Gums and Mucilages

BIOTECHNOLOGICAL APPLICATIONS

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Abstract

Polysaccharides, especially from marine algae, find many major applications in the field of biotechnology. Their applications in biotechnology are due to their gelling properties and encapsulating capabilities. Agar, agarose, alginates and carrageenan are the polysaccharides that are used commonly in biotechnology. The uses of polysaccharides in biotechnology include gel-electrophoresis, molecular sieving, gene fragment separation, gene mapping and immobilization of cells and enzymes. In this article, the common polysaccharides of biotechnological importance, their sources, structural features and their biotechnological applications are reviewed.

Keywords: Polysaccharides, Biotechnological applications, Agar, Carrageenan, Alginate, Electrophoresis, Immobilization.

Introduction

Biotechnology includes a vast variety of techniques—from modifying and culturing living systems to create useful proteins, to human cloning. Biotechnology can be defined as the manipulation and/or use of all or part of a specific biological system to generate a useful product. It is not a science unto itself, but a family of tools and techniques that can be used to create products and processes. These tools include genetic engineering, cell and tissue culture, enzymes, fermentation, immobilized bioreactors, biochemistry and immunology.

Polysaccharides, especially from marine algae, find a vast range of applications in biotechnology. Separation of genes and gene fragments is not possible without agarose gel electrophoresis. Recombinant insulin, interferons, tissue plasminogen activators and other therapeutic agents have all been developed using agarose gels in at least one step of their discovery and development. Recent discoveries in cancer research and understanding of oncogenes would not have been possible without agarose or its derivatives. Polymerase chain reaction (PCR) allows the amplification of a fragment of DNA into millions of copies in a short duration of time. The separation of amplified fragments is done on agarose gels. Agar is the primary medium for growing the genetically transformed bacteria. Bioconversions using microorganisms are most efficiently done by the use of encapsulated organisms, where encapsulation is done using agar, agarose, carrageenan or alginites. In the present review, a detailed account of the biotechnological applications of polysaccharides is given.

Sources of Common Polysaccharides of Biotechnological Importance

Agar and Agarose

Agar consists of the polysaccharides of various species of Rhodophyceae, particularly of the genus *Gelidium*. It is extracted by treatment of seaweeds with boiling water; the extract is filtered hot, then concentrated and dried.

Agar is extracted from thallus of various red algae, especially those belonging to *Gelidium* (*G. corneum*, *G. amansii*, *G. cartilagineum*) and *Gracilaria* (*G. confervoides*, *G. lichenoides*). It is a complex galactan, containing a mixture of two fractions, agarose and agarapectin. Agarose is constructed of (AB) type linear structure with alternate 1→3 and 1→4 bonds, where A units are partially methylated D-galactoses and B units are L-enantiomers of galactose (Percival and McDowell, 1990; Mabeau and Fleurence, 1993).
Agar is soluble in hot water and forms a gel upon cooling to 30-35°C, which liquefies only above 80°C. Agarose forms double helical structures that aggregate into a three dimensional network able to retain water molecules.

**Carrageenans**

Carrageenans are obtained from various *Rhodophyceae* seaweeds, from *Gigartinaeae*, *Solieraceae*, *Hypneaceae* and *Furcellariaceae* families, after treatment with hot water and precipitation by ethanol, methanol, 2-propanol or potassium chloride. They must contain not less than 15% and not more than 40% sulfur, expressed as sulfates (Craigie, 1990; Matsuhiro and Urzua, 1992).

The major source of carrageenans is a seaweed, *Chondrus crispus* (Family: *Gigartinaeae*), also known as Irish moss, which grows in the coasts of Atlantic Ocean and English Channel. It can also be cultivated in basins. Carrageenans are galactans or polymers of D-galactose, are heavily sulfated, and are anions with multiple electrolytes of molecular weight ranging from $10^5$ to $10^6$. All carrageenans have a linear structure of $(AB)_n$ type, with alternating 1→3 and 1→4 bonds, where A and B are galactopyranosyl residues (Percival and McDowel, 1990).

**Alginic Acid and Alginates**

Alginic acid is a mixture of polyuronic acids obtained mainly from seaweeds from the *Phaeophyceae* family. It must contain a minimum of 19% and a maximum of 25% carboxyl groups, relative to the dry matter. Brown algae are the principal algae currently used for the industrial preparation of alginic acid and alginates. Among them, *Fucus serratus*, *Fucus vesiculosus* (Family: *Fucaceae*), *Laminaria digitata*, *Laminaria hyperborea* (Family: *Laminariaceae*) and *Macrocystis pyrifera* (Family: *Lessoniaceae*) are the important sources of alginic acid.

Alginic acid is a linear polymer constructed from two uronic acids, D-mannuronic acid (M) and L-guluronic acid (G). The linkage between monomers is of β-(1→4) type. These acids are present in the polymer under the form of homogeneous poly-M or poly-G blocks separated by regions where they may alternate (G-M-G-M...). The conformations of these blocks are given below. In the native state, alginates occur as mixed salts ($Na^+$, $Mg^{2+}$, $Ca^{2+}$), which must in part be linked to fucans (Bisson-Vidal et al, 1991).
Figure 1. *Gelidium corneum*

a) Habitat of alga

b) A part of alga enlarged

Figure 2. Schematic representation of *Gracilaria lichenoides*

a) Habit

b) Different parts

Figure 3. *Chondrus crispus*

a) Habit

b) Different parts

Figure 4. *Fucus serratus*

a) Habit

b) A portion enlarged
Biotechnological Applications of Polysaccharides

Polysaccharides find applications in biotechnology because of their gelling properties and entrapment capabilities. Some of the important applications are described below:

Gene Fragment Separation

DNA sequence coding for a specific protein can be cut from a gene using enzymes called restriction endonucleases, which cleave the DNA between certain defined nucleotide sequences. After restricting using restriction enzymes, the required fragments are separated using agarose gel electrophoresis (Alexander et al, 1985; Andrews, 1986). In genetic engineering, agarose gel electrophoresis has a major role. Isolation of required gene from genome or a particular chromosome, isolation of 'plasmid' DNA which is used as vector, needs essentially agarose gel electrophoresis. Without this, it would not have been possible to carry out gene manipulation in microorganisms to produce human proteins such as recombinant insulin, interferon, growth hormones and other such proteins.

Chromosome Fragment Separation

Small chromosomes and large chromosomal DNA fragments, greater than 40 kilobases, created by restriction enzyme treatment can only be separated on agarose gels using a technique called pulsed field gel electrophoresis (PFGE). In this technique, an electric current is alternately imposed at a predetermined angle to the direction of electrophoretic migration (Schwartz et al, 1983). A number of modifications of this technique have been developed for genetic disorder-specific chromosomal fragment separations.

Cell and Enzyme Immobilization

Immobilization may be defined as imprisonment of a cell or enzyme in a distinct phase that allows exchange with, but is separated from the bulk phase in which the substrate, effector or inhibitor molecules are dispersed and monitored. Imprisonment refers to physical or chemical trapping of cell or enzyme into the polymer matrix. Most commonly used techniques of immobilization are 'entrapment' and 'encapsulation' using polysaccharides such as agar, agarose, κ-carrageenan, starch gel, cellulose derivatives or alginites as encapsulating agents (Kierstan and Bucke, 1977; Tosa et al, 1979; Rees et al, 1982; Wang and Hettwer, 1982; Trevan, 1987). These techniques are represented diagrammatically in the figure.

After immobilization, the immobilized enzymes can be used as analytical tools. One such examples is of immobilized enzyme electrode, which permits continuous monitoring of small concentrations of specific biochemical. Immobilized enzyme electrodes have been developed for the monitoring of glucose, galactose, amino acids, urea, uric acid and penicillin (Guilbault and Neto, 1985).

Another important application of immobilization is the development of immobilized cell/enzyme bioreactor (Smith, 1996). The cell or enzyme capsules, beads or columns are packed in reactors, substrate molecules in solution are allowed to enter into the reactor, and product molecules are collected from the outlet. A diagrammatic representation of immobilized enzyme/cell reactor is given in the figure.

The immobilized cell/enzyme reactors find application in the biotransformation of certain molecules such as steroids, glycosides and alkaloids, where it is difficult to achieve transformations by chemical methods (Skryabin et al, 1966; Freeman et al, 1982; Medentsyev et al, 1983; Arinbasarova et al, 1985).
Artificial Organ

Experimental implants of insulin and other hormone producing cells and tissues have been shown to reverse the physiological effects caused by lack of these hormones. Hence, methods are being developed to implant the living cells or tissues, yet to keep them isolated to prevent problems like immune rejection. Cages or agarose, its low-gelling-temperature hydroxyethyl derivatives and calcium alginate are compatible with this application (Guiseley, 1976, 1987, 1989). Encapsulated insulin-producing islets of Langerhans cells have been used as model systems with considerable success (Lim and Sun, 1980; Howell et al., 1982; Bouhaddioui et al., 1985; Goosen et al., 1985, 1989; Gin et al., 1987; Iwata et al., 1988). It has been reported that rat hepatocytes retained their enzymatic detoxifying activity when immobilized in alginate droplets (Yarmush et al., 1988). Also, it has been reported that pre-implantation mouse embryos were kept alive by encapsulation in an artificial womb of calcium alginate (Adaniya et al., 1987).

Conclusion

Polysaccharides, especially from marine source, have enabled scientists to develop modern biotechnology tools and techniques, as a result of which, newer therapeutic entities have been produced. They find applications in most of the techniques of biotechnology, such as electrophoresis, molecular sieving, gene fragment separation, gene mapping and immobilization of cells and enzymes. It can be said that without polysaccharides, biotechnology would not have been developed.

References

It must take into consideration the fact, that a raw material base for the agar production is reducing because of its intensive utilization for the industry needs. Search of ways and methods of regenerations of used agar, method for cleaning of agar and exploration of new sources for receiving agar is needed for future biotechnological research laboratories.

Peter Revell, 189 Washington Avenue Hemel Hempstead Hertfordshire HP2 6BB mentioned that "total asepsis on totally sterile substrates lacking all nutrients is the only way forward for plant tissue culture. The nutrients can be added as the culture progresses. Candidates for the substrates include: Perlite, Cotton-wool, Mineral-wool and Glass-wool in that order of success in my limited experiments" (http://plant-tc.coafes.umn.edu/listserv/2000/log0012/msg00145.html).

In the late 1970s, Dr. Jonathan N. Roth began work on a temperature-independent gelling agent to be used in place of agar-agar, the “vegetable gelatin” upon which the growth of microorganisms on solid media depended.

Dr. Roth was one of many scientists who found agar difficult to work with. Yet because agar is derived from marine algae, the availability of agar is dependent upon marine environmental conditions. Additionally, since numerous algal species may be used as sources, agar is also subject to variations which can affect research results.

An even more significant limitation of agar relates to the heating and cooling of the material. To be used, the dried material must be dissolved in 90-100 °C water and dispensed at a temperature higher than 45 °C as it solidifies below that temperature. This characteristic makes agar difficult to use, particularly when it is desirable to mix a test sample with the still-liquid medium before it solidifies in a container, such as a petri plate. The high temperature required for maintaining the liquid state harms many microorganisms. Scientists have observed that using this pour plate technique with agar-based media results in questionable accuracy in the determination of microbial populations of test samples.

Dr. Roth's goal was to develop a temperature-independent gelling agent that would enable scientists to avoid the disadvantages of agar. His years of work and evaluation paid off, and Dr. Roth's work with low methoxyl pectins as agar substitutes resulted in the patented product, Easygel® (Micrology Laboratories L.L.c.206 W. Lincoln Ave.Goshen, IN 46526-3574, http://www.micrologylabs.com/html/contact_us.html).