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Polysaccharases for microbial exopolysaccharides

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Abstract

Microbial exopolysaccharides (EPS) are the substrates for a wide range of enzymes most of which are highly specific. The enzymes are either endoglycanases or polysaccharide lyases and their specificity is determined by carbohydrate structure with uronic acids often playing a major role. The presence of various acyl substituents frequently has little effect on the action of many of the polysaccharases but markedly inhibits some of the polysaccharide lyases including alginate and gellan lyases. The commonest sources of such enzymes can be either microorganisms or bacteriophages. These specific polysaccharide-degrading enzymes can yield oligosaccharide fragments, which are amenable to NMR and other analytical techniques. They have thus proved to be extremely useful in providing information about microbial polysaccharide structures and were routinely used in many such studies. Complex systems containing various mixtures of enzymes may also be effective in the absence of single enzymes but may be difficult to obtain with reproducible activities. Such preparations may also cause extensive degradation of the polysaccharide structure and thus prove less useful in providing information. Commercially available enzyme preparations have seldom proved capable of degrading microbial heteropolysaccharides, although some are active against bacterial alginates and homopolysaccharides including bacterial cellulose and curdlan.

Keywords: Exopolysaccharide; Polysaccharases,Bacteriophage

1. Polysaccharases

Microbial exopolysaccharides may be degraded either by polysaccharide hydrolases (polysaccharases) or by polysaccharide lyases. The latter cleave the linkage between a neutral monosaccharide and the C_4 of a uronic acid with simultaneous introduction of a double bond at the C_4 and C_5 of the uronic acid. Both types of enzyme are commonly found to degrade microbial EPS as well as eukaryotic polymers (Sutherland (1995). Although the enzymes degrading microbial exopolysaccharides may be endo- or exo-acting leading to rapid or slow breakdown of the polymer chain respectively, almost all those studied in detail have proved to be endoglycanases or endo-acting polysaccharide lyases. Only for microbial extracellular homopolysaccharides is a wider range of exo- and endo-acting enzymes usually available. As was pointed out by Mishra and Robbins (1995), β-D-glucanases with a variety of specificities can be used to elucidate the structures of this group of polysaccharides. Thus, even the linear 1,3-β-D-glucan curdlan is degraded by four different enzymes. Two *exo*-glucanases release D-glucose and laminaribiose respectively. Two *endo*-glucanases differ in their mode of action. One attacks randomly, releasing glucose, laminaribiose, laminaritriose and other oligosaccharides in the β-1,3-linked series. The other endoglucanase yields the pentasaccharide laminaripentaose. Scleroglucan, a fungal β-D-glucan carrying 1,6-β-D-glucosyl side-chains, is susceptible to the commercially available (1→3)-β-D-glucanase preparation marketed as 'zymolyase' (Catley and Fraser, 1988). Similarly, degradation of bacterial cellulose (and cellulose from other sources) requires the concerted action of various endo- and exo-β-D-glucanases (Gilbert and Hazlewood, 1993), yielding a complex mixture of glucose and cellodextrins. In contrast to these systems, the hydrolysis of many microbial heteropolysaccharides is commonly achieved by a single, highly specific, endo-acting polysaccharase which yields a series of oligosaccharides representing either the repeating unit structure or multiples of it.

2. Sources of polysaccharases

Polysaccharases may derive from three major sources – endogenously from the polysaccharide-synthesising microorganisms; exogenously from a wide range of other eukaryotic or prokaryotic microorganisms; and thirdly, from bacteriophage particles or phage-induced bacterial lysates. Most of the enzymes acting on microbial EPS

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Table 1
Glycan depolymerases associated with exopolysaccharide synthesis

Bacterial species	Enzyme	Mol. Mass	Reference
Acetobacter xylinum	Endoglucanase (CM-Cellulase)	35.6 kDa	Standal et al. (1994)
Agrobacterium tumefaciens	Endoglucanase (CM-Cellulase)	–	Matthysse et al. (1995)
Azotobacter chroococcum	Alginate lyase (polymannuronate lyase)	43 kDa	Pecina and Paneque (1994)
Azotobacter vinelandii	Alginate lyase (polymannuronate lyase)	–	Kennedy et al., (1992)
Cellulomonas flavigena	1,3-β-D-Glucanase		Voepel and Buller (1990)
Escherichia coli K5	N-acetyl-heparosan lyase	70–89 kDa	Legoux et al. (1996)
Pseudomonas aeruginosa	Alginate lyase (polymannuronate lyase)	39 kDa	Schiller et al. (1993)
Pseudomonas fluorescens	Alginate lyase (polymannuronate lyase)	–	Hughes (1997)
Pseudomonas fluorescens (marginalis)	Galactoglucanase	–	Osman et al., (1993)
Pseudomonas mendocina	Alginate lyase (polymannuronate lyase)	–	Sengha et al.(1989)
Pseudomonas putida	Alginate lyase (polymannuronate lyase)	–	Conti et al. (1994)
Rhizobium meliloti	Succinoglycan depolymerase	–	Glucksman et al. (1993)
Rhizobium meliloti	Polyglucuronic acid lyase		Michaud et al. (1997)
Sphingomonas spp.	Gellan lyase	–	Sutherland and Kennedy (1996).
		–	Yamazaki et al. (1996)
Streptococcus equi	Hyaluronidase	–	

have to be obtained de novo, few are available commercially and few of the commercially available enzymes act on microbial polysaccharides. An exception was demonstrated for xanthan. In its disordered form, the cellulose backbone of the polysaccharide revealed limited susceptibility to some commercial cellulase preparations (Rinaudo and Milas, 1980; Sutherland, 1984).

3. The endogenous production of polysaccharases

It is very rare for a microbial species to use its exopolysaccharide as a source of carbon and energy. The curdlan-synthesising bacterium *Cellulomonas flavigena* is one exception as it produces an extracellular enzyme capable of degrading the EPS to utilisable products (Voepel and Buller, 1990). A more common finding follows recent molecular studies on exopolysaccharide synthesis, which have revealed that glycanases or polysaccharide lyases are gene products which are often associated with the biosynthesis of the exopolysaccharide itself. Such enzymes have now been found in a wide range of exopolysaccharide-synthesising bacterial species but do not allow the microorganism to utilise its own EPS as a carbon source (Table 1). Thorough genetic analysis of more species will probably extend the list further. In many examples which were studied, the genes for the polysaccharases formed part of the operons or gene cassettes regulating synthesis, polymerisation and excretion of the exopolysaccharide (e.g. Glucksman et al., 1993; Matthysse et al., 1995). It is not yet clear whether these enzymes are always expressed or whether they respond to the same physiological conditions, which favour EPS synthesis. If the enzymes are present, they may only be released slowly as cells lyse. This may nevertheless cause a rapid reduction in both the polymer mass and the solution viscosity. The mass of alginates from *Pseudomonas fluorescens* and *Pseudomonas putida* fell approximately 50% per 24h at 30°C in shaken cultures through the action of endogenous poly-D-mannuronate-specific lyases after cell growth had ceased (Conti et al., 1994). This resulted in greatly reduced solution viscosity. In alginate-synthesising strains of *Pseudomonas aeruginosa*, the *algL* gene similarly controls an alginate lyase capable of limited action on the highly acetylated alginate from this bacterium (Schiller et al., 1993).

4. The exogenous production of polysaccharases

The source of exopolysaccharide-degrading enzyme mixtures may either be single microbial species or more commonly mixed cultures. In nature, microorganisms coexist in close proximity and are frequently found as consortia capable of degrading complex substrates, which cannot be utilised by the individual species. Polysaccharases may therefore be produced to enable microorganisms to degrade polysaccharide substrates and utilise their component monomers as carbon and energy sources. The enzymes themselves usually represent a complex mixture of activities even when the substrate is a homopolysaccharide. As well as enzymes capable of degrading the polysaccharide substrate to oligosaccharides, other enzymes capable of acting on these smaller fragments reduce them to smaller

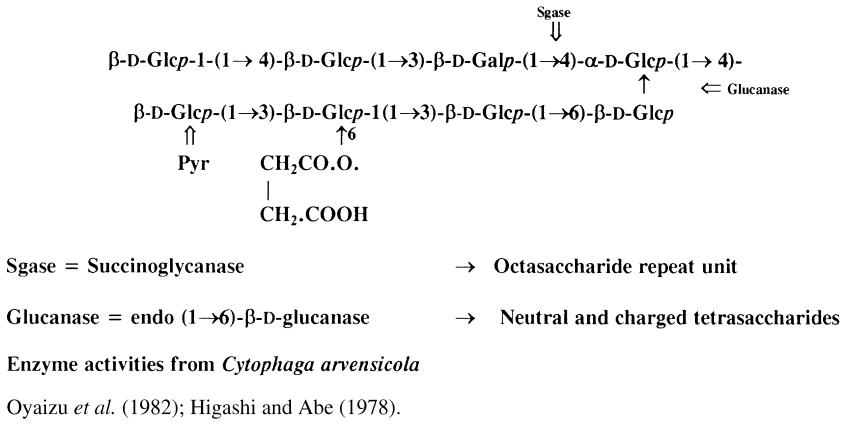

Fig. 1. Enzymes acting on the succinoglycan group of microbial exopolysaccharides

oligosaccharides or to monosaccharides which can then enter the uptake and utilisation systems of the enzyme-producing bacteria. Thus, a mixture of polysaccharases and glycosidases may be present. This appears to be true for homopolysaccharides such as bacterial cellulose, curdlan, scleroglucan and dextrans including mutan or for heteropolymers such as xanthan.

The enzyme systems needed for degradation of cellulose provide a good example of the complexity. Typically such mixtures comprise several examples of each of three types of enzyme -β-1,4-endoglucanases cleaving internal β-1,4-glucosidic bonds; cellobiohydrolases releasing cellobiose from the non-reducing terminus of the cellulose molecule; and β-D-glucosidase degrading the cellobiose so formed (Gilbert and Hazlewood, 1993).

It has proved relatively rare to find a pure bacterial culture capable of degrading an exopolysaccharide following normal enrichment procedures. However, early studies on degradation of the EPS of *Streptococcus pneumoniae* type 8 yielded a bacterium *Bacillus palustris* which excreted a lyase degrading the polymer (Becker and Pappenheimer, 1966). Xanthan lyases were obtained from several mixed bacterial cultures (Sutherland, 1987; Ahlgren, 1991). The preparation described by Ahlgren represented a protein of 33 kDa secreted by a consortium of heat-stable, salt-tolerant bacteria. It only acted on side-chains carrying pyruvate ketal groups on the terminal mannose residues. The problem with such bacterial mixtures is to ensure that they are stable and that the necessary micro-organisms are present in the mixture in sufficient numbers to provide the required enzyme. Although a pure bacterial culture secreting one or more xanthan-degrading enzymes has also been found by Cadmus et al. (1982), more commonly, as was demonstrated in several laboratories, a complex mixture of polysaccharide-degrading micro-organisms was obtained. Mixed microbial cultures were found to synthesise a complex enzyme mixture which achieved extensive degradation (e.g.Hou et al., 1986). Another example of a pure bacterial culture was the succinoglycan-degrading bacterial species *Cytophaga arvensicola* (Abe et al., 1980; Oyaizu et al., 1982; Harada, 1994). This bacterium produced two different enzymes, which acted jointly to yield the octasaccharide repeat units of succinoglycans and the two component tetrasaccharides respectively (Fig. 1). The enzyme degrading the octasaccharide repeat units of succinoglycans may well be of wider specificity than first thought as it also appears to degrade some galactoglucans in addition to succinoglycan (I.W.Sutherland, unpublished results). The enzyme action

Table 2
Examples of polysaccharide depolymerases from heterologous microorganisms

Polysaccharide	Enzyme action	Linkage cleaved	Enzyme source	Reference
Endoglycanases				
Bradyrhizobium sp.	Endorhamnosidase	1,4-β-L-Rha	*Bacillus* sp.	Cadmus et al. (1988)
Dextran	*Endoglucosidase*	1,3-β-D-Glc	*Bacillus* sp.	Bertram et al., (1993).
Dextran	*Endoglucosidase*	1,2-α-D-Glc	*Flavobacterium* sp.	Mitsuishi et al., (1980)
Dextran	*Endoglucosidase*	1,2-α-D-Glc	*Streptococcus mutans*	Pulkownik and Walker (1977)
Streptococcus pneumoniae type 3	*Endoglucosid*ase	1,3-β-D-Glc-1,4-β-D-GlcA	*Bacillus palust*ris	Torriani and Pappenheimer (1962)
Lyases				
Streptococcus pneumoniae type 8		1,4-α-D-Gal-β-D-GlcA-	*Bacillus palustris*	Becker and Pappenheimer (1966)
*Sphingom*onas sp. (Gellan)		1,4-β-D-Glc-β-D-GlcA-	Pseudomonad	Kennedy and Sutherland (1994)
Sphingomonas sp. (Gellan)		1,4-β-D-Glc-β-D-GlcA-	*Bacillus* sp.	Hashimoto et al. (1997)

Table 3
Examples of phage-associated polysaccharide depolymerases

Bacterial host/Polysaccharide	Enzyme action	Linkage Cleaved	Reference
Endoglycanases			
Klebsiella type K3	*Endogalactosidase*		Dutton et al. (1986)
Klebsiella type K8	*Endogalactosida*se		Sutherland (1976)
Klebsiella type K13	*Endogalactosidase*		Niemann et al. (1978)
Klebsiella type K18	*Endogalactosidase*	1,4-β-D-Gal	Dutton et al. (1980)
*Klebsi*ella type K22	*Endogalactosid*ase	1,4-β-D-Glc	Stirm (1994)
Klebsiella type K25	*Endogalactosidase*		Niemann et al. (1977)
Klebsiella type K26	*Endogalactosidase*		DiFabio et al. (1986)
Escherichia coli type K34	*Endogalactosidase*	1,2-β-D-GlcA	Dutton and Kuma-Mintah (1987)
Escherichia col type K36	*Endogalactosidase*	1,3-β-D-GalA	Parolis et al. (1988)
Klebsiella type K36	*Endogalactosidase*	1,3-β-L-Rha	Dutton et al. (1981)
Klebsiella type K43	*Endogalactosidase*	1,3-α-D-Man	Aereboe et al. (1993)
Klebsiella type K51	*Endogalactosidase*	1,3-α-D-Gal	Chakraborty (1985)
Klebsiella sp.	*Endogalactosidase*		Yurewicz et al. (1971)
Klebsiella type K74	*Endogalactosidase*	1,2-β-D-Man	Dutton et al. (1981)
Erwinia amylovora	*Endogalactosidase*	1,3-β-D-Gal	Nimtz et al. (1996)
Escherichia coli K36	*Endogalactosidase*	1,3-β-D-GlcA	Parolis et al. (1988)
Escherichia coli K103	*Endogalactosidase*	1,4-α-D-Gal	Grue et al. (1994)
Klebsiella serotype K11	*Endoglucosidase*	1,3-β-D-GlcA	Stirm et al. (1972); Bessler et al. (1973)
Klebsiella serotype K124	*Endoglucosidase*	1,2-β-D-GlcA	Annison et al. (1988)
Klebsiella serotype K25	*Endoglucosidase*		Niemann et al. (1977)
Klebsiella serotype K39	*Endoglucosidase*	1,3-β-D-Glc	Anderson et al., (1987)
Klebsiella serotype K44	*Endoglucosidase*	1,4-α-D-GlcA	Dutton and Karunaratne (1985)
Klebsiella serotype K60	*Endoglucosidase*		DiFabio et al. (1984)
Klebsiella serotype K63	*Endoglucosidase*		Dutton and Merrifield (1982)
Escherichia coli serotype 29	*Endoglucosidase*	1,3-β-D-GlcA	Fehmel et al. (1975)
Escherichia coli serotype 39	*Endoglucosidase*	1,6-β-D-Glc	Parolis et al. (1989)
Klebsiella pneumoniae SK1	*Endoglucanase (Endoglucosida se*)	1,3-β-D-Glc	Cescutti and Paoletti (1994)
Klebsiella serotype K2	*Endoglucosidase*	1,4-β-D-Glc	Geyer et al., (1983)
Klebsiella serotype K6	*Endoglucanase*		Elsässer-Beile and Stirm (1981)
Escherichia coli Type 8	*Endomannosidase*	1,3-α-D-Man	Prehm and Jann (1976)
Klebsiella serotypeK30	*Endomannosidase*		Ravenscroft et al. (1988)
Klebsiella serotypeK69	*Endomannosidase*	1,4-β-D-Glc	Hackland et al. (1988)
Escherichia coli Type 44	*Endo-N-acetyl-β-D-galactosaminid*ase 1,	β-D-GlcA	Dutton et al. (1988)
Acetobacter methanolicus	*Endorhamnosidase*		Grimmeke et al., (1994a)
Klebsiella serotype K17	*Endorhamnosidase*	1,4-β-D-Glc	Dutton et al. (1981)
Klebsiella serotype K19	*Endorhamnosidase*	1,2-α-D-Glc	Beurret and Joseleau (1986)
Pseudomonas syringae pv. morsprunorum	*Endorhamnosidase*		Smith et al. (1994)
Escherichia coli	*Endofucosidase*	1,3-β-D-Glc	Sutherland (1971)
	Neuraminidases/Sialidases		
	Neuraminidase		Hallenbeck et al. (1987)
Escherichia coli	*Endo-N-acetylneuraminidase*	2,8-α-NeuNAc	Kwiatkowski et al. (1983)
Escherichia coli K1 Phage E	*endosialidase*		Long et al. (1995)
Escherichia coli	KDO-KDO glycanase		Nimmich (1997)
Escherichia coli	KDO-KDO glycanase	β-D-KDO*f* $\rightarrow$ Ribp	Altmann et al. (1986) andAltmann et al., (1987)
		α-D-KDO*f* $\rightarrow$ Ribp	
Lyases			
Azotobacter vinelandii	*Alginate lyase*		Davidson et al. (1977)
Streptococcus	Hyaluronidase		Niemann et al. (1976)
Klebsiella serotype K5	Polysaccharide lyase	β-D-Manp-(1 $\rightarrow$ 4)-β-D-GlcpA	van Dam et al. (1985)
Klebsiella serotype K14	Polysaccharide lyase	β-D-Manp-(1 $\rightarrow$ 4)-β-D-GlcpA	Parolis et al. (1988)
Escherichia coli K5	Polysaccharide lyase	α-D-GlcpNAc-(1 $\rightarrow$ 4)-β-D-GlcpA	Hänfling et al. (1996)
Rhizobium spp.	Polysaccharide lyase		McNeil et al. (1986)

appeared to be largely unaffected by the different acyl groups found in succinoglycans from a wide range of bacterial sources. In contrast, the endogenous succinoglycan depolymerases (gene products *Exo*K and *Exs*H) from *Rhizobium meliloti*, proved to be affected by the presence of both acetyl and succinyl groups on their succinoglycan substrate (York and Walker, 1998). Acetylation inhibited the action of both polysaccharases while the presence of succinyl groups stimulated action.

Generally speaking, mixed cultures yield a range of both polysaccharases and glycosidases. Many of the enzymes produced in mixed culture only act on the initial degradation products, causing further breakdown of the oligosaccharides to monosaccharides, disaccharides or trisaccharides, fragments utilisable by the microbial cells.

As well as the endogenous polysaccharide lyase found in gellan-synthesising *Sphingomonas* spp. (Sutherland and Kennedy, 1996), other sources of similar enzymes were found. A group of Gram negative rods produced a gellan lyase acting on deacylated gellan (Kennedy and Sutherland, 1994) as did a red-pigmented Gram positive *Bacillus* sp. (Hashimoto et al., 1997; Hashimoto et al., 1998). In the *Bacillus* sp., intracellular glycosidases further degraded the tetrasaccharide released by the lyase. Neither of the lyase preparations acted on any of the closely related polysaccharides produced by other *Sphingomonas* sp. (or on the chemically deacylated derivatives). They were also strongly inhibited by the acyl groups present on the native gellan polysaccharide. Some of these enzymes are listed in Table 2.

5. Bacteriophage

Many of the bacteria which are surrounded by polysaccharide slime or capsules are also hosts for virulent bacteriophage. As demonstrated byStirm and Freund-Mölbert, 1971) and Eichholtz et al. (1975), the phages themselves vary greatly in their structures. To gain access to their primary receptors on the bacterial walls, most of the phage possess associated polysaccharide-degrading enzymes. As with polysaccharide-degrading enzymes in general, these enzymes may act either hydrolytically, cleaving specific linkages in the polysaccharides, or they may be polysaccharide lyases acting by eliminative cleavage at a monosaccharide-uronic acid linkage and introducing an unsaturated bond at the C_4 and C_5 of the non-reducing uronic acid terminal. The polysaccharide depolymerase activity may be associated with small spikes attached to the viral base-plate. Several studies have separated the spikes and demonstrated enzyme activity. In addition to the phage-associated enzyme, further activity is usually found in the soluble proteins in the cell lysates following viral maturation. Bacteriophage have thus provided a very extensive range of highly specific polysaccharases which were widely used in structural studies on bacterial EPS (e.g. Rieger-Hug and Stirm, 1981). Although many EPS-producing bacteria have proved to be hosts for polysaccharase-inducing bacteriophage (see e.g. Stirm, 1994), others including *Xanthomonas campestris* have proved recalcitrant and viruses of this type were difficult to isolate. Stirm (1994) has provided excellent protocols for the isolation of polysaccharase-inducing phages and the subsequent preparation of the enzymes and of their products.

Similarly, phages destroying their lipopolysaccharide (LPS) receptors are well known and in one example, the tail spike protein was fully characterised and functions in both adhesion to the host cell surface and receptor destruction (Baxa et al., 1996; Steinbacher et al., 1997). The enzyme in this phage is an endorhamnosidase that cleaves the 1,3-α-*O*-glycosidic bond between L-rhamnose and D-galactose, yielding octasaccharide fragments (2 repeat units) from the LPS side-chains as the major product. As no enzymes acting on EPS have yet been characterised in such detail with regard to their protein structure, it is not clear whether this enzyme protein represents the typical structure and configuration for phage-associated polysaccharide depolymerases.

Phage enzymes were used both in crude bacterial lysates and after extensive purification. Protocols for their use can be found in Dutton et al. (1981) or in Stirm (1994). These phage-induced enzymes acting on exopolysaccharide substrates have revealed a very wide range of specificities as indicated by the examples inTable 3. Although several *endoglucosidases*, *endogalactosidases*, or *endorhamnosidases* are listed, each is distinct in its specificity. It is clear from the table, however, that the majority of the enzymes, which were reported, are either endoglucosidases or endogalactosidases. Cleavage of other types of glycosidic linkage was less commonly described. Acyl groups seldom affect the action. An example was reported in which the same phage enzyme (from *Klebsiella* type 5) degrades both the EPS of the host strain which is acetylated on position 2 of a glucose residue and that of *E. coli* K55 in which the same polysaccharide structure contains an O-acetylated mannose (Anderson and Parolis, 1989). Enzyme activity is generally greatly influenced by the residues adjacent to the bond cleaved. Side-chains and charged groups appear to play an important role in determining the specificity. It is perhaps of interest that many of the phage-induced enzymes appear to preferentially attack either (1 → 3)-α- or (1 → 3)-β-bonds. Further, the residue targeted is very often adjacent to an anionic residue such as glucuronic acid, which may be part of the main chain or attached as a side-chain. In some EPS however, including that of *E. coli* K103, uronic acids are absent but charge is conferred by the presence of a pyruvate ketal (Grue et al., 1994). The enzymes are usually highly specific. It is rare for one such enzyme to act on more than one polysaccharide substrate unless the structures are very similar, although a few examples are known. Chemical modification of the substrate such as carboxyl reduction of uronic acids to the corresponding neutral hexose results in

loss of polysaccharase activity, indicating that the polyanionic nature of the polymers appears to be an important feature of enzyme specificity. However, removal of acyl groups by mild alkali treatment usually has little if any effect. Recent work in our laboratory using a phage originally isolated on a Pseudomonad host, isolated from a freshwater biofilm has shown that this phage is unusual. It can also form plaques on *Enterobacter cloacae* NCTC 5920 and some colanic acid-producing *E. coli* K12 strains (Lopez and Sutherland, unpublished results). On each host, the plaques are surrounded by large haloes. Assays using viscometry and measurement of the reducing sugar released, clearly demonstrated enzyme action on the polysaccharides from each bacterial host strain. Reducing sugar release was greatest from the polysaccharide of the original host. This result appears to be exceptional although one other example of a phage enzyme attacking two different EPS is known. The Klebsiella K5 phage enzyme cleaves the main chain of the polysaccharide from this strain (van Dam et al., 1985). It does also have slight activity against xanthan in which the same linkages are present in the side-chain of the polymer (I.W.Sutherland, unpublished results).

6. Effect of enzymes on exopolysaccharides

The effect of the phage enzyme attached to the viral base plate, is to carve a path through polysaccharide capsules as demonstrated by Bayer et al. (1979) for *E. coli* K29. As most of the polysaccharases described in this article are endoglycanases they rapidly destroy solution viscosity along with release of oligosaccharides representing the repeat units of the polysaccharides. This allows the phage particles to reach their primary receptors on the bacterial walls, inject their nucleic acid and complete the lytic cycle.

In a study of attached growth of alginate-producing *Pseudomonas aeruginosa*, Boyd and Chakrabarty (1994) observed that increased expression of alginate lyase caused alginate degradation and increased cell detachment. As the enzyme probably has limited activity against its substrate, it probably caused cell detachment through reduced mass and viscosity. This would correspond to the observations of Conti et al. (1994) in other alginate-synthesising *Pseudomonas* spp. The uncontrolled action of the enzymes does however greatly reduce the molecular mass of bacterial alginates and thus reduce their potential value as products of biotechnology. Similar problems are encountered in the commercial production of bacterial hyaluronic acid from *Streptococcus* spp. As has already been mentioned, alginate lyases present in *Pseudomonas* spp. caused a considerable drop in the mass and solution viscosity of these polymers. Similar problems were encountered during attempts to use *Azotobacter* spp. as a source of commercial alginate production (Deavin et al., 1977). The rapid loss of polymer integrity lead to the abandonment of the project. The same type of problems were encountered in commercial production of hyaluronic acid by strains of *Streptococcus* spp. but have apparently been overcome.

Much depends on the nature of the enzyme action. Endo-acting polysaccharases will cause a rapid reduction in the degree of polymerisation (DP) of the polymer substrate. The glycan chains may however remain associated with one another or with other macromolecules to some extent after a single bond is cleaved. Only when extensive bond breakage has occurred will the polysaccharide be totally dispersed. The environment of the polysaccharide will also be of major importance. In biofilms, the proximity of polysaccharides and micro-organisms may allow much greater action than would be the case with individual polymers and single enzymes (Hughes, 1997). One of the few successful examples of commercial enzymes acting on microbial EPS was observed by Johansen et al. (1997) on biofilms from several bacterial species. The preparation contained a range of different activities. Similarly, polysaccharides in flocs such as those found in waste–water purification may be affected by the enzyme activities associated with these complexes (Frølund et al., 1995).

The physical properties of the polysaccharide may occasionally be enhanced, as is the case with the levans and dextrans associated with dental plaque. *Streptococcus mutans* secretes an extracellular endo-dextranase that may play a significant role in modifying the physicochemical properties of mutans (Lawman and Bleiweis, 1991). The enzymes attack α-1,6 linkages. The net effect is thus to increase the proportion of α-1,3 linkages and enhance the hydrophobicity of the polysaccharide, rendering it increasingly insoluble in water and more adherent to the dental surface.

Exo-acting enzymes only cause a slow reduction in DP and a slow gradual release of oligosaccharide products. An unusual bacterial dextranase from an *Achromobacter* sp. proved to be an exo-glucanase releasing isomaltose from its substrate (Sawai et al., 1974). Unlike the well documented degradation of cellulose in which there is combined attack of endo- and exo-1,4-β-D-glucanases (Gilkes et al., 1991), most exopolysaccharides subjected to enzymic degradation are subjected to the action of a single endo-acting enzyme. Although action may be slow and incomplete, the presence of exo-acting enzymes may lead to alteration of the exposed surfaces over a prolonged period. Such enzymes could cleave exposed monosaccharide termini from either exopolysaccharides or glycoproteins. Removal of the terminal sugars would also lead to possible changes in physical properties. A debranching enzyme from a *Flavobacterium* sp. was shown to be highly specific for (1 → 2)-β-D-glucosidic linkages in dextrans (Mitsuishi et al., 1980).

7. Other enzymes acting on microbial exopolysaccharides

Many microbial exopolysaccharide are acylated, the

commonest substituents being ketal-linked pyruvate or ester-linked acetyl groups. Removal of the acyl groups, especially acetate (Sutherland, 1997) may greatly affect the physical properties of the polysaccharides. Esterase activities capable of removing these substituents have not yet been demonstrated although deacetylation of pectin was recently reported in a strain of *Erwinia chrysanthemi* (Shevchik, 1997) and an esterase acting on acetylated xylans was obtained from *Bacillus pumilis* (Degrassi et al., 1998). Phage-induced enzymes acting on the 'Vi' (poly2-amino-2-deoxy-D-galacturonic acid) antigen of Enterobacterial strains, also revealed esterase activity against acetylated poly-D-galacturonic acid (Kwiatkowski et al., 1975). As the pectic derivative is an analogue of the natural substrate, this finding is not unexpected. Phage-induced esterases removing acetyl groups from LPS are also known (Iwashita and Kanegasaki, 1976), while Shabtai and Gutnick (1985) demonstrated esterase activity against emulsan, the surface-active lipopolysaccharide-like polymer synthesised by *Acinetobacter calcoaceticus* strain RAG-1. The emulsan esterase appeared to show specificity for this unusual polymer but also cleaved nitrophenyl esters, a feature that was also observed for the acetylated xylan esterase. Now that several prokaryotic exopolysaccharides are known to carry sulphate groups, sulphatases similar in action to those acting on glycosaminoglycans (Shaklee et al., 1985) may also be found.

A small number of glycosidases do act on polymeric substrates to release some terminal sugar residues. An example of such action was demonstrated by Gidley et al. (1992) who used an α-D-galactosidase to remove almost 50% of the (1 → 2)-linked α-D-galactose side-chains of Rhizobium capsular polysaccharide. This caused progressive elimination of helix-helix aggregation and its resultant gel formation. A similar study was made on a *Xanthomonas campestris* mutant yielding EPS partially defective in the terminal β-linked D-mannosyl residues of the xanthan-type polysaccharide (Tait and Sutherland, 1989). Prolonged exposure to a commercial β-D-glucuronidase removed many of the terminal uronic acid residues. Ion binding and interchain associations were thus affected. Further removal of the exposed D-glucuronosyl residues enhanced the solution viscosity.

Some examples are known in which chemical modification of microbial EPS was shown to increase their susceptibility to enzymes. Anderson and Stone (1978) demonstrated that after deacetylation and carboxyl reduction, EPS from several species of *Rhizobium* could be hydrolysed by several endoglucanases. Through the use of enzymes specifically acting on (1 → 4)-β-D-glucans and (1 → 3)-β-D-glucans, further structural information was obtained.

8. Potential applications of enzymes

Several of the enzymes hydrolysing microbial exopolysaccharides can be used to produce oligosaccharides. Pullulanase can thus be used for the quantitative preparation of maltotriose from pullulan (Catley, 1994), β-D-glucanases may allow production of laminaridextrins from curdlan (or laminaran). To ensure that there is no further degradation of the product, the enzyme must be free of other glucosidase activities. It has also been suggested that oligosaccharide products of alginate lyases might promote growth in plants. Root elongation was demonstrated in barley (Tomoda et al., 1993). Alginate lyase preparations active against either marine algal alginate or deacetylated bacterial alginate are routinely used to degrade algal alginates in the walls of marine algae to form protoplasts but thus is not strictly an application to microbial exopolysaccharides. However, it is clear that the most widespread application of these enzymes was in studies of EPS structures. Release of relatively large quantities of oligosaccharide repeat units without the degradation and loss of acyl substituents associated with acid hydrolysis, has proved extremely useful in determination of microbial exopolysaccharide structures. This will no doubt continue to be the case! With the increasing interest and sophistication available in the modelling of oligosaccharide and polysaccharide conformation, the oligosaccharides produced by enzymes from phage or other sources may also prove useful model systems.

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