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Solving the problem of how to eat something as big as yourself: diverse bacterial strategies for degrading polysaccharides

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Polysaccharide digestion by bacteria is an important activity in many ecosystems, and a number of bacterial genera can perform this function. Although many papers have been published about the properties of isolated polysaccharide-degrading enzymes, relatively little is known about how intact bacteria degrade polysaccharides. This review summarizes recent findings suggesting that there are at least three different strategies. The most familiar one is the excretion of extracellular polysaccharidases, which diffuse to and degrade nearby polysaccharides. An example of this type of strategy is provided by the plant pathogen, *Erwinia* spp. A second strategy is to have the enzyme exposed to the extracellular medium but attached to the surface of the cell. Examples of this strategy are provided by the pullulanase system of *Klebsiella oxytoca* and the cellulosomes of *Clostridium thermocellum*. A strategy that could be seen as a combination of the extracellular enzyme strategy and the surface organelle strategy is provided by *Vibrio harveyi*, which attaches to its substrate, chitin, via proteins that appear to be specialized for attachment and produces extracellular enzymes that attack the chitin. A third strategy is to import the polysaccharide, as appears to be done by *Bacteroides* spp. In this instance, the polysaccharide is bound to an outer membrane receptor, then passes into the periplasm where the degradative enzymes are located. The ecological advantages and disadvantages of these systems are discussed, and areas where further research is needed are defined.

Keywords: polysaccharide-degradation; bacterial; mechanisms; ecology

Ecological roles of polysaccharide-degrading bacteria

Environmental and agricultural importance

We are not up to our ears in plant, animal and microbial biomass because bacteria and fungi continuously and efficiently break down polysaccharides. In modern industrialized societies, it has become increasingly important to learn how to speed up this process in a number of settings. For example, much of the paper and yard waste in landfills remains undegraded. Improved microbial recycling of paper and plant polysaccharides would help solve this waste disposal problem. Also, food processing results in the accumulation of plant skins and other wastes that need to be recycled. Increasing the efficiency of microbial digestion of such biomass will not only reduce waste accumulation but could also help to produce alternative fuels and chemicals. Many approaches to energy production from biomass are already under study, but breakdown of polysaccharides remains the most problematic step.

Polysaccharide-degrading bacteria also play a critical role in meat production. Cows and other ruminants depend on ruminal bacteria to ferment polysaccharides to short chain fatty acids, which are a readily absorbable form of carbon and energy [8,21]. If the efficiency of the rumen fermentation of polysaccharides could be improved, less feed would be necessary to produce the same final weight and reliance on expensive high quality feed for finishing could be reduced. Even in non-ruminant animals, polysaccharide fermentation by colonic bacteria contributes to animal nutrition and is thus a factor in feed efficiency.

Importance for human nutrition

Polysaccharide fermentation in the human colon, long neglected by nutritionists, has attracted increased attention in recent years because the increasing popularity of high fiber foods has increased the flow of polysaccharides into the human colon. New low-fat, low-calorie foods also contribute to this trend. In these new foods, the bulk previously taken up by sugar has been replaced with plant polysaccharides that are not digested in the human small intestine. Fat substitutes are also indigestible polysaccharides and even starch, a digestible polysaccharide, is being treated to make it less digestible by small intestinal enzymes. All of these polysaccharides are readily fermented by colonic bacteria however, thus moving their digestion from the small intestine into the colon. Whether this increased colonic fermentation will have a significant impact on human health remains to be seen.

Holes in the information database

Polysaccharide fermentation by microbes, especially bacteria, is clearly a very important process. Yet there is remarkably little information about how this process occurs in an intact microbe. Most of the papers published on polysaccharide breakdown during the last two decades have focused on the properties of polysaccharide-degrading enzymes, treated as isolated molecules, when in fact these enzymes are usually part of a complex system that mediates

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polysaccharide binding and breakdown (see, for example [8,24]). Another major group of studies has used polysaccharidase excretion as a model for studying protein export by Gram-negative bacteria [15]. The purpose of the present review is to shift the focus of attention back to the biology and ecology of polysaccharide utilization, with emphasis on how this process occurs in living bacteria. As will become evident, bacterial strategies for utilizing polysaccharides are diverse, a fact that is not surprising since the ability to degrade polysaccharides has evolved in many different phylogenetic groups of bacteria. This review will focus on bacteria to the exclusion of fungi, because there is even less information about how intact fungi utilize polysaccharides than there is about how bacteria accomplish this activity. Where possible, the references used are review articles or recent papers that summarize earlier work, and no attempt is made to include all papers published in this area. Also, attention will be focused primarily on those systems where the most detailed biochemical and genetic analyses have been done.

Diversity of polysaccharide-degrading bacteria

Some examples of polysaccharide-degrading bacteria from different bacterial phyla are given in Table 1. This table almost certainly underestimates the phylogenetic diversity of polysaccharide-degrading bacteria. Table 1 also lists the type of strategies used by bacteria in the different phylogenetic groups. So far, there appear to be three main strategies, which are illustrated in Figure 1. Some bacteria excrete polysaccharidases into the surrounding medium, then take up the monosaccharide or oligosaccharide products of enzyme action (extracellular enzyme strategy). Other bacteria digest polysaccharides by using enzyme-containing complexes, which are attached to the bacterial surface (surface complex strategy). Still other bacteria appear to have their degradative enzymes in the periplasmic space, and internalize the polysaccharide prior to enzymatic breakdown (polysaccharide import strategy). Although Table 1 makes it appear that different strategies tend to be associated with different phylogenetic groups, it is still too soon to say whether particular polysaccharide utilization strategies are phylum-specific.

Table 1 Some examples of well-studied polysaccharide-degrading bacteria from different phylogenetic groups

Phylogenetic group	Examples of polysaccharide-degrading species	Type of strategy used by the bacteria
E. coli-Pseudomonas group	*Erwinia* spp (plant cell wall polysaccharides)	Extracellular enzymes
	Vibrio harveyi (chitin)	Extracellular enzymes Chitin-binding proteins
	Klebsiella oxytoca	Cell surface enzyme
Gram-positive bacteria	*Clostridium thermocellum* (cellulose)	Cellulosomes
	Cellulomonas fimi (cellulose, xylan)	Extracellular enzymes Cellulose-binding proteins (?)
Bacteroides-cytophaga group	*Bacteroides thetaiotaomicron*	Polysaccharide import

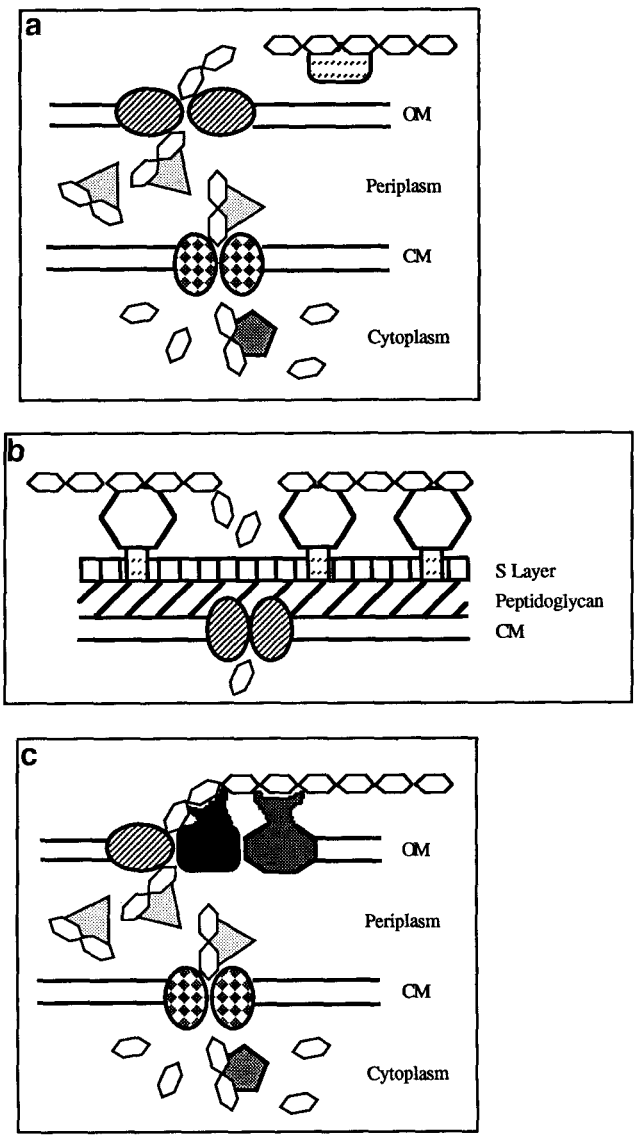

Figure 1 Features of different bacterial strategies for polysaccharide utilization. The polysaccharide being degraded is denoted by joined hexose rings. (a) The extracellular enzyme strategy. The polysaccharide is bound and degraded by extracellular enzymes. In the case of Gram-negative bacteria, the products of polysaccharide breakdown diffuse through porins in the outer membrane (OM). In the periplasm, the products are bound by sugar-binding proteins and conveyed to the transporter in the cytoplasmic membrane (CM). (b) The cellulosome strategy of clostridia. Protein complexes embedded in the S layer that covers the peptidoglycan cell wall bind the polysaccharide and degrade it. What happens to the products at this point is not known. (c) The polysaccharide import strategy proposed for *Bacteroides* spp. The polysaccharide is bound to OM proteins, which translocate it across the OM, possibly beginning to digest it in the process. In the periplasm, the polysaccharide is further digested by enzymes linked to the OM complex and the products are sequestered by periplasmic-binding proteins, which convey the products to the CM transporter.

A major impediment to elucidating bacterial strategies for polysaccharide utilization is that many polysaccharide-degrading bacteria have not been easy to manipulate genetically. Of the bacteria listed in Table 1, only the *E. coli-Pseudomonas* group and *Bacteroides* spp have relatively-

well understood genetic systems. As a result, work on polysaccharide digestion by Gram-positive bacteria such as *Clostridium* spp and *Cellulomonas* spp or by Gram-negative bacteria such as cytophagas and *Fibrobacter* spp has been limited to biochemical description of component proteins or to cloning and sequencing of genes encoding these proteins. Since the only way to determine the function of a particular polysaccharide-utilization protein in the intact cell is to create a disruption in the gene that encodes the protein, the lack of genetic techniques for gene disruption essentially precludes the determination of function.

Taking an ecological perspective: diversity and complexity of substrates attacked by polysaccharide-degrading bacteria

One feature shared by all of the polysaccharide utilization strategies is that they are complex, and frequently appear to contain an unnecessarily large number of enzymes and other proteins. This is particularly apparent in the case of the cellulose-degrading bacteria, but it is also true of bacteria that degrade other polysaccharides. One explanation for the plethora of enzymes may be found in a frequently neglected, but very important, characteristic of the substrates which bacteria actually encounter in natural settings: the complexity of such substrates as the cell walls of plants, the glycocalyx of mammalian cells, and the chitinous exoskeletons of invertebrates. A plant cell wall, for example, consists of cellulose, hemicelluloses and pectins joined to each other by covalent and noncovalent bonds (Figure 2; [23]). These polysaccharides are also attached to proteins and to lignin by covalent and noncovalent bonds. The extracellular matrix of mammalian cells and the chitinous coverings of invertebrates are similarly complex, consisting of an interlocking meshwork of polysaccharides and proteins. Clearly, nature abhors a pure polysaccharide. It may be that some of the apparently redundant cellulases produced by cellulolytic bacteria, for example, are designed to attack cellulose molecules that are tightly associated with lignin or with xyloglucans, just as some cellulases seem to work better on amorphous as opposed to crystalline cellulose.

Another important ecological consideration, which is discussed in more detail in a later section, is that bacteria rarely grow as pure cultures in nature. Instead, they must make a living in an ecological niche they share with many competitors. Polysaccharide utilization systems may thus have evolved to have other functions in addition to the central one of breaking down the polysaccharide. One obvious function is adherence to polysaccharide-rich particles. Another possible function is sequestering the products of the degradative enzymes. A bacterium that synthesizes a complex system to degrade cellulose to glucose and cellobiose and then lets these products simply diffuse away into the surrounding environment is not likely to survive long in a competitive ecosystem. Viewed from an ecological perspective, it is not surprising that bacterial polysaccharide utilization systems include a large number of proteins, nor is it surprising that they would be as diverse in their organization as the ecological niches in which they were evolved.

Bacterial strategies for polysaccharide utilization

Adherence to particulate material—a presumably important, but poorly understood, step in polysaccharide utilization

Although individual polysaccharides may be smaller than the bacterium which utilizes them, the polysaccharide complexes normally encountered in nature are much larger than a bacterium. Thus, it is not surprising that adherence to a polysaccharide matrix appears to be a common theme of many bacterial polysaccharide utilization strategies. Relatively little is known about this adherence step. In electron micrographs of the intestinal contents of animals and humans, plant fragments are coated with bacteria [21]. Binding to chitinous material has also been described [13,14], but so far the question of how adherence is coupled to polysaccharide digestion has been generally neglected. Surface-exposed enzymes and polysaccharide-binding proteins may function as adhesins. Also, enzymes that appear to be extracellular when the bacteria are grown in liquid cultures could actually be trapped between the adherent bacterium and its substrate. Also, the conditions under which bacteria are grown in the laboratory could result in aberrant localization of enzymes. Whether this is a problem has not yet been established, but it is clear that the stage of growth can affect the localization of enzymes. For example, the *Klebsiella oxytoca* pullulanase is attached to the outer membrane until the bacteria reach stationary phase, at which time the enzyme is released into the extracellular fluid [15]. Also, some Gram-negative ruminal bacteria were initially thought to produce outer membrane blebs containing the polysaccharide-degrading enzymes, which diffused away from the bacterium and attached to the substrate being attacked. More recent work has shown that blebbing occurs only during late stages of growth and is not necessary for polysaccharide utilization [9].

Excretion of polysaccharide-degrading enzymes

The strategy for polysaccharide utilization usually presented in textbooks is the extracellular enzyme strategy. Since polysaccharides can be very large, this strategy is certainly an intuitively obvious one. An example of this strategy is provided by *Erwinia* spp that cause plant diseases [15]. *Erwinia* spp excrete a variety of polysaccharidases into the extracellular fluid, including pectinases, hemicellulases and cellulase. This mixture of enzymes makes sense when one considers the components of the plant cell wall matrix (Figure 2). The effect of these enzymes on plants is striking and consists of a spreading zone of rot on the plant surface.

In theory, there is a potentially serious problem with this strategy, from the bacterial point of view. Enzymes that diffuse away from the bacterial cell will yield products far removed from the bacterium, and these products could be acquired by other bacteria in the same site, which cannot degrade polysaccharides themselves. For plant pathogens, however, this may not be a serious problem because they are usually the predominant species in the infected portion of the plant. Also, it is possible that extracellular enzymes produced by plant pathogens are not intended simply to yield food for the bacteria but also to allow movement of bacteria by breaking down the structure of plant tissue.

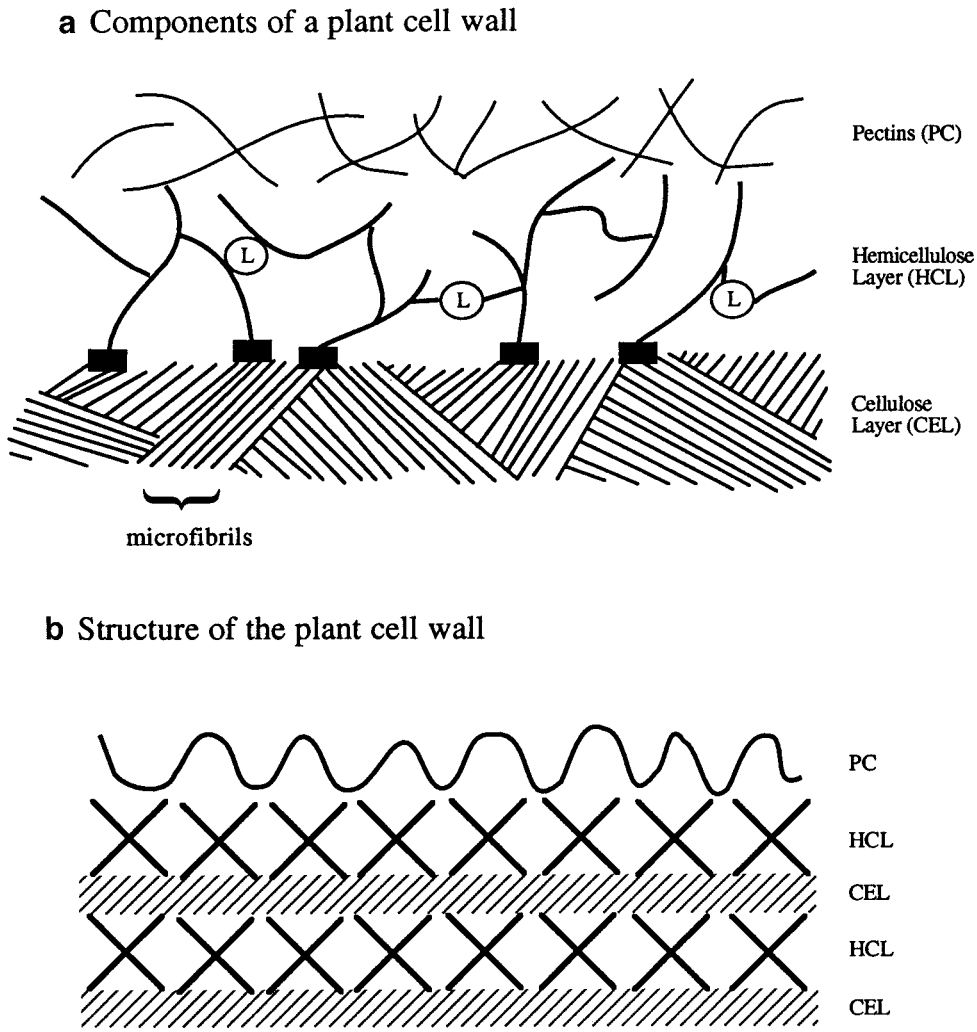

Figure 2 Features of a plant cell wall. (a) Components of a typical plant cell wall [23]. Cellulose fibrils are arranged in random arrays to form the cellulose layer. Attached to this layer are xyloglucans and other cellulose-binding polysaccharides. Covalently linked to these polysaccharides are other hemicelluloses, such as xylans and arabinogalactans. These are cross-linked by lignin monomers (L). Over the hemicellulose layer is the pectin layer, which is attached to the hemicelluloses by noncovalent linkages. (b) A plant cell wall. An actual plant cell wall actually contains layers like the one shown in panel (a), with the pectin layer at the surface.

Polysaccharidases such as hyaluronidases, which degrade the extracellular matrix of mammalian tissue, may have a similar function. It is interesting that the polysaccharidases of *Erwinia* spp are regulated by a quorum-sensing system [5]. That is, the enzymes are only produced when the number of bacteria present on the plant surface rises to a certain level. This could have the effect of assuring that *Erwinia* is the predominant organism on the plant surface before degradation begins, ensuring that *Erwinia* will be the primary benefactor of degradation.

Surface-attached polysaccharidases

One way to keep the products of polysaccharide breakdown near the bacterium that produced the enzymes is to anchor the enzymes to the bacterial surface. Such enzymes are free to interact directly with substrates too large to be readily internalized, but the digestion products are released close to the bacterial surface. The best-studied systems of this type are the pullulanase system of *Klebsiella oxytoca* [15] and the cellulosomes of clostridia [2,7].

Pullulan is a large polymer consisting of repeating maltotriose units linked by α-1,6 bonds. There are two types of enzymes that degrade pullulan, pullulanases that attack the α-1,6 linkage to produce maltotriose and neopullulanases that attack the α-1,4 linkage to produce trisaccharides with mixed α-1,4 and α-1,6 bonds. The pullulanase of *K. oxytoca* is a classical pullulanase, which is excreted to the outer membrane where it is tethered to the bacterial surface by a lipid moiety [15]. Although many papers have been published on the *K. oxytoca* pullulanase, little attention has been paid to other parts of the pullulan utilization system, because the main focus of work on this enzyme has been to identify the proteins responsible for its surface localization. This is unfortunate because the well-understood genetic system available in this case would make it an ideal system with which to address the question of how the bacteria solve the problem of sequestering products of pullulanase action. An obvious strategy would be to have the pullulanase tightly associated with the outer membrane porins that admit maltotriose to the periplasm. If *K. oxytoca*

utilizes maltotriose similarly to *E. coli*, to which it is very closely related, the maltose-maltotriose porins are specific for this type of substrate and the di- and tri-saccharides that enter the periplasm through them are quickly bound to sugar-binding proteins that convey the sugars to cytoplasmic membrane transporters. The question of what happens to the products of enzymatic breakdown is one of the most neglected aspects of polysaccharide utilization systems. Yet it is of critical importance to the polysaccharide-utilizing bacteria. For this reason, it seems likely that all polysaccharide utilization systems of bacteria contain oligosaccharide-binding proteins that bind the products of polysaccharidase action and facilitate the trapping of these by the bacterium that produced the polysaccharidase.

A well-studied example of surface-localized polysaccharidases in Gram-positive bacteria is the cellulosome complex of cellulolytic *Clostridium* spp. Cellulosomes are large protein aggregates, consisting of at least 14 different proteins, which are responsible for cellulose binding and cellulose breakdown [7,10,12]. The proteins in cellulosomes have not been fully described, and it is thus possible that some cellulosome functions remain to be discovered. For example, one might expect cellulosomes to contain proteins that bind the products of cellulose digestion. The cellulosome is thought to be anchored in the S layer, a crystalline array of proteins that covers the peptidoglycan layer. If the product-binding proteins were in the part of the cellulosome that was exposed to the peptidoglycan layer, they could communicate with other sugar-binding proteins similar to the maltose-binding protein of *E. coli* that would convey the sugar to cytoplasmic transporters. A recent study of the starch utilization system of the thermophilic Gram-positive anaerobe, *Thermoanaerobacterium thermosulfurigenes*, has shown that the amylase gene is genetically linked to genes encoding a maltose-binding protein and a maltose transporter [18], raising the possibility that the proteins encoded by these genes could be physically linked as well. Possibly, further analysis of the cellulosomes will reveal that cellobiose-binding proteins are part of the cellulosome complex.

In addition to mediating cellulose digestion, cellulosomes may also mediate the adherence of the cellulolytic bacteria to plant cell wall fragments [2]. A look at the structure of the typical plant cell wall shows that the bacteria would have to burrow through the pectin and hemicellulose layer in order to reach the cellulose layer of an intact plant cell. This may be the reason that some cellulosome complexes contain hemicellulases (eg xylanases) as well as cellulases [2].

Some bacteria may utilize both the free extracellular enzyme strategy and the surface-bound enzyme strategy. For example, *Erwinia* spp produce a pectinase that is embedded in the outer membrane as well as extracellular pectinases that diffuse away from the cell [20]. The authors suggest that this outer membrane enzyme is mainly used to degrade oligosaccharides produced by the extracellular enzymes, but it is also possible that the bacteria use extracellular enzymes to break down the structure of plant tissue and surface-anchored enzymes for taking up and utilizing polysaccharides and large polysaccharide fragments that are released in this process.

Extracellular enzymes produced by bacteria that adhere to their substrate

A polysaccharide utilization strategy that could be considered as intermediate between the extracellular enzyme strategy and the cell surface polysaccharidase strategy is the one used by *Vibrio harveyi* for degrading chitin [12,13]. Chitin is a linear polymer of N-acetyl-glucosamine residues. It is ubiquitious in the exoskeletons and intestinal linings of insects and in the coverings of shellfish. It is also one of the components of fungal cell walls. *Vibrio harveyi*, a marine bacterium, lives in environments that are rich in chitin. Thus, it is not surprising that *V. harveyi* has developed the ability to attach to and degrade chitin. Free-swimming bacteria produce low levels of a chitinase, which is excreted into the medium. When *V. harveyi* encounters a chitinous surface it attaches via outer membrane proteins. The secreted enzyme presumably releases chitobiose and other fragments, which are then internalized by the bacterium and lead to an increase in production of chitinase and other chitin-associated proteins, which probably increase the binding of bacteria to the chitin surface. It is not yet clear whether binding to the chitin affects the localization of the chitinase, ie whether the chitinase produced after binding is localized primarily between the bound bacterium and the chitin surface. Since *V. harveyi* is easily manipulated genetically, genetic analysis of this system should be possible and should prove quite interesting results.

The Gram-positive bacterium, *Cellulomonas fimi* could also be considered to fall in this category. *C. fimi* produces a variety of cellulases, at least some of which are extracellular, and attaches to its substrate, but does not produce cellulosome-like surface organelles [2,24]. A great deal of work has been done to characterize the multiple cellulases of *C. fimi*, but relatively little is known about how cellulose digestion is carried out by the intact organism.

Polysaccharide import by Bacteroides spp

Still another type of strategy is illustrated by the polysaccharide utilization systems of human colonic *Bacteroides* spp. *Bacteroides* is the numerically predominant genus in the microbiota of the human colon, and some *Bacteroides* spp, notably *Bacteroides thetaiotamicron* and *Bacteroides ovatus*, can ferment an impressive variety of plant and animal polysaccharides [19]. In contrast to most of the other bacteria mentioned in this review, *Bacteroides* spp appear to specialize in utilizing soluble or well-hydrated polysaccharides, rather than insoluble polysaccharides, but *Bacteroides* spp are capable of fermenting very large, branched polysaccharides like xylan and galactomannans. Intact cells of *Bacteroides* spp can even degrade polysaccharides such as chrondroitin sulfate that are tied up in complexes such as mammalian proteoglycan (a mixture of hyaluronic acid, proteins and chondroitin sulfate), as long as the complex is well-hydrated [11]. Nothing is known about whether *Bacteroides* spp normally attach to plant cell walls or intestinal mucin when they degrade polysaccharides in the colon.

The best-studied *Bacteroides* polysaccharide utilization system is the starch utilization system of *B. thetaiotaomicron*. *B. thetaiotaomicron* can utilize amylose (a linear α-1,4 linked glucan), amylopectin (amylose chains connected

in a large branching structure by α-1,6 linkages) and pullulan (maltotriose units connected by α-1,6 linkages). Utilization of these polysaccharides is very efficient, as is evident from the fact that *B. thetaiotaomicron* grows nearly as rapidly on these polysaccharides as it does on glucose or maltose. Early studies of the starch-degrading enzymes of *B. thetaiotaomicron* revealed that these enzymes were not extracellular but appeared to be localized in the periplasm [19]. This suggested the hypothesis that the polysaccharide substrate has to transit the outer membrane to come into contact with the periplasmic enzymes.

Biochemical and genetic studies have so far identified five outer membrane proteins that are produced only when *B. thetaiotaomicron* is grown on maltose or starch. Genetic analysis has so far shown that at least two of these proteins, and possibly more, are essential for growth on starch [16,17,22]. By contrast, genes encoding the neopullulanase and α-glycosidase can be disrupted without affecting growth on starch significantly [6]. In our experience, it is generally the case that individual polysaccharidases are dispensible, presumably due to the existence of multiple redundant polysaccharidases, where disruption of genes encoding membrane proteins is much more likely to abolish growth on the polysaccharide [4,19].

Genes encoding the starch utilization outer membrane proteins (*susC-susG*) are located in the same operon [16,17]. This operon also contains a gene encoding an α-glycosidase, and is adjacent to a gene encoding the main neopullulanase activity [6]. These enzymes and outer membrane proteins might form a complex that binds and degrades starch, but there is still no direct evidence that SusC, SusD, SusE, SusF and SusG actually form a complex with each other or with the starch-degrading enzymes. Cross-linking studies are currently underway to determine whether these proteins interact with each other. Preliminary results suggest that SusC and SusD proteins are essential for starch binding, whereas SusE and SusF make a contribution to binding but are not essential for binding. SusG appears not to be directly involved in binding and may be an enzyme.

Our recent experience with SusG provides a good example of why it is essential to supplement biochemical analyses with genetic experiments. In early studies of the starch-degrading enzymes of *B. thetaiotaomicron*, we identified and characterized biochemically a pullulanase, a neopullulanase and an α-glucosidase. These were the major enzyme activities detected in our assay system. As already mentioned, we now know that the enzymes encoding the neopullulanase (SusA) and the α-glucosidase (SusB) are closely linked to SusC-SusG. The gene encoding the pullulanase (*pulI*) is located somewhere else on the chromosome. A mutant with a disruption in *pulI* grew normally on pullulan, amylose or amylopectin [19]. A mutant with a disrupted *susA* grew, but more slowly on starch than the wild type [6]. Thus, neither PulI nor SusA was essential for growth on starch despite their high starch-degrading activity *in vitro*. SusA proved to be responsible for about 90% of the total starch-degrading activity detected in our assay system. Eliminating SusA in the *susA* disruption mutant allowed us to detect two enzymes with very low activity enzymes, one of which proved to be encoded by *susG*. SusG, in contrast to SusA and PulI, proved to be essential for growth on starch because a disruption in *susG* eliminates growth on all forms of starch (unpublished results). This mutant, however, still grows normally on maltose (G2) and oligosaccharides as large as maltoheptaose (G7). This phenotype suggests that SusG takes part in the initial attack on the full-length starch molecule. We do not yet know whether SusG is exposed on the cell surface.

The results of our genetic analyses of the *B. thetaiotaomicron* starch utilization system illustrate two critical points. First, the activity of an enzyme in an *in vitro* assay system is not a good indicator of the importance of that enzyme in the polysaccharide utilization system. Second, non-enzymatic proteins (eg SusC, SusD) can be as important as, or more important than, polysaccharide-degrading enzymes. Moreover, the proteins that mediate the initial binding of the substrate could well carry out the rate limiting step in polysaccharide breakdown. In our experience, increasing production of a polysaccharide-degrading enzyme does not always affect the rate of digestion of the polysaccharide. Thus, scientists interested in improving the efficiency of polysaccharide breakdown would do well to consider the possibility that binding proteins rather than enzymes could be the key to more efficient utilization.

So far, work on the *Bacteroides* starch utilization system has focused primarily on the early steps in polysaccharide utilization. Presumably, the system also includes periplasmic proteins, which bind the monosaccharides and oligosaccharides produced by the starch-degrading enzymes and prevent them from diffusing back out of the cell, and transport proteins that convey the monosaccharides and oligosaccharides across the cytoplasmic membrane and into the cytoplasm. No information is yet available about these later steps in polysaccharide digestion.

Other polysaccharide utilization systems of *Bacteroides* spp, although less well characterized than the starch utilization system, appear to function similarly to the starch utilization system. A recent paper on amylose digestion by the Gram-negative anaerobe *Ruminobacter amylophilus* reports evidence that *R. amylophilus* uses a similar system for import and digestion of starch [1]. A 1977 paper on a cellulolytic cytophaga, a Gram-negative bacterium, also suggested that the degradative enzymes were cell-associated [3]. This cytophaga could have a *Bacteroides* type utilization system or a *K. oxytoca* type pullulanase system.

The more things change, the more they remain the same

Clearly, bacterial strategies for degrading polysaccharides exhibit considerable diversity. The localization and organization of the enzymes, the type of enzymes produced, how bacteria attach to the substrate they are attacking, the preference for insoluble versus soluble substrate, and even the nature of the inducer, can all vary from one system to another. Nonetheless, amid all this diversity there are also some common themes. Perhaps the most important common theme is the fact that polysaccharide digestion systems are highly complex, involving not just the polysaccharide-degrading enzymes but also polysaccharide-binding pro-

teins, proteins that bind the products of enzymatic digestion and transporters that convey the digestion products into the cell. The complexity of these systems presumably reflects the need of the bacterium to bind the polysaccharide, sequester the products generated by its polysaccharide-degrading enzymes and prevent these products from being acquired by competing microbes in the same ecosystem. An interesting question for the future is whether polysaccharide-degrading bacteria also exhibit diversity in how they interact with other bacteria in the same ecological niche. The subject of polysaccharide-degrading consortia of bacteria has received little attention, despite the fact that the structure of plant cell walls and other natural sources of polysaccharides would seem to be obvious targets for a polymicrobic attack. Also, polysaccharide-degrading bacteria carry out the first steps in an anaerobic food chain that ends with the production of methane or acetate. This food chain may be coupled to the reduction of sulfate or the utilization of ammonia in the case of sulfate-containing or hexosamine-containing polysaccharides. Nothing is known about how, or even if, the polysaccharide-degrading bacteria are closely linked physically to the microbes that utilize their end-products.

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