the polysaccharides in plant-based food¹ into single sugar molecules, but some bacteria among the human gut microbiota do so instead. But how are the complex components of fruit cell walls, such as RGII, decomposed in the human gut, and which microbes are responsible? On page XXX, Ndeh *et al.*² report that, surprisingly, bacterial consortia are not required — single bacterial strains common in most human gut microbiota possess the entire enzymatic system needed to break down and metabolize RGII.

It has become clear that human nutrition affects the diversity of the gut microbiota and directly influences health³. But many questions remain about the interplay of different microbial strains in polysaccharide digestion and the underlying molecular mechanisms. RGII, for example, constitutes up to 15% of the cell wall components of some fruit,

particularly grapes. Do different types of mutually beneficial microbes work together to cleave the 21 glycosidic bonds⁴ that connect the individual sugars in RGII from wine? Or have highly specialized bacterial phyla developed an enzyme system that breaks down all the bonds?

Ndeh and colleagues used a combination of biochemical, crystallographic and microbial studies to work out the key mechanistic and functional aspects of RGII decomposition. Their detailed analyses led to the discovery of seven families of glycoside hydrolases (GHs, the enzymes that cleave glycosidic bonds) that have previously unreported catalytic functions, and several new specificities for existing GH families. Strikingly, the authors' detailed study of the specificity and mode of action of the newly discovered enzymes revealed that some of the chemical structures thought to be present in RGII were incorrect (Fig. 1).

The tremendous complexity of naturally occurring polysaccharides is one of the main reasons why the biochemical characterization of these compounds still lags way behind that of other naturally occurring polymers. The polysaccharide 'alphabet' is much more complicated than those of DNA or proteins, consisting of hundreds of naturally occurring monosaccharides that can be linked and branched in many different ways. We do not yet have the methods and techniques to easily access the sequences and detailed structures of polysaccharides, so such analyses remain hard and time-consuming.

One approach that helps simplify sequencing and structure determinations, and which was used by Ndeh *et al.* in their study, is to expose polysaccharides to GH enzymes

that break a known type of glycosidic bond. Characterization of the products of several different GH reactions provides clues about the structure of the original polysaccharide. This strategy nevertheless still requires a lot of effort because many different enzymes and characterizations are needed, especially for complex polysaccharides like RGII, as the authors found in this case.

A particularly impressive aspect of Ndeh and colleagues' work was that they produced and isolated short polysaccharides (oligosaccharides) formed as intermediates in the depolymerization of RGII. They did this either by feeding RGII to mutant bacterial strains that lacked one of the key enzymes in the proposed depolymerization pathway, or by incubating RGII (or oligosaccharides produced from RGII by the mutants) with purified enzymes from the pathway. Once purified and characterized, the oligosaccharides could themselves be used as substrates to probe the specificity of other enzymes along the reaction pathway. This approach allowed Ndeh *et al.* not only to decipher the entire depolymerization pathway, but also to propose a model for the bacterial deconstruction of RGII.

The huge and rapidly growing CAZy database⁵ groups enzymes that cleave glycosidic bonds into families based on their amino-acid sequences, and provides valuable information about the key amino-acid residues in active sites, catalytic mechanisms and overall three-dimensional structures of these enzymes. Nevertheless, assigning which glycosidic bonds are cleaved by which family remains challenging, because most families contain members with diverse substrate specificities. Moreover, even though the database currently contains more than 700,000 sequences, full biochemical characterization is available⁵ for only about 2 % of

the enzymes. Ndeh and colleagues thus also provide new and useful enzymatic tools to further decipher and understand biological functions of RGII.

Notably, Ndeh and coworkers identified and described the sophisticated machinery that metabolizes RGII in the genome of the human-gut bacterium *Bacteroides thetaiotaomicron*. Bacteroidetes are major members of the microbiota of animals, especially in the gastro-intestinal tract. These physiologically diverse bacteria express many enzymes that use carbohydrates as substrates, and are generally considered to be the main players in polysaccharide degradation. They live in many ecological niches where carbohydrate use is inextricably linked with the ability of microbes to survive.

Bacteroidetes genomes contain polysaccharide utilization loci (PUL)⁶ — strictly regulated, co-localized gene clusters that encode all the enzymes needed to break down a given polysaccharide. Since the discovery⁷ of PUL in 1980, considerable progress has been made in working out their functions and regulation to gain insight into their impact on human health, and into their link with human nutritional habits (see ref. 8 for review). For example, pioneering work⁹ on the differential use of polysaccharides known as fructans by several symbiotic *Bacteroides* species of the human gut showed that the bacteria rely on enzymes that cleave specific glycosidic linkages, and that these enzymes are instrumental in defining nutritional preferences. The preference of different bacteria for degrading certain polysaccharides might have a central role in shaping the relationships between organisms of the microbiota.

Ndeh and co-workers show that three different PULs induced by RGII in *B. thetaiotaomicron* are adapted to cope with structural variations of the polysaccharide associated with RGII's diverse plant origins. One allows the microbes to grow on apple RGII, while another is needed to break down wine RGII. These findings show that possessing all of the PULs gives a net advantage to these microbes, in the battle for the coveted substrates.

Several questions arising from the new work remain unanswered. For example, do the fragments of RGII released by its depolymerization regulate or influence the presence of other bacterial phyla in the microbiota? And are some of the rare monosaccharides produced from RGII degradation beneficial to humans? Future work is needed to address these issues.

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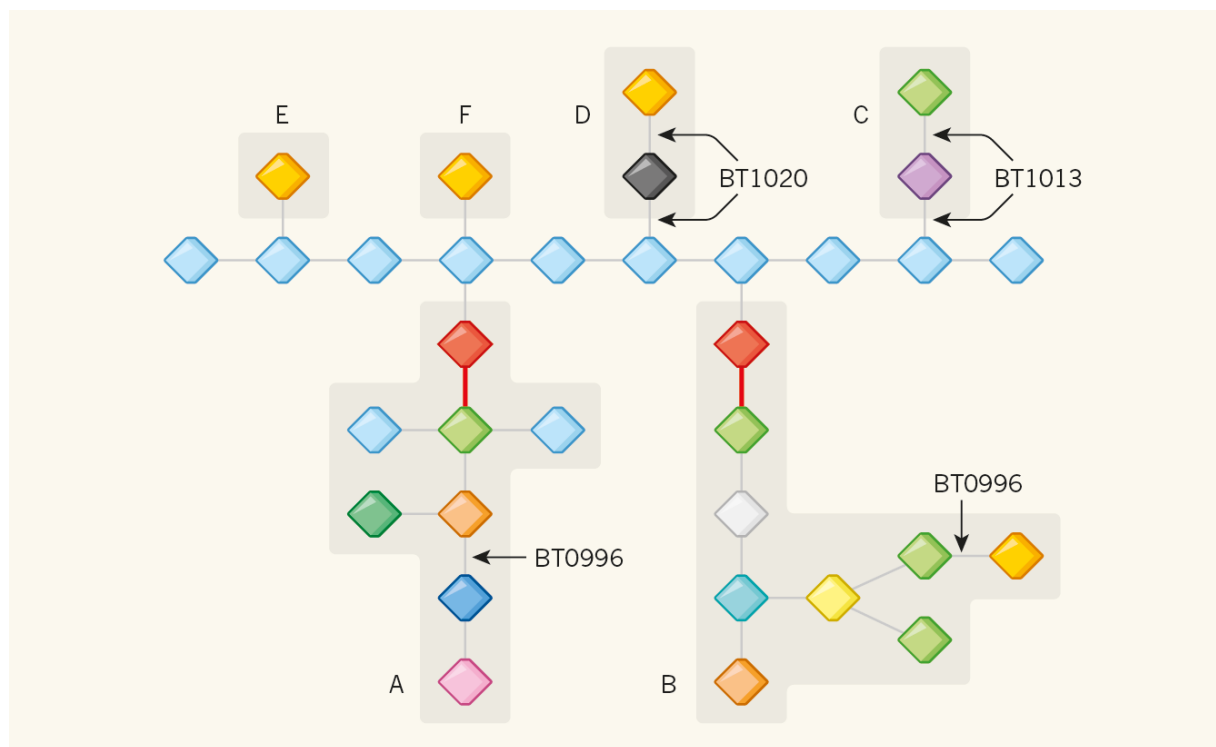


Figure 1 | The structure of rhamnogalacturonan II (RGII). The polysaccharide RGII is produced by some fruits, and is digested in humans by the gut microbiota. Ndeh *et al.*² report that a single bacterium, *Bacteroides thetaiotaomicron*, is responsible for this digestion, and identify the enzymes and reactions by which RGII is broken into its constituent monosaccharides. A key finding is that three enzymes, BT1020, BT1013 and BT0996 are bifunctional — they each cleave two of the glycosidic linkages that connect the monosaccharides. The authors also report a previously unknown chain of RGII (chain F), and find that one of the linkages in chain A and B has a different structure to what was previously thought. Different symbols represent different types of monosaccharide.