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Microbial Polysaccharides: Current innovations and Future trends in Medical science.

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Abstract
Microbial polysaccharides are water-soluble polymers gained an industrial importance over the past 20 years. They are an emerging class in several biomedical fields such as tissue regeneration, particularly for cartilage, drug delivery devices and gel entrapment systems for the immobilization of cells. Most of the polysaccharides derive from natural sources; particularly, alginate and chitin, which have an extensive use in medicine, pharmacy and basic sciences, and can be easily extracted from marine plants (algae kelp) and crab shells, respectively. Although there are numerous microbially produced polysaccharides, by focusing on two major commercial products, Xanthan and Dextran, an insight into their behavioral characteristics is unveiled. This was influenced by the fact that these microbial polysaccharides are considered to be classed as GRAS (generally regarded as safe) by the FDA. Their use as good emulsifying, gelling and viscosifying agents is an indication of their diverse capabilities in both industry and research. The recent rediscovery on microbial polysaccharides is also attributable to new synthetic routes for their chemical modification, with the aim of promoting new biological activities and/or to modify the final properties of the biomaterials for specific purposes. New technologies have revolutionized the production of Polysaccharide by use of microbes. Thus, leading to improved yields, higher throughput and making their production economically viable. This review is focused on the production strategy and applications of microbial polysaccharides in medical field.

Key Words
Microbial polysaccharides, Xanthan, Gellan and Polysaccharide based biomaterials.

Introduction
Polysaccharides are relatively complex carbohydrates consisting of multiple monosaccharides joined together and often branched. Properties include solubility in water and no formation of crystals. Polysaccharides carry a phenomenon, which allows them to produce a material that leads them to sticking to each other and other surfaces. Microbial polysaccharides have many practical applications in various industries as food, pharmaceutical, medical technology and cosmetics.[1] They might function in foods as viscosifying agents, stabilizers, emulsifiers, and gelling agents. Polysaccharides derived from plants and seaweeds have been in use for thousands of years. However, over the past 20 years a new class of microbial products, the microbial polysaccharides have grown in industrial importance. These products can be used as alternatives to other synthetic or natural water-soluble polymers Many yeasts fungi and bacteria produce them.

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These microbial polysaccharides can be produced in either one of two forms[2].
1. Capsular polysaccharides (C.P.S)
2. Exo-polysaccharides (E.P.S)

Capsular polysaccharides (C.P.S) protect pathogenic micro-organisms from immune system defenses by providing a physical barrier to infection by bacteriophage. For the production of microbial polysaccharides the main interest is in that of EPS. EPSs of microbial origin have unique properties in their capability of forming very viscous solutions at low concentrations and their pseudoplastic nature.[3] Those produced by lactic acid bacteria have been considered very important in use for food emulsifiers and thickeners throughout the years.

Classification of Microbial Polysaccharides[2]
Microbial polysaccharides are generally divided into three groups, namely-
(1) The cell wall polysaccharides,
(2) The intercellular polysaccharides and
(3) The exocellular polysaccharides.

The polysaccharides in-group (1) and (2) are integral parts of cell wall or in some cases of a structurally demonstrable microcapsule and hence these are also

called capsular products. These are difficult to separate from cell biomass and are commercially unimportant. The exocellular polysaccharides, which constantly diffuse into the cell culture medium making it slimy and viscous, are easy to isolate from the culture media, free from protein and cell debris. Yeast glucan, which is somewhat similar to starch and fungal chitin are typical examples of capsular polysaccharides, while xanthan, dextran, curdlan, welan and gellan are typical exocellular polysaccharides. Microbial polysaccharides can be either homo or hetropolysaccharides. Table-2 lists some of the important microbial polysaccharides, currently produced industrially or in the process of development. Microorganism source, monosaccharide constituents, and mode of linkage and some of their other features are also given.

Biological functions of Microbial Polysaccharides[2]

Capsular polysaccharides are normal functional constituents (structural components or energy source) of bacterial cell. Attempts have also been made to explain the functions of exocellular microbial polysaccharides. It has been suggested that these 4 products act in creating a more favorable environment for cell growth by providing a high level of hydration, particularly at the period of water strain. In general exocellular polysaccharides do not appear to be the reserve energy source, but they can be catabolised and serve as a potential energy reserve, when the nutrients in the culture media are used up. This is why the fermentation conditions during their production are adjusted to give a high yield of polysaccharide, rather than the cell-biomass. It is necessary to stop the fermentation process at an appropriate time to have a high yield of these products, i.e. when the nutrients in a culture media have reached the stage of near exhaustion. They also act as a barrier against bacteriophages and their function to act as a host-recognition system has also been suggested.

Production Process of Microbial Polysaccharides[3]

Production of most microbial polysaccharides involves growth in stirred tank fermenters using media with glucose or sucrose as the carbon and energy source. Synthesis is often favoured by high C: N ratios. Because of the high viscosity of the fermentation broths, efficient mixing and aeration are required together with considerable energy input. Fed-batch fermentations may be preferable to the use of high initial sugar concentrations. After pasteurization of the broth, recovery by precipitation with iso-propanol is followed by drying and grinding to yield a fine powder. Filtration or centrifugation and other downstream processing add to the final cost.

Typical examples of commercial microbial polysaccharides: **Dextran**

Dextran is a microbial fermentative linear polysaccharide made of many glucose molecules. It is water soluble gum and the first microbial polysaccharides to be commercialized. Dextran is commercially produced from two specific strains, *L-mesentecosides* and *L- dextranicum*. It is most commonly used in confectionary to improve moisture retention, viscosity and inhibit sugar crystallization. In gum and jelly confectionery it acts as a gelling agent. In ice cream it acts as a crystallization inhibitor, and in pudding mixes it provides the desirable body and mouth feel.[4] It is now more commonly used as a blood plasma extender in blood transfusions. Dextran (Sephadex^{Tm}) is also used in as matrices in size-exclusion chromatography. It is normally manufactured in a bead form and most commonly used for gel filtration columns.

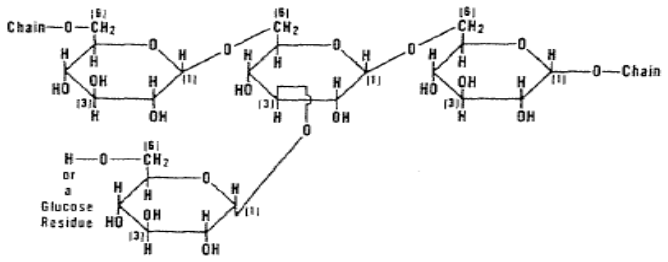

Fig 2: Structure of Dextran

Xanthan

Xanthan gum is a polysaccharide used as a food additive and rheology modifier.[5] It is produced by fermentation of glucose or sucrose by the plant pathoen, *Xanthomonas campestris* bacterium. After a fermentation period, the polysaccharide is precipitated from a growth medium with isopropyl alcohol, dried, and ground into a fine powder. Later, it is added to a liquid medium to form the gum.[6] Xanthan is a product from the plant pathogen *Xanthomonas campestris*. It has a cellulosic backbone on every second glucose residue of which a trisaccharide side chain is attached. This unusual structure confers physical properties to the polymer which are utilized in food and other industries. Xanthan is stable at both acid and alkaline pH and forms pseudoplastic dispersion in water. Relatively low polysaccharide concentrations produce highly

viscous solutions and the viscosity does not change greatly on raising the temperature. The solutions are compatible with many other ingredients in food and give good flavor release. Xanthan is also a good suspending and stabilizing agent for oil/water emulsions such as salad dressings.[7]

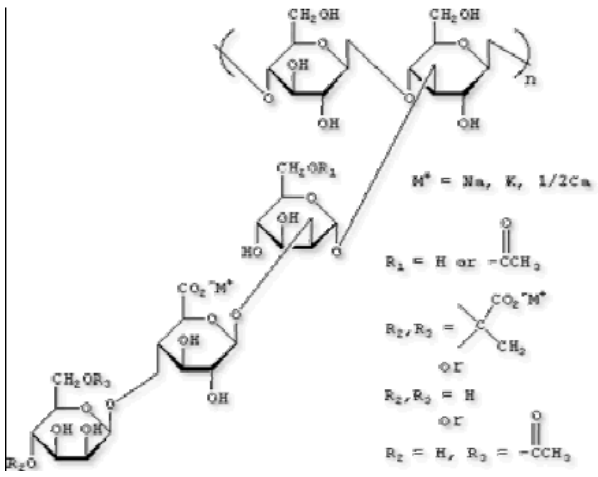

Fig 3: Molecular structure of Xanthan [8]

Xanthan's .shear thinning. characteristics can be utilized in the paint industry. Paints containing Xanthan's are highly viscous at low shear rates, and thus will not drip from a brush. A Molecular structure of Xanthan is shown in Figure 3. However, the shear stress produced by brushing, thins the paint and allows for easy application.[9] Xanthan solutions are often included as components of drilling muds used in drilling oil wells because of their shear-thinning and particle suspending properties. These muds are used to suspend sand particles from the oil well and carry them to the surface. They are also useful lubricants for the drill bit. In cosmetics, xanthan gum is used to prepare water gels, usually in conjunction with bentonite clays. It is also used in oil-in-water emulsions to help stabilize the oil droplets against coalescence. It has some skin hydrating properties. Xanthan gum is a common ingredient in fake blood recipes, and in gunge or slime.

Gellan

Gellan gum is an extracellular polysaccharide produced commercially as a fermentation product of the bacterium *Sphingomonas elodea* (ATCC 31461) previously referred as *Pseudomonas elodea*. It is a linear anionic heteropolysaccharide with a molecular weight of 500kDa. Gellan gum is a linear polysaccharide with a tetrasaccharide repeat unit of glucose, glucuronic acid and rhamnose in the molar ratio of 2:1:1. The polymer is produced with two acyl substituents present on the 3- linked glucose, namely L-glyceryl, positioned at O (2) and acetyl at O (6). On average there is one glycerate per repeat unit and acetate per every two repeats. Chemical analysis proved that glycerate substitution predominates over that with acetate. Moreover, glycerate substitution dramatically influences gellan properties since its bulk hinders chain associations and accounts for the change in gel texture brought about by de esterification. Gellan gum has wide applications in various fields e.g., in microbiological media, tissue-culture media, foods and pet foods, deodorant gels, films and coatings and capsules, bakery products, photographic emulsions and microcapsules. Gellan gum is used in low calorie jams and jellies because in addition to provide good acid stability, clarity and flavour release. In some starch-based products, it is possible to replace a portion of the starch with gellan gum and improve flavour release. Gellan gum can be used as a fining agent for alcoholic beverages including beers, wines and fortified wines. The delicate texture, acid stability and intense flavour impact imparted by gellan gum is utilized in citrus flavoured desert gels, while gel clarity is a key feature in car deodorant gels. In soft gelatin capsules and photographic emulsions, blends of gelatin and low acyl gellan gum have been found to be a reasonable replacement for gelatin alone. Gellan gum is also used as a solidifying agent in substitution of agar. The advantages of gellan gum over agar relate to their thermostability, enabling long incubations at higher temperatures. Gellan culture medium is advantageous in that it reduces the time required for plate preparation, it produces a drier medium and in case of some mesophilic species, it reduces required incubation time. The presence of sulphur or other impurities in the agar affect plant tissue culture's growth. Gellan gum is pure enough to use it in tissue culture. Transparency of gel is also another advantage in tissue culture. Gellan gum exhibits good resistance to contamination by molds, easy washing from the plant tissue for transplantation and the ability to observe stages in culture development. Gellan gum is likely to become more popular in the near future.

Curdlan

It is an extracellular (1,3)-ß-D-glucan given its name because of its ability to "curdle" when heated. Curdlan is produced by Alcaligenes sp. and Agrobacterium sp.

Curdlan is characterized by repeating glucose subunits joined by a ß-linkage between the first and third carbon of the glucose rings.[10-14] In its natural state, curdlan is poorly crystalline and is found as a granule, much like that of starch. Curdlan polysaccharide consist of as many as 12,000 glucose units[15] and is insoluble in water, alcohols and most organic solvents, but dissolve in dilute bases (0.25 M NaOH), dimethylsulfoxide (DMSO) and formic acid.[16] Curdlan forms a weak gel on heating above 55°C followed by cooling. Further heating to 80-100°C increases the gel strength and produces a firm, resilient gel, while autoclaving at 120°C converts the molecular structure to a triple helix. The gel formed by this high-temperature treatment no longer melts when heated. It is very susceptible to shrinkage but resistant to degradation by most ß -(1, 3)-D-glucanases. Gelation involves aggregation of the rod-like triple helices through non-covalent associations.

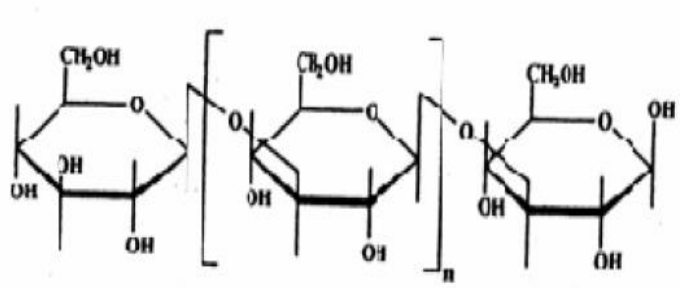

Fig 4: Structure of curdlan

Medical and Pharmaceutical applications of Microbial Polysaccharides

Microbial Polysaccharides in Health and disease: Polysaccharides are well known for their ability to form vaccines if coupled with a suitable protein the reason for this being that the protein can stimulate T cell help for polysaccharide-specific B cells. Take for example one of the most frightening and most common diseases that we vaccinate against, especially amongst infants is that of meningitis, which bacteria that live in the back of the nose and throat cause. It is an infection of the fluid and lining of the brain and spinal cord. Many people are carriers of the meningococcal bacteria, but their bodies have built up immunity against the bug. In people with no antibodies, however, one to two out of every 100,000 exposed to the bacteria develop a serious illness such as meningitis. Polysaccharides have played a part in the vaccination of Group C meningitis for the past 25 years. However the first vaccine developed was derived from the purified complex sugars of the bacteria's surface so, therefore it contained no protein, This caused a major problem in that even though it provided immunity in adults and older children it did not affect the immature immune response of young infants as infants make poor T-independent responses to polysaccharide antigens making the vaccine ineffective towards them. This was quite a concern as over 50% of all cases of group C meningitis occur in young infants. Another problem was also associated with the first vaccine in that even in older children and adults, the antibodies, which the vaccine produced, diminished over time, thus yielding only short-term immunity from group C meningitis. To overcome this, the covalent linkage of a suitable protein to the polysaccharide is required to convert them into Tindependent antigens inducing protective immunity. It was not until 1982 that a Dr. Harold Jennings[13] successfully and safely conjugated a protein with the complex sugars of the bacteria they are converted into T-dependent antigens and protective immunity is induced resulting in the development of what's known as conjugate vaccine for Group C Meningitis that not only was effective in young infants but also provided long-lasting immunity in adults and older children. A quadrivalent polysaccharide that includes serogroups A, C, Y and W135 is produced and used in the US. The Bivalent A and C polysaccharide vaccines are being used in other parts of the world.

Anti-Tumorigenic Effects

Many polysaccharides from mushrooms, particularly those derived from *Lentinus edodes* (lentinan), *Grifold frondosa* (grifolan), *Sclerotinia sclerotiorum* (scleroglucan) and *Schizophyllum commune* (schyzophyllan) have showed anticarcinogenic effects in both animals and humans. Although the mechanism of their anti-tumour action is still not completely clear, ß-glucans appear to mediate their anti-tumour activity by activation or augmentation of the host's immune system, via activation of leukocytes and production of inflammatory cytokines.[17] Glucan-specific receptors are present on phagocytic cell membranes of several species and potent *in vitro* activation of neutrophil function, including an increase in phagocytosis and killing has been described *in vitro*.[18,19] Nevertheless, in animal experiments, ß--glucans have shown varying activity against sarcomas, mammary cancer, some chemically induced cancers, adenocarcinoma, colon cancer and some leukemias. Lentinan has already

been shown effective in gastric carcinomas [20,21]. Moreover, lentinan was reported to induce apoptosis (programmed cell death) in murine skin carcinoma cell-lines[22].

Table 1: List of Microbial polysaccharides and bacteria's from which they manufactured by fermentation[2].

Polysaccharide	Bacteria	Functional use
Curdlan	A.facclia Agrobacterium spp	Non-thermo-Reversible Gels
Dextran	Leuconoatoc spp	Low viscosity,
Pullulan	Aureobasidium Pullana	Blood plasma expender Flocculating Agent
Scleroglucan	Scleretium Rolfail	High Suspending Agent
Gellan	Aureomans spp	Strong Food Gels
Welan	Acaligenes spp	Thermostable Suspending agent

Table 2: List of marketed microbial polysaccharides, their manufacturer.

Product	Tradenamer	Manufacturer name
Gellan gum	Kelco gel Gelrite	Kelco Biopolymers
Curdlan	Pureglucan®	Takeda Chemical Industries Ltd., Japan
Xanthan gum	Keltrol	CP Kelco

Conclusion

The development Microbial polysaccharides are best viewed in the context of their maximum utility in food industry and medical industry. The polymers actually available as products in the market are very few compared to the large number of polysaccharides whose structures are either published or patented. The main drawback for the commercialization of new polysaccharides lies in the identification of new or superior properties compared to the one possessed by traditional products. The second obstacle for improvement of original structures is the cost of production and development that may be again a limiting factor. In food industry, only three bacterial polysaccharides are commonly employed despite the hundreds of structures known. One of them is the curdlan which has been authorized for food in 1996 because of its unique features and two others are the xanthan and the gellan. These polysaccharides could have significative development in the next few years, notably in non-food sectors. The most promising developments seem possible in therapeutic and cosmetic applications as these compounds have been described as immunomodulators, antitumorogenic, antiviral (AIDS), agents for treatment of hypercholesterolemia and agents for stabilization of glycemia. Moreover, specific action as food additives has been ascribed for glucan oligosaccharides. In future years it will be largely driven more carbon for chemical processes from renewable resources and to preserve the ecosystem. Fermentation industry supported by developments of genetic engineering can yield microbes that more efficiently convert inexpensive raw materials to neutral polysaccharides. Now the technical background of food industry permits to develop environmentally friendly products based on starch and composite biomaterials. On the basis of economic and environmental considerations, the commercialization of microbial polysaccharides continues and shows increasing markets of products that have relatively short use lifetime.

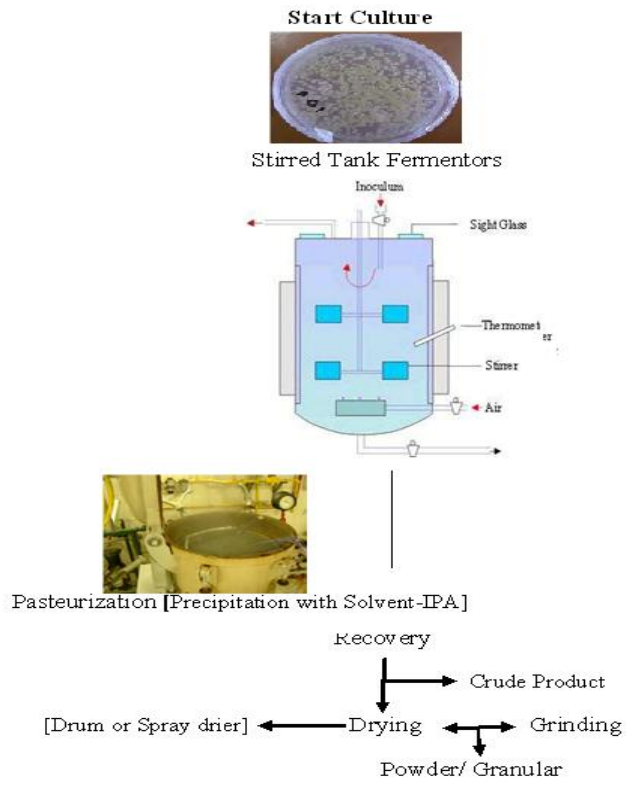

Fig 1: Sequential steps involve in the production process of Microbial Polysaccharides.

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