Polysaccharides, Microbial

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Glossary

capsular polysaccharide (CPS) An exocellular polysaccharide secreted in such a large amount that it encapsulates a microorganism, facilitating adhesion and helping screen from immune response. Sometimes a distinction is made with more loosely attached polysaccharides 'released polysaccharide' (RPS).

epimerase An enzyme that converts mannuronic acid residues to guluronic acid residues in alginates.

intrinsic viscosity, [η] (ml g^{-1}) A measure of a particle's conformation and volume occupancy in solution.

Mark–Houwink 'a' parameter A measure of the conformation of a macromolecule, and determined from measurements of solution viscosity and molecular weight. Limits are 0 (sphere), 1.8 (rod), with values 0.5–0.8 for a flexible coil-like molecule.

molecular weight (Da or g mol^{-1}) Popular terminology for 'molar mass' – the mass of 1 mol (6.023×10^{23}) molecules.

molecular weight – number average, M_n (Da or g mol^{-1}) In a mixture of macromolecules M_n is the molecular weight averaged over the number concentrations of each species. It can be determined by techniques such as osmotic pressure, size exclusion chromatography coupled to multiangle light scattering (SEC-MALLs) or analytical ultracentrifugation.

molecular weight – weight average, M_w (Da or g mol^{-1}) In a mixture of macromolecules M_w is the molecular weight averaged over the weight concentrations of each species. It can be determined by techniques such as size exclusion chromatography coupled to multiangle light scattering (SEC-MALLs) or analytical ultracentrifugation.

non-Newtonian fluid A fluid in which viscosity changes with applied strain, an example of which is shear thinning.

persistence length, L_p (nm) A measure of the flexibility of a linear macromolecule ranging from ~2 nm for a very flexible molecule to ~200 nm for very stiff.

polydispersity index The ratio of the weight average molecular weight to the number average molecular weight, M_w/M_n. Minimum value = 1.

Abbreviations

CPS	capsular polysaccharide
GDP	guanosine diphosphate
GRAS	generally regarded as safe
HA	hyaluronic acid
RPS	released polysaccharide

Defining Statement

Microbial polysaccharides are used for food, pharmaceutical, and medical applications: this wide range of usefulness derives from the great diversity in structural and functional properties. We consider the structure, properties, extraction, production, modification, and applications of commercially available microbial polysaccharides including xanthan, xylinan, gellan, curdlan, pullulan, dextran, scleroglucan, schizophyllan, and cyanobacterial polysaccharides.

Introduction

Over the last two decades there has been an expanding interest in polysaccharides produced extracellularly by microorganisms for food, pharmaceutical, and medical use, including vaccines. The wide range of their usefulness derives from the great diversity in structural and functional properties even though they are built up from very similar building blocks: the pyranose or furanose carbohydrate ring structure. One can have rod-shaped molecules like schizophyllan and scleroglucan from the fungi *Schizophyllan commue* and *Sclerotium rolfsii*, respectively, linear random coil type structures like pullulan from the fungus *Aureobasidium pullulans*, and intermediate structures such as alginates from the bacterium *Azotobacter vinelandii*. One can have highly electronegative or 'polyanionic' molecules like xanthan secreted by *Xanthomonas campestris* or neutral polysaccharides like dextrans from the bacteria *Leuconostoc mesenteroides*.

Polysaccharides made by microrganisms are secreted from the cell to form a layer over the surface of the organism, often of substantial depth in comparison with the cell dimensions (**Figure 1**). Because of their position they are characterized as exopolysaccharides, to distinguish them from any polysaccharides that might be found within the cell. The functions are thought to be mainly protective, either as a general physical barrier preventing access of harmful substances, or more specific as a way of binding and neutralizing bacteriophage. In appropriate environments they may prevent dehydration.

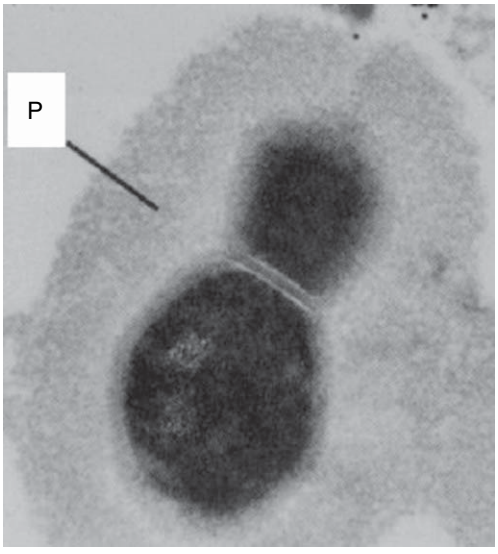

Figure 1 Exocellular or 'capsular' polysaccharide layer (labeled P) from *Streptococcus pneumoniae*. Adapted from Skov-Sørensen UB, Blom J, Birch-Andersen A, and Henrichsen J (1988) Ultrastructural localization of capsules, cell wall polysaccharide, cell wall proteins and F antigen in pneumococci. *Infection and Immunity* 56: 1890–1896. Reproduced with permission from the American Society for Microbiology.

They may also prevent phagocytosis by other microorganisms or the cells of the immune system. The capsular polysaccharides (CPSs) are often highly immunogenic, and may have evolved their unusual diversity as a way of avoiding antibody responses: advantage of this feature can be taken in the development of vaccines. They also have a role in adhesion and penetration of the host. In the case of plants this involves interaction with polysaccharide structures in the cell walls and there are possibilities of specific interactions. Plant lectins (glycoproteins) that have specific binding properties with respect to carbohydrate structures may play a part in this and in the general defense of plants against bacterial infection.

Some secreted polysaccharides can be involved in pathogenicity. *Pseudomonas aeruginosa*, commonly found in respiratory tract infections, produces alginate which contributes to blockage in the respiratory tract, and leads to further infection, and similar blockages of phloem in plants have also been described. However, the secreted polysaccharides themselves present virtually no known toxicity problems and many are harvestable at low cost in large quantities, making them attractive for biotechnological use.

Microbial products are very important in all aspects of polysaccharide biotechnology. They range from bulk materials such as xanthan, priced at about US$14 per kg, used mainly in food applications, to cyclic dextrans, valued at about US$50 per kg and used in high-value applications in research and pharmaceuticals.

Certain microbes are known to produce nearly all the major plant polysaccharides such as glucans, alginate-like materials, and even cellulose – as well as the complex bacteria-specific materials. Genetic manipulation of bacteria has been studied for longer and is in general much easier than for higher organisms, so that they are an obvious target for both manipulation of biosynthetic pathways and the expression of heterologous genes to produce especially desirable enzymes. Polysaccharides are not of course under the direct control of genetic material in the way that enzymes are, and must be approached indirectly by manipulating the biosynthetic or degradation pathways by way of the enzymes responsible: there is currently great scope for this approach. In addition, since there are now fermentation processes involving the large-scale growth of bacteria, all the advances in this field can be achieved by biotechnology.

There are also limitations. It is unlikely that large-scale fermentation could ever compete with existing processes for pectin manufacture, for example, since processors currently use waste by-products from other processes as their raw material. This is quite apart from the inherent costs of large-scale fermenters in comparison with simple extraction processes. It follows that bacterial products must have particularly useful properties in order to justify their probable greater cost.

This article looks at the production, properties, and biomodifications where appropriate of a selected range of important microbial polysaccharides because it is impossible to cover each substance comprehensively. Instead, we focus on xanthan as our main example because of its commercial importance but also consider xanthan-like exopolysaccharides such as gellan, xm6, curdlan, xylinan, and the increasingly important group of cyanobacterial polysaccharides – also xanthan-like – as well as pullulan, dextrans, scleroglucan, schizophyllan, microbial hyaluronic acid (HA), and bacterial alginates as representative examples. We complete our review with a look at the use and potential further use of exopolysaccharides as vaccines against serious diseases such as meningitis in infants.

Xanthan

Structure

Xanthan (or, as commonly called, xanthan gum) at US$14 per kg falls in a similar price range as other gums and exudates and has similar functional properties. It is made by the organism *X. campestris* (which in its existence in the wild is responsible for cabbage blight) grown largely on glucose, itself derived from maize starch, which it converts with high efficiency (80%) to the xanthan gum structure. Typically, for a fermentation product, the raw material costs are a small part of the total, which are mainly to do with recovering the gum from the culture medium. Production is around 9000 tonnes a year. It has a $\beta(1\rightarrow4)$-linked glucan main chain with alternating residues substituted on the 3-position with a trisaccharide chain containing two mannose and one glucuronic acid residue (**Figure 2**). It is thus a charged polymer. Some of the mannose residues may also carry acetyl groups. It is useful because it forms relatively rigid rod-like structures in solution at ambient temperatures, though they convert to the random configuration on heating. These rods are able to align themselves, rather like agarose and the κ- and ι-carrageenans, with the unsubstituted regions of galactomannans, such as guar and its derivatives and locust bean gum, to produce fairly rigid mixed gels with applications in food manufacture.

The primary structure (as worked out by sequential degradation and methylation analysis) consists of a repeating unit of β-D-glucose:

$$\ldots \rightarrow 4)\beta\text{-D-Glc}p(1\rightarrow \ldots$$

and in this respect it resembles cellulose, with the important difference that with the substitution at C3 (i.e., the third position on the carbon ring) every alternate Glc*p* residue is substituted by a negatively charged trisaccharide involving mannose and acetylated mannose:

$$\beta\text{-D-Man}p(1\rightarrow4)\beta\text{-D-Glc}p\text{A}$$
$$(1\rightarrow2)\alpha\text{-D-Man}p\text{-6-OAcetyl}(1\rightarrow3)$$

with some of the terminal β-D-Man*p* residues substituted at positions C4 and C6 by pyruvate ($CH_3.CO.COO^-$). Thus xanthan (except under highly acidic conditions) is, like alginate, a highly charged polyanion. **Figure 2(a)** shows the Haworth projection of a (pyruvalated) alternate repeat section of xanthan.

It is not only charge repulsion effects that convey on xanthan an extended rigid rod conformation. X-ray fiber diffraction studies have shown the presence of some helical structure although there is still uncertainty as to whether this helix is a coaxial duplex or just a single chain one, with the projecting trisaccharides folded back along the axis of the helix giving a chain rigidity effectively similar to that of a double helix. Using a combination of electron microscopy (contour chain length, L), and light scattering (weight average molecular weight, M_w), Stokke and coworkers evaluated a mass per unit length, M_L, of $1950 \pm 250\,\text{g mol}^{-1}\,\text{nm}^{-1}$. From X-ray fiber diffraction studies, a corresponding value of $950\,\text{g mol}^{-1}\,\text{nm}^{-1}$ was obtained, and a duplex or double-helical structure was inferred for the native structure. High temperatures reversibly melt this helix to give a more random structure.

Extraction and Production

The primary laboratory and commercial fermentation medium – as has been known for over 50 years – for *X. campestris* growth and xanthan production is a phosphate-buffered (pH ~ 7) broth containing D-glucose ($30\,\text{g l}^{-1}$) (or sucrose, starch, hydrolyzed starch), NH_4Cl, $MgSO_4$, trace salts with $5\,\text{g l}^{-1}$ casein (or soybean) hydrolysate, and the fermentation process takes place aerobically at a temperature of ~28 °C. Xanthan production is further stimulated by the presence of pyruvic, succinic, or other organic acids. The xanthan produced in this way is very similar to the naturally produced xanthan made by the microbes living on a cabbage. In the commercial process the oxygen uptake from the broth is controlled to a rate of $1\,\text{mmol l}^{-1}\,\text{min}^{-1}$. Treated in this way the bacterium is an extremely efficient enzyme minifactory converting >70% of the substrate (D-glucose or related) to polymeric xanthan. The bacterium having done its work is then removed in a rather undignified way by centrifugation and the xanthan precipitated with methanol or 2-propanol at 50% weight concentration. The xanthan slurry is then dried and milled for use. The original commercial producer of xanthan was Kelco Ltd (now CPKelco) and together with other suppliers the annual worldwide production is now over 10 000 tonnes.

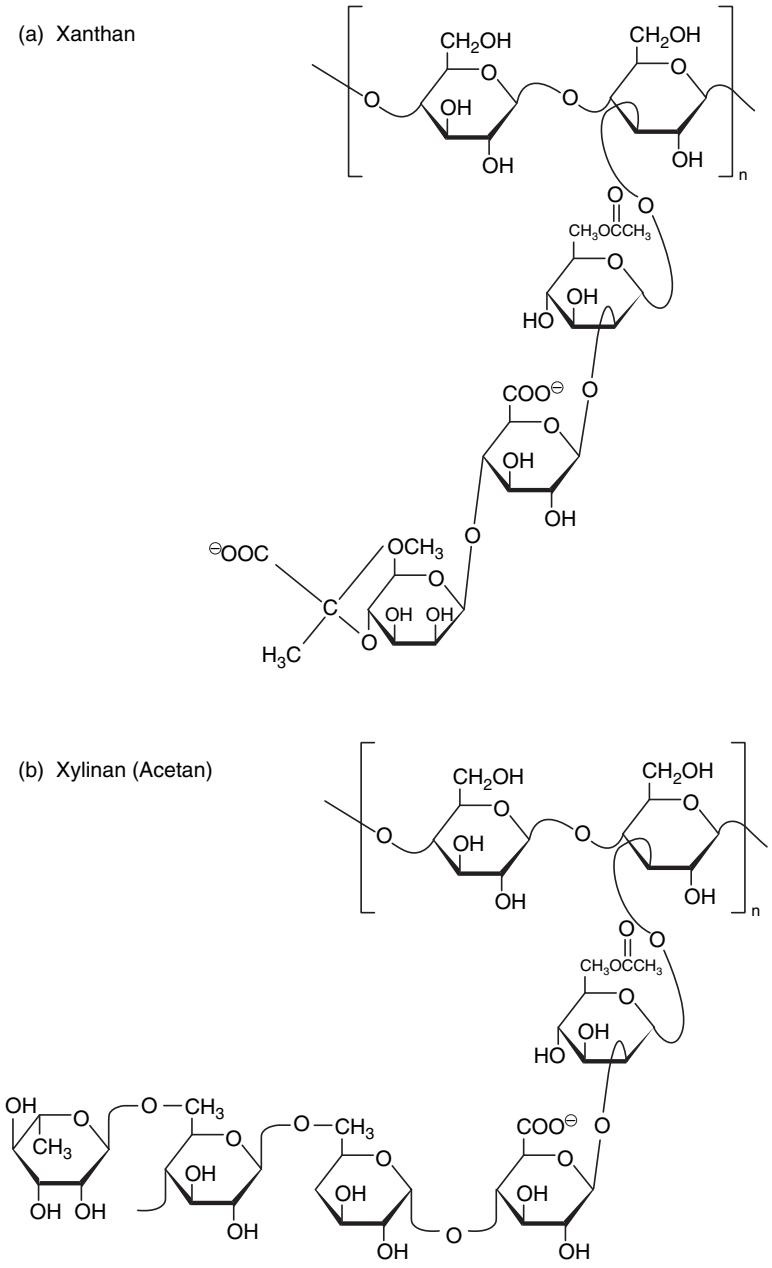

Figure 2 (a) Xanthan repeat unit showing a trisaccharide side chain with pyruvalated end mannose unit. Not all the terminal side chain residues are pyruvalated. (b) Xylinan (acetan) repeat unit showing a pentasaccharide side chain.

The biosynthetic process by the bacterium worked out by Sutherland and later confirmed by others follows the same basic pattern proposed for other microbial polysaccharides: (1) substrate uptake; (2) substrate metabolism; (3) polymerization (4) modification and extrusion, and involves lipid carriers (although there is still uncertainty over their precise role and how the whole process is controlled).

Properties

These molecules are extremely large, for example, the weight average M_w of keltrol xanthan has been found by ultracentrifuge techniques to be $\sim 6 \times 10^6$ g molecular persistence mol^{-1}, and stiff, with a molecular persistence length L_p of $\sim$150 nm (the practical limits for linear molecules are $\sim$2 nm for a very flexible, or 'random coil' structure, and $\sim$200 nm for a rigid rod structure). Xanthan gives very viscous solutions (intrinsic viscosities, $[\eta]$, approximately several thousand milliliters per gram) and indeed is one of the largest of the aqueous soluble polysaccharides. The very high viscosity at low concentrations (e.g., at 5 mg ml^{-1} a viscosity of $\sim$1000 cP has been observed at room temperature) makes it ideal as a thickening and suspending agent.

Modification

The key sites for modification are the first and terminal mannose residues on the trisaccharide side chains (particularly extent of acetylation of the first and pyruvalation of the latter) and the helical backbone formed by noncovalent interactions with galactomannans: these interactions also appear to be affected by the substitutions in the side chains. Insofar as the trisaccharide side chains are concerned there are two approaches for alteration or control: one is changing the physiological conditions of fermentation; the other is the use of different pathovars or strains of *X. campestris*. The pathovars pv phaseoli and oryzae yield virtually acetyl-free and pyruvate-free xanthan, respectively.

The other approach has been to look at the genetics of the enzymes controlling the biosynthetic pathway and to attempt to produce and isolate in sufficient quantity genetic mutants deficient or defective in one or more of these enzymes to give 'polytetramer' (i.e., lacking in the terminal mannosyl or pyruvalated mannose group) and 'polytrimer' (lacking in addition the adjacent glucuronic acid residue). Although yields of 50% polytetramer have been found, corresponding attempts for polytrimer and other xanthan variants have thus far been disappointing.

There has been considerable interest in improving the weak gelation characteristics of xanthan by inclusion of galactomannans such as locust bean gum and gum into mixtures. As with carrageenan and agarose, excellent synergistic interaction occurs between xanthan and the galactomannans such as locus bean gum or guar. Xanthan gels are very weak and transient but made in the presence of guar or locust bean gum give stronger gels with an optimum mixing ratio ~50:50 by weight. Deacetylating the xanthan side chains seems to enhance these synergistic interactions.

Uses of Xanthan

Xanthan was approved as food grade by the US Food and Drug Administration nearly 30 years ago. This makes it not only attractive as a food product but also useful in packaging material in contact with food and also for use in pharmaceutical and biomedical applications that involve ingestion. Its uses chiefly derive from its solubility in hot and cold water and its very high thickening and suspending potential, which in turn derives from the very high viscosity of its suspensions. Despite its high viscosity, xanthan suspensions exhibit high shear thinning, which means they also flow easily (i.e., good pourability). For food applications, xanthan suspensions have high acid stability besides high viscosity and thickening and suspending ability. This makes it highly popular in sauces, syrups and toppings, and salad dressings. In drinks, the addition of xanthan together with carboxymethylcellulose adds body to the liquid and assists with uniform distribution of fruit pulp. It is also used to add body to dairy products. The high freeze–thaw stability of xanthan suspensions makes it particularly attractive for the frozen food industry. The high suspending and stabilizing properties are also taken advantage of by the animal feed industry for transporting liquid feeds with added vitamins and other supplements, which would otherwise sediment out during transport or with storage time. It has also been suggested as an additive to fruit drinks to reduce tooth decay. For pharmaceuticals, xanthan has recently been added to the list of hydrophilic matrix carriers, along with chitosan, cellulose ethers, modified starches, and scleroglucan. Tablets containing 5% xanthan gum under low shear conditions were shown to enable the successfully controlled release of acetaminophen into stomach fluid and tablets containing 20% xanthan successfully carried a high loading (50%) of the drug theophylline. The high suspension stability is made use of in pharmaceutical cream formulations and in barium sulfate preparations. This high cream stability is also taken advantage of by the cosmetic industry, including toothpaste technology, facilitating the suspension of ingredients (high viscosity) and the easy brushing onto and off the teeth (high shear thinning). Uniform pigment dispersal along with other ingredients and long-time stability make xanthan a good base for shampoos. The high suspension stability of xanthan makes it an ideal base for suspending adhesive agents for wallpapers. Just to reinforce the diversity of application, the suspension-stabilizing property of xanthans makes it ideal for producing sharp prints from dyes, with a minimum risk of running in textiles and (in conjunction with guar) carpets.

Xylinan (Acetan)

Structure

Xylinan (or acetan) is a complex anionic exopolysaccharide produced by the Gram-negative bacterium *A. xylinum*. The structural repeat unit is similar to that of xanthan and consists of a cellulosic backbone (... →4) β-D-Glc*p*(1→ ...) where alternate glucopyranose residues are substituted at the 3-position with a pentasaccharide side chain (**Figure 2(b)**):

α-l-Rha*p*-(1→6)-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-
β-D-GlcA*p*-(1→2)-α-D-Man*p*-(1→3)

The branched glucopyranose residues together with the mannopyranose residue are partially O-acetylated at the 6-position. The pentasaccharide side chain protects the cellulosic backbone from enzymatic degradation by cellulases.

Analysis from X-ray diffraction and atomic force microscopy show that xylinan adopts a helical structure

in both the solid and solution phases. Hydrodynamic characterization using sedimentation velocity in the analytical ultracentrifuge, viscometry, and multiangle laser light scattering have shown that double-stranded helices are predominant although single- and multistranded chains are also present. Xylinan chains upon heating undergo thermoreversible helix (ordered state)–coil (disordered state) transition.

Extraction and Production

Xylinan is extracted as a water-soluble by-product during the commercial production of bacterial cellulose by *A. xylinum*.

Properties

These molecules are extremely large and a weight average M_w of $\sim 2.5 \times 10^6\,\mathrm{g\,mol^{-1}}$ has been found by ultracentrifuge and light scattering techniques. Furthermore, the double-helical structure results in a stiff macromolecular chain with a molecular persistence length L_p of $\sim 100\,\mathrm{nm}$ and a mass per unit length M_L of $\sim 2500\,\mathrm{g\,mol^{-1}\,nm^{-1}}$, resulting in viscous solutions, $[\eta]$, approximately several thousand milliliter per gram. A Mark–Houwink '*a*' parameter of 0.90 was found, which is consistent with a rigid conformation. Intermolecular binding has been demonstrated between xylinan and the industrially important glucomannan (konjac gum) and galactomannan (carob gum), resulting in the formation of synergistic gels (or increased viscosity at lower concentrations).

Modification

Chemical modification of xylinan has been through deacetylation of the 6-linked *O*-acetyl groups. It has been shown that acetylation does not prevent helix formation but it does reduce the synergistic interaction with glucomannans or galactomannans.

Xylinan synthesis involves several genes including *ace*A, *ace*B, *ace*C and it has recently been shown that chemical mutagenesis of the native bacterium results in a strain that produces a polysaccharide in which the side chain is a tetrasaccharide. Furthermore, this novel xylinan variant has higher viscosity. A further novel polysaccharide with a trisaccharide side chain was designed by deactivating the *ace*P gene.

Uses

Xylinan is used in the food industry as a viscosifier and gelling agent. It is also a component in nata, a confectionery popular in Japan and the Philippines.

Gellan, XM6, and Curdlan

Inspired by the example of xanthan, other bacterial polysaccharides with useful properties have been developed, for example, gellan, the result of a systematic search for a polysaccharide having the required properties, followed by the identification of the organism.

Gellan is obtained from cultures of *P. elodea* found growing on the *elodea* plant. It is isolated by ethanol precipitation from the culture medium, and may be partially deacetylated by alkali treatment. It has a linear structure with a repeat unit of a tetrasaccharide (**Figure 3**), each with one carboxyl group and in the native state one acetyl group. It is therefore sensitive to calcium levels but has some rheological properties similar to those of xanthan, which has a similar charge density and adopts a double-helical conformation in solution. The double-helix molecular weight is reported to be $\sim 5 \times 10^6$, with an intrinsic viscosity of $\sim 3500\,\mathrm{ml\,g^{-1}}$.

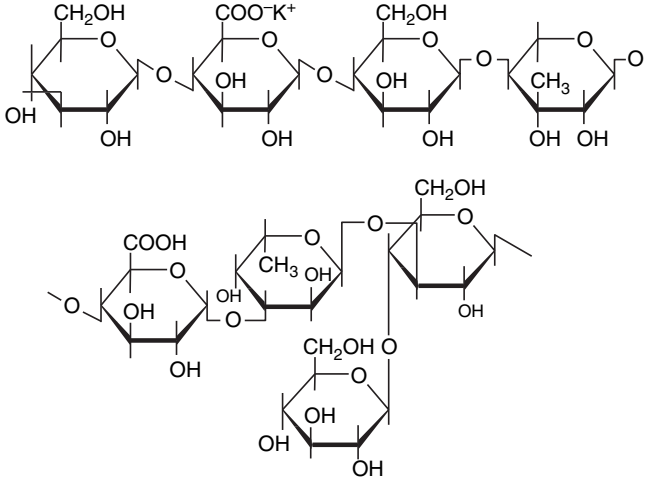

Figure 3 Repeat units, at the top for gellan in deacetylated form and below for the XM6 polysaccharide.

It was clearly intended to be a xanthan competitor, though before permission for food use was obtained it was promoted as an agar substitute, particularly for use in growth media. Gels produced on untreated gellans are weak and rubbery although deacylation produces hard, brittle gels, and after prior removal of all multivalent cations and addition of Ca^{2+} ions, gellan forms gels in rather the same way as with alginates. It is one of many film-forming polysaccharides being considered for use in implants for insulin in the treatment of diabetes.

The XM6 repeat unit structure is also shown in Figure 3 and is a polysaccharide made by an *Enterobacter* discovered at Edinburgh University. It lacks acetyl groups but is classed along with xanthan and gellan, and can be induced to gel by adding calcium ions. It is included here as a representative of many similar bacterial polysaccharides all of which have interesting and potentially useful rheological properties, although it is not commercially available, and as far as we are aware has not been approved for food use, and probably never will.

Curdlan is one of two polysaccharides produced by *A. faecalis* with a repeat unit of three $\beta(1\rightarrow3)$-linked D-glucose residues and unlike the examples above is not charged. Production is now from *Agrobacterium* mutants, which make only curdlan in high yield, and is mostly confined to Japan. Curdlan suspensions gel on heating, apparently irreversibly. There are a number of potential applications for it in the food industry, mostly as a replacer for existing gums, and it is in use in Japan in some products. Novel products based on its heat set ability may also appear.

Cyanobacterial Polysaccharides

Another group of polysaccharides with reputed xanthan-like properties are the exopolysaccharides from Cyanobacteria – blue-green algae – known to produce large amounts of these substances.

Structure

Like xanthan, the cyanobacterial polysaccharides are relatively complex containing on average 6–10 different saccharide residues, with the neutral sugars xylose, arabinose, fucose, galactose, rhamnose, mannose, glucose, and uronic acids, with glucose normally being the dominant residue. Charged groups such as sulfate and pyruvate together with hydrophobic groups, for example, acetates have also been reported. All the cyanobacterial polysaccharides studied so far have been branched structures.

Extraction and Production

Due to their diversity, cyanobacteria are found growing under a wide variety of conditions. Nitrogen seems to have a negative effect on polysaccharide production whereas temperature, salinity, illumination, and exposure to UV radiation can have either positive or negative effects on exopolysaccharide production in cyanobacteria. It is worth noting that optimal exopolysaccharide production does not necessarily coincide with those for cell growth. The daily production of cyanobacterial polysaccharides from different sources is up to $2\,g\,l^{-1}$, which is small in comparison to xanthan production, which can be up to $10\,g\,l^{-1}$.

Properties

Cyanobacterial exopolysaccharides have been shown (Figure 4) to exhibit xanthan-like shear-thinning properties. Polysaccharide molecular weights have been measured in the range of $1–2 \times 10^6\,g\,mol^{-1}$ and 'gross' macromolecular conformations have been estimated to be a random coil for the exopolysaccharide from *Cyanospira capsulata* (CC-EPS), a rigid rod type polysaccharide from *Aphanothece halophytica* GR02 (AH-EPS), or intermediate between random coil and rigid rod for the polysaccharide from *Annabaena* sp. ATCC 33047.

Uses

These exopolysaccharides are of potential biotechnological importance as conditioners of soils as they improve water-holding capacity; as emulsifiers, viscosifiers, medicines, bioflocculants; and for heavy metal removal. A pectin-like polysaccharide that is able to absorb metal cations has also been described.

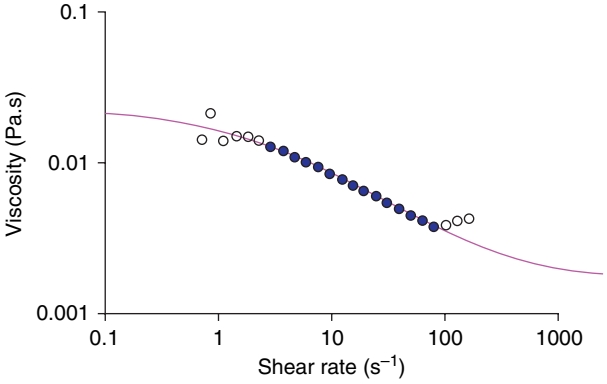

Figure 4 Viscosity and fitting for the shear-thinning cyanobacterial exopolysaccharide from *Aphanothece halophytica* GR02 (AH-EPS) at $0.6\,g\,l^{-1}$ measured at 25 °C. Circles: points not used for fitting. Square: points used for fitting. Line: fitted curve. From Morris GA, Li P, Puaud M, *et al*. (2001) Hydrodynamic characterisation of the exopolysaccharides from the halophilic cyanobacteria *Aphanothece halophytica* GR02: A comparison with xanthan. *Carbohydrate Polymers* 44: 261–268.

Pullulan

Pullulan is an α-glucan made up from maltotriose units linked by $\alpha(1\rightarrow6)$ bonds. It is obtained from *A. pullulans* and is hydrolyzed by pullulanase to yield maltotriose. It is not attacked by digestive enzymes of the human gut, and is used to form films. Production is now substantial, and has found particular application in formulating snack foods in Japan based on cod roe, powdered cheese, and as a packaging film for ham. It is slowly digested in humans.

It is water soluble, with molecular weights in the range 5000–900 000 g mol^{-1}, with straight unbranched chains, and behaves as a very flexible molecule, with properties of a 'random-coil' (with a molecular persistence length L_p of approximately 2 nm) depending on the combination of sedimentation coefficient and intrinsic viscosity measurements. It has been proposed as a 'standard polysaccharide' because its behavior resembles random coil behavior, and it is readily obtainable in a very reproducible form so that it can be used for comparative tests with other polysaccharides. The idea is presumably that deviation from pullulan type behavior must imply a more complex structure. It might also be used to standardize instruments. The solutions in water are liable to bacterial attack, with rapid degradation of the molecular weight, and preservatives such as azide may be needed.

Dextrans

Bacterial dextrans are produced in substantial quantities by *L. mesenteroides* and are familiar to laboratory workers as the basis for cross-linked dextran beads used in gel filtration columns. They are mostly $\alpha(1\rightarrow6)$D-glucopyranosyl polymers with molecular weights up to $\sim1\times10^6$ g mol^{-1} more or less branched through $1\rightarrow2$,$1\rightarrow3$, or $1\rightarrow4$ links. In most cases the length of the side chains is short, and branched residues vary between 5% and 33%. The major commercial dextran is about 95% $1\rightarrow6$ linked and 5% $1\rightarrow3$ linked, and is made from selected strains of *Leuconostoc*. After ethanol precipitation from the culture medium, acid hydrolysis is used to reduce the overall molecular weight, though fungal dextranases can also be used. The product with an average molecular weight of about 60 000 g mol^{-1} is used in medicine as a blood extender, while fractions of defined molecular weights (e.g., the Pharmacia T-series, e.g., T500 Dextran, which stands for dextran of weight average molecular weight 500 000 g mol^{-1}) are familiar in laboratories and to some extent, like the pullulan P-series, serve as polysaccharide standards in molecular weight calibrations. 'Blue dextran' is a well-known marker for the void volume in gel filtration studies. Dextrans are also used as part of incompatible phase separation systems, usually with polyethylene glycol. Their solubility in both aqueous and nonaqueous media renders them good membrane formers using electrospinning techniques.

Dextrans have found very wide application in laboratory work because they are particularly free from positive interactions with proteins. The interactions can be almost entirely characterized as coexclusion. This has found application, like in the case of *pullulans*, in calibrating gel filtration media, but cross-linked dextran gels show other effects. For example, they swell and shrink in a way related to the osmotic pressure of the solvent system, and can be used to make miniature osmometers.

Scleroglucan and Schizophyllan

A consideration of microbial polysaccharides would not be complete without at least a brief consideration of two fungal polysaccharides that are attracting increasing commercial interest: the weak-gelling scleroglucan and schizophyllan systems. They are both large, neutral polysaccharides of weight average molecular weights, M_w, (as largely established by light scattering techniques) $\sim$500 000 g mol^{-1}, with a greater diversity being reported for scleroglucan. X-ray diffraction studies indicate that they exist as hydrogen-bond-stabilized triple helices, stabilized by hydrogen bonds with resultant extrarigid rod-like properties in solution; they have virtually the largest persistence lengths known for polysaccharides: $\sim$150 nm for schizophyllan and 200 nm for scleroglucan. The existence of the triple helix for scleroglucan has been further supported by electron microscopy/light scattering measurements of the mass per unit length, $M_L = 2100 \pm 200$ g mol^{-1} nm^{-1}, along similar lines to that which supported the duplex model for xanthan as discussed above. The Mark–Houwink viscosity '*a*' parameter (a measure of how the intrinsic viscosity of a substance changes with molecular weight and a parameter that reflects the conformation of a macromolecule) of 1.7 for schizophyllans of $M_w < 500\,000$ g mol^{-1} is again the highest known for a polysaccharide and is on the rigid rod limit, and these molecules have been used to flocculate small colloidal particles. For chains of $M_w > 500\,000$ g mol^{-1} the '*a*' parameter falls to $\sim$1.2 and corresponds to slightly more flexibility as the polymer length increases.

Chemically also they are very similar, with a backbone of repeating $\beta(1\rightarrow3)$-linked glucose residues:

$$\ldots \rightarrow 3)\beta\text{-D-Glc}p(1\rightarrow \ldots$$

In scleroglucan every third residue has a $\beta(1\rightarrow6)$-linked D-glucose branch that protrudes from the triple helix. Using electron microscopy, Stokke and coworkers have demonstrated that certain denaturation–renaturation treatments cause the formation of interesting ring

structures or 'macrocycles'. In common with other branched glucans they appear to stimulate an immune response against tumor cells, and, particularly scleroglucans, have been considered for use in cosmetics (as part of skin and hair products), for application in pesticides (to assist binding to foliage), and, along with xanthan and other polysaccharides, for binding water and providing high heat stability in oil well drilling fluids.

Bacterial Alginates

Structure and Production

Alginates are copolymers of the residues guluronic acid (abbreviated as GulA or just G) and mannuronic acid (ManA or M), (**Figure 5**) and hence under normal solution conditions (except in acidic environments) these molecules are highly charged polyanionic polyelectrolytes. Although commercial alginates derive largely from algal sources there is a large potential for producing tailor-made alginates from bacterial sources, especially if advantage is taken of the genetic tools for controlling the production of the enzymes that are responsible for the synthesis and epimerization (conversion of D-mannuronic(M) to L-guluronic residues (G)) of the polymeric alginate chain (**Figure 6**). There appears to be considerable structural diversity (poly-M, poly-G, and poly-MG residues) and our understanding of the genes and the enzyme gene products is much greater for bacterial alginate production compared to the case for seaweed.

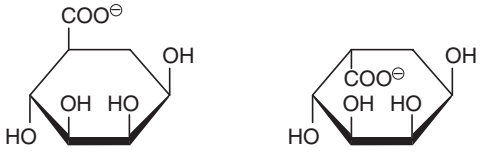

Figure 5 Guluronic and mannuronic acid residues of alginates.

The main alginate-producing bacteria that have been studied are *P. aeruginosa* and *A. vinelandii*. *P. aeruginosa* has been the subject of particular attention because of its association with respiratory disease and is found in patients suffering from cystic fibrosis. *A. vinelandii* appears to be the most promising in terms of industrial production because of its stable output of alginate. *P. aeruginosa* alginate has no poly-G residues, (and hence has a low G content) whereas *A. vinelandii* can, like seaweed alginate, possess all three block sequences (poly-M, poly-G, and poly-MG residues). However, all bacterial alginates are O-acetylated to varying degrees:

Ac Ac
| |
-G-G-G-M-M-M-G-M-G-

Structural Enzymes

Starting from fructose-6-phosphate the following enzymes are involved: hexokinase; phosphomannose isomerase; D-mannose-1-phosphate guanyl transferase; GDP (guanosine diphosphate)-mannose dehydrogenase; transferase; acetyl transferase; and mannuronan C-5-epimerase. Schemes involving these have been worked out for *A. vinelandii* and *P. aeruginosa*. Key structural genes have been identified in a gene cluster for *P. aeruginosa* with algG encoding for the epimerase and algF the acetylase (**Figure 7**).

Possibilities

It may be possible to treat alginate with epimerases to increase the poly-G content, producing a stiffer polymer. In principle this can be done *in vivo* by the incorporation and expression of genes from a plasmid in an alginate-producing

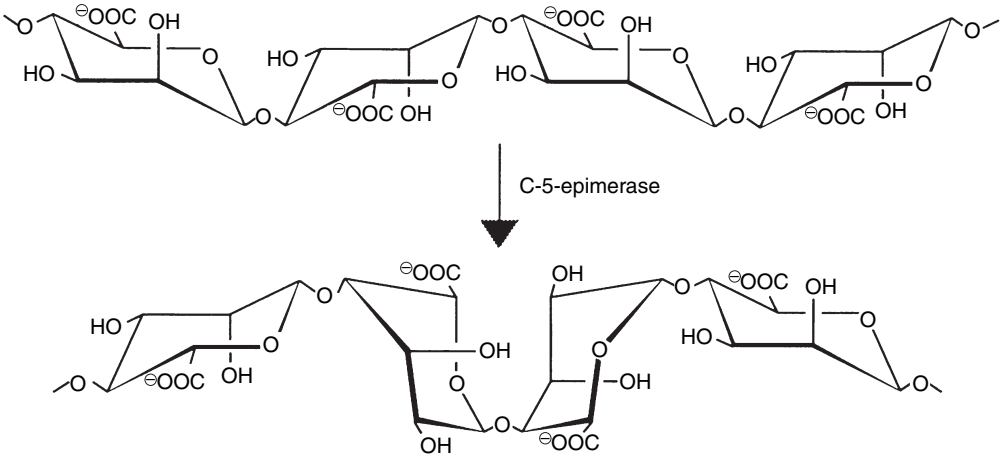

Figure 6 Action of epimerases. Reproduced from Skjåk-Braek G and Espevik T (1996) Application of alginate gels in biotechnology and biomedicine. *Carbohydrates in Europe* 14: 19–25, with permission from the Carbohydrate Research Foundation.

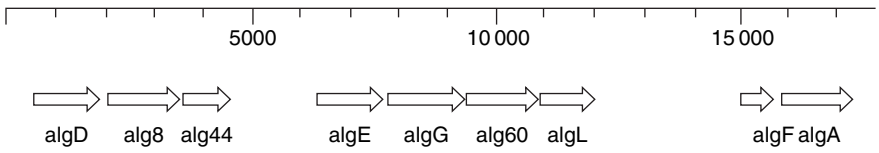

Figure 7 Gene cluster organization encoding most of the alginate structural enzyme genes in *Pseudomonas aeruginosa*. Scale is in base pairs. Reproduced from Ertesvag H, Valla S, and Skjak-Braek G (1996) Genetics and biosynthesis of alginates. *Carbohydrates in Europe* 14: 14–18 and May TB and Chakrabarty AM (1994) *Pseudomonas aeruginosae*: Genes and enzymes of alginate synthesis. *Trends in Microbiology* 2: 151–157, with permission from the Carbohydrate Research Foundation.

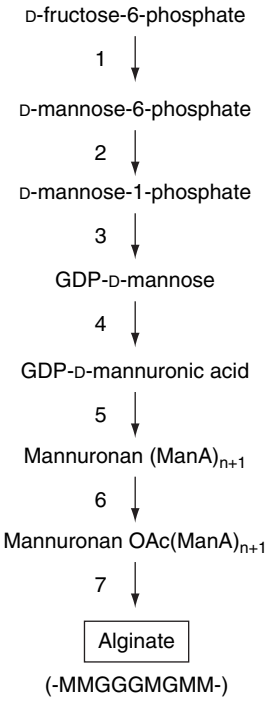

Figure 8 Alginate biosynthesis in *Azotobacter vinelandii* and the principal enzymes involved. 1, hexokinase; 2, phosphomannose isomerise; 3, D-mannose-1-phosphate guanyl transferase; 4, guanosine phosphate mannose dehydrogenase; 5, transferase (*n* is the number of uronic acid residues in the polymer chain, i.e., the degree of polymerization); 6, acetyl transferase; 7, mannuronan C-5 epimerase. Reproduced with permission, from Pindar DF and Bucke C (1975) The biosynthesis of alginic acid by *Azotobacter vinelandii*. *The Biochemical Journal* 152: 617–622. © the Biochemical Society.

bacterium (Figure 8). The degree of acetylation is another control point: acetylated mannuronic acids cannot be converted by epimerases from M→G.

Bacterial Hyaluronic Acid

The HA industry is worth an estimated US$1000 million per year and medical grade HA sells at US$40 000–60 000 per kg. Potentially the most interesting of the glycosaminoglycans, this biopolymer is found in the joints, skin, vitreous humor of the eyes, and umbilical cord of vertebrates; it is also produced by *Staphylococcus* and some *Streptococci*. The HA layer may enable virulent stains of *Streptococci* to attack immune systems of higher organisms unrecognized.

Structure

HA is a linear polymer consisting of alternating β-(1→4) *N*-acetyl-D-glucosamine and β-(1→3) D-glucuronic acid, resulting in stiff chains due to strong interchain hydrogen bonding and water bridges between acetamido and carboxylate groups. HA therefore adopts a conformation between that of a random coil and a rigid rod in dilute solution.

Extraction and Production

HA is commercially extracted from rooster combs or by microbial fermentation by *Streptococcus* bacteria (e.g., *Streptococcus zooepidemicus*). The fermentation medium typically includes yeast, glutamine, mineral salts (sodium chloride, potassium hydrogen phosphate, and magnesium sulfate), and glucose.

HA quality, in terms of productivity and molecular weight, has been studied under various conditions: temperature; pH; agitation speed; and aeration rate. These experiments demonstrated that under nonoptimal growth conditions HA is produced in larger amounts and is of higher molecular weight. It should be noted that HA production greater that $\sim 5–10\,g\,l^{-1}$ is impractical due to the high viscosity of the medium.

Properties

Due to its high molar mass of $\sim 1–3 \times 10^6\,g\,mol^{-1}$ and semiflexible coil conformation, HA behaves as a non-Newtonian solution even at low concentrations and the critical coil overlap concentration c^* has been reported to be molecular weight dependent where $c^* \times M_w \approx 2800\,g^2\,ml^{-1}\,mol^{-1}$ and therefore we enter the semidilute regime at approximately $1.5\,g\,l^{-1}$ and the concentrated regime (independent of molecular weight) at $>15\,g\,l^{-1}$. HA molecules of high molecular weight have also been shown to have mucoadhesive and anti-inflammatory effects and patients require less frequent injections of

higher molecular weight material for arthritis treatment by viscosupplementation, which is important in patient's acceptance of a treatment.

Modification

Chemically modified HAs are available commercially, examples of which include HA benzyl esters (Fidia farmaceutici, Albano Terme, Italy) and divinyl sulfone cross-linked HA (Genzyme, Cambridge, USA). Internally cross-linked (or autocross-linked) HA molecules have also been prepared. HA nanofibers for wound healing are also commercially available (CPN, Dolní Dobrouč, Czech Republic). Modification in terms of molecular weight and molecular weight distributions has also been achieved by means of changes to the growth medium (see above).

Genetic modification of the non-hyaluronic-acid-producing bacteria is an important development in bacterial HA production. The introduction of the *hasA* gene into *Escherichia coli* or *Bacillus subtilus* results in the production of HA and although there is no improvement in quality (in terms of molecular weight and yield) any possible contaminations by streptococcal exo- and endotoxins are eliminated and HA produced by *B. subtilus* has received GRAS (generally regarded as safe) status and should be released in the market in the near future.

Uses

HA is used extensively in the medical, cosmetic, and food industries and its applications have been reviewed recently. The most important industrial applications include the following:

1. Viscosurgery – protects delicate tissues during surgical interventions, for example, ophthalmological surgery. HA-based products have been used in $\sim 60 \times 10^6$ patients and there is an annual market of ~US$140 million.
2. Viscosupplementation – supplements tissue fluids, for example, the replacement of synovial fluid in arthritis. Higher molecular weight material requires fewer injections.
3. Viscoaugmentation – used widely in cosmetic surgery to fill tissue spaces. In the United States $\sim 1.6 \times 10^6$ cosmetic procedures utilized HA-based products costing a total of ~US$850 million.
4. Viscoseparation – prevention of connective tissue surface adhesion after injury or surgery, thereby causing less scarring.
5. Drug delivery – HA microspheres for controlled drug delivery.

Although not strictly speaking HA, the high molecular weight HA-like bacterial exopolysaccharide from *Vibrio diabolicus*, a bacterium from deep-sea hydrothermal vents, shows great potential in bone healing.

Polysaccharide Vaccines

No survey of microbial polysaccharides would be complete without reference to the use of polysaccharides in the production of vaccines against serious diseases. Certain types of pathogenic bacteria such as from *Streptococcus pneumoniae*, *Neisseria meningitidis* (Meningococcus), and *Haemophilus influenzae* type B, besides producing harmful or dangerous toxins also produce high molecular weight CPSs, which themselves appear harmless. They do though help the bacterium establish an infection and help hide cell surface components from immune recognition and complement activation. Purified extracts of polysaccharides may themselves be immunogenic and can be used at least in principle to produce immunity against the organism that is producing them. As a result, vaccines against some of these organisms are now available.

The polysaccharides themselves consist of repeat sequences of saccharide residues. One residue that appears frequently is *N*-acetyl neuraminic acid (**Figure 9**), a type of 'sialic acid'.

This residue is common on the surface of membrane glycoproteins and also on mucosal surfaces, so considerable care has to be taken against possible autoimmunity problems. A more serious problem with polysaccharide vaccines is that their effects are not generally long lasting: the repeat sequences produce a T-cell-independent IgM rather than IgG response in infants with little immunological memory effect and are ineffective for infants <2 years. To counter this there has been a lot of recent work on the development of conjugate vaccines where the polysaccharides – or repeat sequences from them – are covalently attached via a linker to an appropriate protein carrier usually based on a bacterial toxoid from diphtheria or tetanus (**Figure 10**).

Such conjugates have been successful in enhancing IgG antibody production and stimulating T-cell-dependent immunity against diseases such as meningitis. Two recent articles from the National Institute of Biological Standards in London provide an excellent description of current developments in this area.

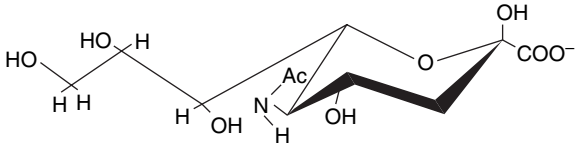

Figure 9 *N*-acetyl neuraminic acid.

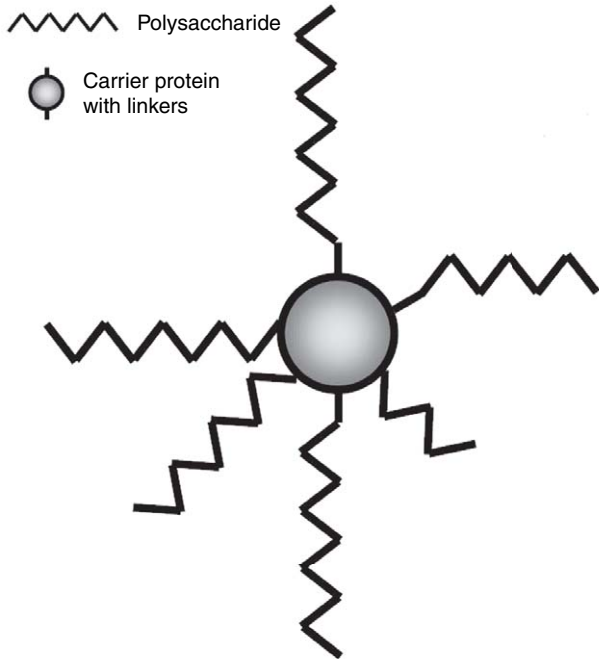

Figure 10 Schematic conjugate vaccine showing capsular polysaccharide (CPS) fragments coupled to an appropriate carrier protein. Reproduced from Ward J, Lieberman JM, and Cochi SL (1994) Haemophilus influenza vaccines. In: Plotkin SA and Mortimer EA (eds.) *Vaccines*, pp. 337–386. Philadelphia: Saunders & Co, with permission from W.B. Saunders & Co.

In generating these vaccines important issues have to be addressed about the reproducibility of preparations with regard to not only antigenicity and chemical purity but also physical properties including molecular weight and molecular weight distribution limits, and in this regard the use of techniques – with correctly established operating procedures – like SEC-MALLs, analytical ultracentrifugation, viscometry for physical characterization, and NMR and GC mass spectroscopy for chemical characterizations will become increasingly important. This highlights the increasing difference in characterization requirements for a microbial polysaccharide for food use, such as xanthan, and those for biomedical pharmaceutical use, with vaccines as the outstanding example.

Conclusion

The microbial production of polysaccharides is providing a valuable and cost-effective supply of valuable materials for a wide range – potential wide range – of uses, including food and pharmaceuticals because of the absence of or virtually no known toxicity problems. The relative simplicity of bacterial genetics as compared to that for higher organisms renders the manipulation of the production system much more easy as we have seen for alginates. Furthermore, the virtual lack of toxicity is providing hope in the development of effective conjugate vaccines against serious disease, provided, issues concerning autoimmunity and product reproducibility are properly taken care of.

See also: Cosmetics Microbiology; Enzymes, Industrial (overview); Xylanases

Further Reading

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Relevant Website

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