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Review

# Pullulan: Microbial sources, production and applications

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**Abstract**

Pullulan is a water-soluble glucan gum produced aerobically by growing a yeast like fungus *Aureobasidium pullulans*. It is a regularly repeating copolymer with the chemical structure {→ 6)-α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 →}$_n$. Thus the polysaccharide is viewed as a succession of α-(1 → 6)-linked (1 → 4)-α-D-triglucosides i.e. maltotriose ($G_3$). Pullulan have a wide range of commercial and industrial applications in many fields like food science, health care, pharmacy and even in lithography. Due to its strictly linear structure, pullulan is also very valuable in basic research as well as a well-defined model substance. This review attempts to critically appraise the current literature on fungal exopolysaccharide (EPS) 'pullulan' considering its microbial sources, structural geometry, upstream processing, downstream processing, peculiar characteristics and applications.


*Keywords:* *Aureobasidium pullulans*; Pullulan; Maltotriose; Upstream processing; Downstream processing

## 1. Introduction

A variety of exopolysaccharides (EPSs) are produced by a number microorganisms as an extracellular or cell surface-attached material in the form of amorphous slime (Sutherland, 1998). These EPSs can be categorized as homopolysaccharides and heteropolysaccharides (Byrom, 1991; Garcia-Ochoa, Santos, & Alcon, 1995; Jorris & Vandamme, 1993; Weiss & Ollis, 1980; Yuen, 1974). Homopolysaccharides are generally neutral glucans, while most of the heteropolysaccharides are polyanionic due to the presence of uronic acid. Microbial EPSs are propitious substitutes for the plant polysaccharides due to their unique and superior physical properties. Microbial EPSs extensively studied now a days include xantham from *Xanthomonas campesteris*, succinoglycan from *Rhizobium*, bacterial alginates from *Pseudomonas* spp.; *Azotobacter vinelandii* and pullulan from *Aureobasidium pullulans* with potential industrial applications. These polysaccharides (group of macromolecules) possess diverse applications in food, chemical, energy production, and pharmaceutical industries. A few fungal EPSs with interesting industrial properties are well known. Pullulan is one of such commercially emerging biopolymers and synthesized by a yeast-like fungus *A. pullulans*. It is a water soluble random coil glucan that serves as a paradigm for the behavior of aqueous polysaccharides (Morris, 1995; Tsujisaka & Mitsuhashi, 1993; Yalpani, 1998).

It is well established that regularly repeating structural unit of pullulan is a maltotriose trimer α-(1 → 4)Glu*p*-α-(1 → 4)Glu*p*-α-(1 → 6)Glu*p*-, produced extracellularly by *A. pullulans* (de Bary) G. Arnaud, a mitosporic fungus formerly known as *Pullularia pullulans* (de Bary) Berkhout (Syn: *Dematium pullulans* de Bary) (Gibbs & Seviour, 1996; Leathers, 2003). However, other structures particularly the tetramer or maltotetraose α-(1 → 4)Glu*p*-α-(1 → 4)Glu*p*-α-(1 → 4)Glu*p*-α-(1 → 6)Glu*p*-, may be present in the pullulan polymeric chain (Wallenfels, Keilich, Bechtler, & Freudenberger, 1965). So far, the maximum extent to which maltotetraose subunits have been detected

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is 7% (Catley, Ramsay, & Servis, 1986). This is the main reason why currently and frequently in the literature the term "pullulan" is used for both the "polymaltotriose" produced by *A. pullulans* and the polysaccharide varieties, similar to the pullulan, produced by other microbes. A couple of theories as to how pullulan is elaborated have been proposed by various researchers. Catley (1971a) proposed that a lipid is used as a carrier in which pullulan is taken to the outside of the plasmalemma. This view was supported by other research groups i.e. Lee et al. (1999) and Simon et al. (1995). Rho, Mulchandani, Luong, and LeDuy (1988) proposed two pathways, one allowing direct conversion of glucose, whereas the second pathway used an identified precursor. A thorough review of the peculiarities of the pullulan biosynthesis with various *A. pullulans* strains was published in 1981 (Kondratyeva, 1981). Simon, Caye-Vaugien, and Bouchonneau (1993) proposed that both pullulan and the insoluble polysaccharide are localized on an outer surface of the chlamydospores on the basis of an electron microscopic study. Simon, Bouchet, Bremond, Gallant, and Bouchonneau (1998) revealed an inverse correlation between the concentration of pullulan and the content of intracellular glycogen though the mechanism according to which glycogen is transformed into pullulan is not well understood.

The regular alternation of (1 → 4) and (1 → 6) bonds results in two distinctive properties of structural flexibility and enhanced solubility (Leathers, 1993). The unique linkage pattern also endows pullulan with distinctive physical traits along with adhesive properties and its capacity to form fibers, compression moldings, and strong, oxygen-impermeable films. Pullulan's solubility can be controlled or provided with reactive groups by chemical derivatization. Consequently, pullulan and its derivatives have numerous potential for food, pharmaceutical and other industrial applications. Pullulan is water soluble, insoluble in organic solvents and non-hygroscopic in nature. Its aqueous solutions are stable and show a relatively low viscosity as compared to other polysaccharides. It decomposes at 250–280 °C. It is moldable and spinnable, being a good adhesive and binder. It is also non-toxic, edible, and biodegradable. Its main quality parameters are summarized in Table 1. A number of reviews on pullulan have appeared (Israilides, Smith, Scanlon, & Barnett, 1999; Leathers, 2002; Leathers, 2003; Shingel, 2004) and this review focuses on microbial sources, structural geometry, upstream processing, downstream processing, peculiar characteristics and applications of pullulan.

Table 1
Main quality parameters of pullulan

| Parameter | Specification |
|---|---|
| Appearance | White or yellowish-white powder |
| Water solubility (25 °C) | Easily soluble |
| Specific optical activity [α]$D_2O$ (1% in water) | Minimum +160° |
| Polypeptides (%) | Maximum 0,5 |
| pH of solution | 5–7 |
| Mineral residue-ash (sulphated, %) | Maximum 3 |
| Moisture (loss on drying, %) | Maximum 6 |
| Molecular weight (range, kDa) | 100–250 |

A survey of the World patent Index in 1983 revealed that over 150 inventions were related to this polysaccharide, mostly on its new applications (LeDuy, Choplin, Zajic, & Loung, 1988). Literature has been surveyed from 2000 to 2007 and it reveals ten new inventions related to production and pharmaceutical applications of the pullulan (Boyd, Xu, Gaffar, & Visco, 2006; Cade, Scott, & He, 2003; Gaddy & Patton, 2006; Ikewaki, Fujii, & Onaka, 2005; Leung, Leone, Kumar, Kulkarni, & Sorg, 2006; Scott, Cade, & He, 2005; Thorne, Pollock, & Armentrout, 2000; Thorne, Pollock, & Armentrout, 2002; Wolf, 2005). A number of methods for pullulan production have been reported (Kato & Shiosaka, 1974; Ozaki, Nomura, & Miyake, 1996; Wallenfels & Bender, 1961; Zajic, 1967). Many applications of pullulan in food have been documented (Hiji, 1986; Hijiya & Shiosaka, 1975a, 1975b; Kato & Shiosaka, 1975a). Applications of pullulan in personal care products have been summarized by Nakashio, Tsuji, Toyota, and Fujita (1976b). Numerous derivatizations of pullulan have also been patented, including esterification (Hijiya & Shiosaka, 1975c), etherification (Fujita, Fukami, & Fujimoto, 1979), hydrogenation (Kato & Shiosaka, 1976) and carboxylation (Tsuji, Fujimoto, Masuko, & Nagase, 1978).

## 2. Historical background

Currently only a small number of exocellular fungal α-glucans are reported in the literature. Pullulan is the best studied α-linked glucan produced by the polymorphic fungus *A. pullulans*. Bauer (1938) was pioneer who observed the polysaccharide production by *A. pullulans*. First isolated and characterized by Bernier (1958) from culture broths of *A. pullulans*, pullulan has become the object of an ever increasing research effort. Thorough study of this novel polysaccharide was done by Bender, Lehmann, and Wallenfels (1959) and named as "Pullulan" by the same group. A number of research groups worked for elucidating the pullulan structure and reported that the structure of pullulan could not be narrated as consisting solely of α-(1 → 4)- and α-(1 → 6)-linkages, because a small proportion of α-(1 → 3)-linkages are also reported in the structure (Bouveng, Kiessling, Lindberg, & McKay, 1962; Sowa, Blackwood, & Adams, 1963). Wallenfels et al. resolved the pullulan structure in the beginning of 1960s (Bender & Wallenfels, 1961; Wallenfels, Bender, Keilich, & Bechtler, 1961; Wallenfels et al., 1965). Catley et al. (1986) evidenced that pullulan may be branched and possess maltosyl or glucosyl groups instead of maltotriosyl residues. Wallenfels et al. (1961) reported the action of enzyme pullulanase on pullulan. The enzyme specifically hydrolyzes the α-(1 → 6)-linkages in pullulan yielding quantita-

tively the oligomers $(G_3)_n$, where $G_3$ represents the α-(1 → 4)-linked repeating trimer maltotriose, and n is the number of trimeric repeating units in the oligomer. Catley et al. (1986) suspected that there is, perhaps, no unique structure of pullulan, as reported by Cately (1970). Interesting reviews on the structural elucidation of pullulan done so far included those by Gibbs and Seviour (1996), Leathers (2003) and Shingel (2004).

Hayashibara Company Limited, in Okayama, Japan is the commercial producer of pullulan since 1976 (Tsujisaka & Mitsuhashi, 1993). Hayashibara adjusted the growth conditions of the source fungus to produce pullulan products of particular molecular weight and specification. These include food grade (designated as PF) and deionized (PI) products with mean molecular weight of 100,000 (PI-10 and PF-10) or 200,000 (PI-20 and PF-20). Pullulan films were commercialized by Hayashibara in 1982. Today, this company is the principle commercial producer of pullulan. Hayashibara Biochemical Labs. (HBL) has granted Pfizer a worldwide license to commercialize and market film-based oral care products containing HBL's patented pullulan and has commenced supplying the pullulan powder to Warner-Lambert Company, a subsidiary of Pfizer Inc. Pfizer has introduced the film form oral care product in Canada under the brand name Listerine.

## 3. Structural geometry

The characteristic dimeric segments of pullulan are [ → *x*)-α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1→] and [ → 4)-α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1→], where *x* may be either 4 or 6 for the (1 → 4)-linked segment. The trisaccharide $G_3$ (maltotriose), the fundamental repeating unit of pullulan contains two (1 → 4)-linkages and no (1 → 6)-linkages. For the first time pullulan was isolated from cultures of *A. pullulans* in 1958 and D-glucose was reported to be the main product of acid hydrolysis (Bernier, 1958). Bender et al. (1959) evidenced the polymer to be an α-D-glucan with predominantly α-(1 → 4) linkages on the basis of its positive optical rotation and infra-red spectrum. Elemental analysis of pullulan suggested the compound to have chemical formula $(C_6H_{10}O_5)_n$. In the beginning of 1960s, the research group of Wallenfels and other researchers concluded that pullulan is a linear α-D-glucan possessing (1 → 4) and (1 → 6) linkages in a ratio of 2:1. This conclusion was drawn on the basis of IR spectroscopic data, periodate oxidation and methylation data (Bouveng et al., 1962; Sowa et al., 1963; Wallenfels et al., 1961; Wallenfels et al., 1965). The structural formula of pullulan corresponds to:

$$[\alpha\text{-D-Glc}_p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Glc}_p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Glc}_p\text{-}(1 \rightarrow 6)]_n$$

However, it was stated that the linear chain of pullulan also contains maltotetraose subunits (Wallenfels et al., 1965) that should be randomly distributed throughout the molecule (Carolan, Catley, & McDougal, 1983). In addition, according to other authors (Catley et al., 1986), 7% represents the maximum extent to which maltotetraose subunits have been detected. Moreover, α-(1 → 3) and even β-(1 → 3) as well as β-(1 → 6) linkages were found in the main backbone of pullulan produced by some strains, in addition to the α-(1 → 4) linkages (Fujii, Shinohara, Ueno, & Imada, 1984; Sowa et al., 1963).

Discovery of an extracellular enzyme from *Aerobacter aerogenes* i.e. pullulanase proved to be a critical tool for the analysis of pullulan structure (Bender & Wallenfels, 1961). Pullulanase converted a yeast (*A. pullulans*) α-glucan containing α-(1 → 6) bonds into maltotriose, thereby describing pullulan as a polymer of (1 → 6) linked maltotriose subunits (Fig. 1). Sometimes, partial acid hydrolysis of pullulan yields isomaltose, maltose, panose and isopanose (Bender et al., 1959; Bouveng, Kiessling, Lindberg, & McKay, 1963; Sowa et al., 1963). Thus, pullulan is often viewed as a polymer of panose or isopanose (Figs. 2 and 3). Panose [*O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranose] and isopanose [*O*-α-D -glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranose] is the D-glucose containing trisaccharides having (1 → 4)- and (1 → 6)-α-D-glucosidic linkages.

The black yeast *A. pullulans* (de Bary) Arnaud is known to synthesize occasionally additional aubasidan-like EPS along with pullulan. In a series of publications, Elinov and Matveeva (1973) and Elinov et al. (1974, 1975) reported the aubasidan-like components, a related group of glucans with α-1,4-D-; β-1,6-D- and β-1,3-D-glycosidic bonds. This highly branched polysaccharide contains a main core of β-1,3-linked D-glucopyranosyl residues, to which the side chains of α-1,4-D-glucosyl residues are attached through β-1,6-D-glucosidic bonds (Elinov, Marikhin, Dranishnikov, Myasnikova, & Maryukhta, 1975). Methylation and periodate oxidation data showed that pullulan-like EPS contained a high amount of 1,4-linkages (33.8–59.0%) and aubasidan-like EPS proved to contain a higher amount of 1,3-linkages. Specific rotation $[\alpha]_D$ of pullulan is positive and high indicating the predominance of α-glycosidic bonds. $[\alpha]_D$ of aubasidan-like EPS is positive but lower than that of pullulan-like EPS, indicating a predominance of β-glycosidic bonds. Infra-red spectroscopy also confirmed the presence of α- and β-glycosidic bonds in the polymers. In IR-spectra of pullulan-like EPS, the peak at $\lambda = 850\ \text{cm}^{-1}$ is significant, while in aubasidan-like EPS, a significant peak is obtained at $\lambda = 890\ \text{cm}^{-1}$ (Yurlova & de Hoog, 1997). Madi, Harvey, Mehlert, and McNeil (1997) also reported a peak at $\lambda = 859.6\ \text{cm}^{-1}$ for pullulan from *A. pullulans* which is interpreted as an α-configuration. The co-existence of α-(1 → 4)- and α-(1 → 6)-glycosidic linkages in the pullulan structure can be established by the appearance of a band at $\lambda = 935\ \text{cm}^{-1}$ (Petrov, Shingel, Scripko, & Tsarenkov, 2002).

Due to co-existence of both α-(1 → 4)- and α-(1 → 6)-linkages in a single compound, pullulan structure is often seen as an intermediate between amylose and dextran structures. Consequently, the segmental mobility of pullu-

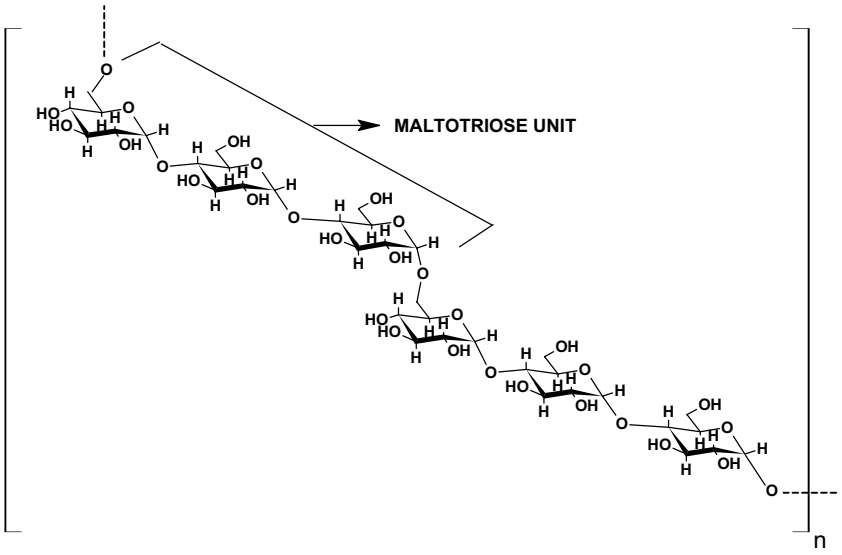


Fig. 1. Schematic chemical structure of pullulan with maltotriose as repeating unit.

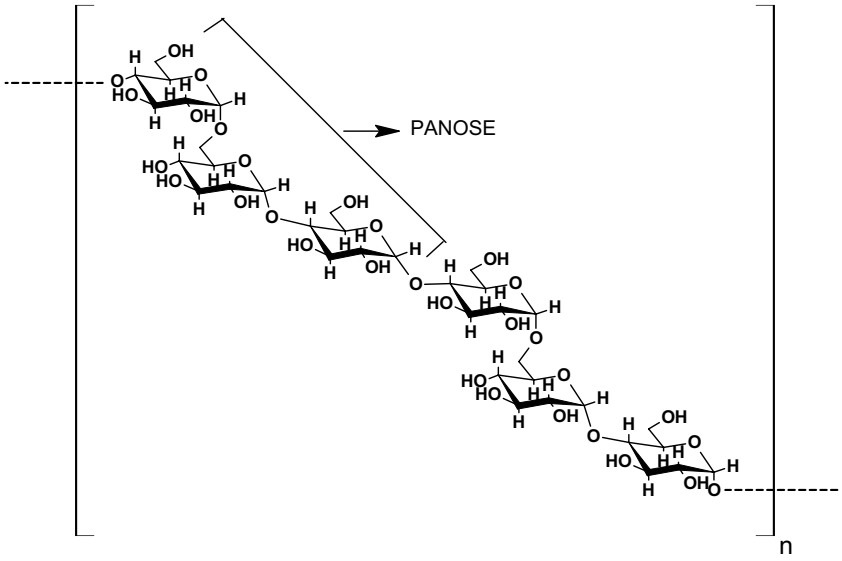


Fig. 2. Schematic chemical structure of pullulan with panose as repeating unit.

lan backbone is not uniform, with the regions of increased mobility centered on the α-(1 → 6) linkages (Dais, 1995; Dais, Vlachou, & Taravel, 2001). Pullulan structure has also been determined unambiguously by employing proton and carbon-13 NMR spectroscopy (Arnosti & Repeta, 1995; McIntyre & Vogel, 1993). The number of α-(1 → 4)- and α-(1 → 6) linkages could be quantified for pullulan by NMR spectroscopy. Arnosti and Repeta (1995) evidenced the occurrence of α-(1 → 6) linkage between every third glucose ring by $^{13}C$ NMR spectra. Raman spectroscopy has also been employed to establish pullulan structure. For pullulan, bands at 543 and 480 $cm^{-1}$ appear corresponding to the presence of α-(1 → 4)- and α-(1 → 6) glycosidic linkages in the biopolymer (Zhbankov, Andrianov, Ratajczak, & Marchewka, 1995).

## 4. Microbial sources

Pullulan is produced as a water-soluble, extracellular polysaccharide by certain strains of the polymorphic fungus *A. pullulans* (De Bary) Arnaud (formerly known as *Pullularia pullulans* De Bary) *Berkhout* or *Dematium pullulans* (De Bary). The microbial production of pullulan by *Pullularia pullulans* was discovered by R. Bauer in 1938. *A. pullulans* is a ubiquitous fungus isolated commonly from the environment (Cooke, 1959; Hermanides-Nijhof, 1977). It is found in soil, water and as saprophyte on decaying leaf

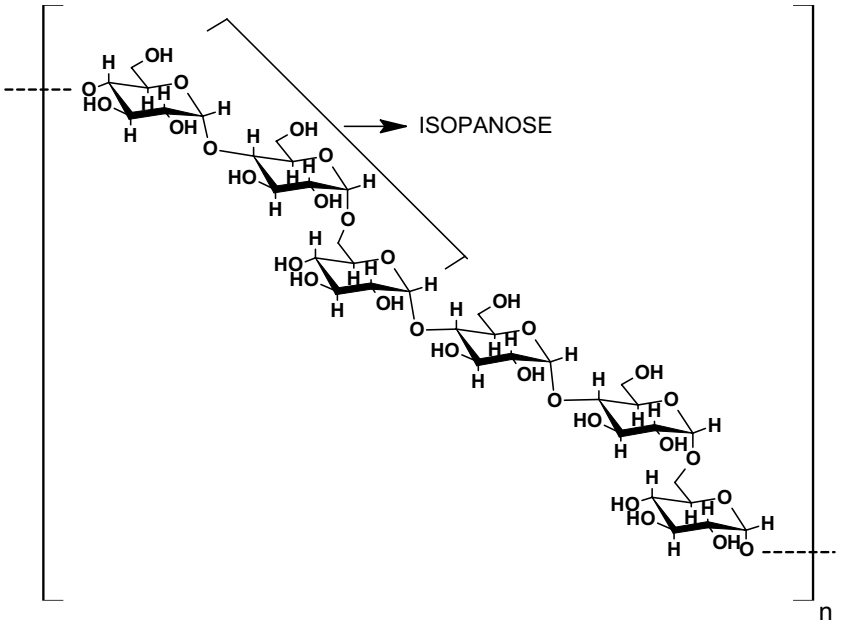


Fig. 3. Schematic chemical structure of pullulan with isopanose as repeating unit.

litter, wood and many other plant materials. *A. pullulans* has a role in the deterioration of both exterior and interior house paints (Brand & Kemp, 1973). *A. pullulans* has also been reported as a slime-producing contaminant of paper mills and to colonize optical lenses (Webb et al., 2000). The fungus has been described to be omnivorous and its isolates produce an impressive array of degradative enzymes, including amylases (Federici, 1982; Leathers, 1987; Leathers, 1993; Linardi & Machado, 1990; Saha, Silman, & Bothast, 1993), proteases (Federici, 1982; Ahearn, Meyers, & Nichols, 1968), esterases (Federici, 1982), pectinases (Dennis & Buhagiar, 1973; Federici, 1982; Finkelman & Zajic, 1978) and hemicellulases including xylanase (Biely, Kratky, Kockova-Kratchilova, & Bauer, 1978; Biely, Kratky, Petrakova, & Bauer, 1979; Flannigan, 1970; Saha & Bothast, 1998) and mannanase (Kremnicky & Biely, 1997; Kremnicky, Slavikova, Mislovicova, & Biely, 1996). Some of the pullulan producing color-variant strains of *A. pullulans* are also known as natural overproducers of endoxylanase (Leathers, 1989; Leathers, Kurtzman, & Detroy, 1984). The color-variant strains are differentiated from typically pigmented (off-white to black in appearance) strains of *A. pullulans* by their brilliant pigments of red, yellow, pink or purple color and their low DNA relatedness (Leathers, Nofsinger, Kurtzman, & Bothast, 1988; Wickerman & Kurtzman, 1975).

*Aureobasidium pullulans* is a polymorphic fungus and its three distinctive forms are: elongated branched septate [i.e. the septa, or "cross-walls," that divide the hyphae into numerous uninucleated or multinucleated cells] filaments, large chlamydospores and smaller elliptical yeast-like cells. An undesirable characteristic feature of *A. pullulans* is the production of a dark pigment, which is a melanin-like compound and appears dark green to black in color. Another undesirable characteristic of *A. pullulans* occurs during its submerged growth; as the fermentation progresses, the culture viscosity decreases due to a decrease in the average molecular weight of the accumulated extracellular pullulan (Catley & Whelan, 1971; Kaplan, Wiley, Arcidiacono, Mayer, & Sousa, 1987). *A. pullulans* is genetically imperfect and traditionally has been considered to be among the Fungi Imperfecti or Deuteromycetes (Hermanides-Nijhof, 1977; Wynne & Gott, 1956; Cooke, 1962). Recently, *Aureobasidium* has been described as a filamentous ascomycetes (Euascomycetes, order Dothideales, family Dothideaceae) capable of growing yeast-like in culture (de Hoog, 1998; de Hoog & Yurlova, 1994). The specie is of practical interest due to production of extracellular polysaccharide present widely applied in biotechnology. Two varieties of this specie i.e. var. *pullulans* and var. *aubasidani* exists usually distinguished by molecular characteristics, nutritional physiology and the structure of exopolysaccharide elaborated by them. *A. pullulans* varieties show medium-dependence differing with EPS structure. The production of pullulan is stimulated with ammonium sulpahte as nitrogen source by *A. pullulans* var. *pullulans* (Bulmer, Catley, & Kelly, 1987; Imshenetskii, Kondrat'eva, & Smut'ko, 1981b), while sodium nitrate is optimal for the production of aubasidan by *A. pullulans* var. *aubasidsani* (Elinov et al., 1974).

Keen interest has been shown to relate pullulan production with a particular morphological form of yeast-like fungus *A. pullulans*. Catley (1973) was first to notice that the pullulan formation appeared to coincide with the shift in morphology from mycelial to unicellular forms and consequently blastospores were held responsible solely for pullulan elaboration. This proposal was supported by frequent reports of maximum polysaccharide production occurring

under culture conditions favoring a unicellular morphology (Gibbs & Seviour, 1996; Reeslev, Storm, Jensen, & Olsen, 1997). Some researchers reported that unicellular forms other than blastospores are the major pullulan producers. Data is available where swollen cell as well as chlamydospore formation parallels pullulan formation (Simon, Bouchet, Caye-Vaugien, & Gallant, 1995). Simon and coworkers also suggested that whatever polysaccharide might be synthesized by hyphal forms of *A. pullulans*, it is not pullulan-like. When a significant amount of chalamydospores are present, pullulan is always elaborated, whatever be the culture conditions. Yurlova, Gun, Sintiskaja, Kashkina, and Khmara (1993) showed that hyphal cells can also produce extracellular polysaccharides, while Seviour, Kristiansen, and Harvey (1984) supposed that polysaccharide production did not coincide with any major morphological transformation at all. Recent developments by Campbell, Siddique, McDougall, and Seviour (2004) showed polysaccharide secretion by chlamydospores and swollen cells but not by conidia or hyphae.

Many, but not all, strains of *A. pullulans* are capable of producing pullulan (Augustin, Kuniak, & Hudecova, 1997; Leathers et al., 1988; Ueda, Fujita, Komatsu, & Nakashima, 1963). Apart from *A. pullulans*, a number of other microorganisms summarized in Table 2 are also reported as pullulan producers. Pullulan has also been isolated from the saprophytic (sometimes mycoparasitic) fungus *Tremella mesenterica* (Fraser & Jennings, 1971), from obligate tree parasitic fungi *Cytaria harioti* and *C. darwinii* (Waksman, De Lederkremer, & Cerezo, 1977; Oliva, Cirelli, & De Lederkremer, 1986) and from the fungal agent of chestnut blight, *Cryphonectria parasitica* (Corsaro et al., 1998). It is important to note that this is the first report of *C. parasitica* as a pullulan producer *in vitro*. Jennings and Smith confirmed the relative composition of the *T. mesenterica* glucan and obtained its sequence by $^{13}C$ NMR spectroscopy. Sequence information came from the sensitivity of some resonances to the nature of linkages to the next glucosyl moieties (Jennings & Smith, 1973). An α- and β-glucan have been isolated from the lichenised ascomycetous *Teloschistes flavicans*. The α-glucan structure obtained has been classified as a pullulan, similar to that has been isolated from the fungi *A. pullulans*, *Tremalla mesenterica* and *Cyttaria hariotii*, but with different ratios of the component glycosidic linkages (Reis, Tischer, Gorrin, & Iacomini, 2002). Its 1:1 linkage ratio is different from those of known pullulans i.e. 2:1. *Rhodotorula bacarum*, an isolate from the Chinese plant leaves collected from the south China has been reported to produce a large amount of pullulan and melanin has not been produced during the fermentation (Chi & Zhao, 2003). Delben, Forabosco, Guerrini, Liut, and Torri (2006) and Forabosco et al. (2006) also reported the production and characterization of pullulans from virulent and hypovirulent strains of *Cryphonectria parasitica* (Murrill) M.E. Barr, formerly called *Endothia parasitica* (Murrill) P.J. Anderson and H.W. Anderson. The spectral properties of the pullulan-like exopolysaccharides produced by *C. parasitica* agree with the presence of both maltotriose and maltotetraose sequences in the polysaccharide backbone.

Table 2
Microbial sources of pullulan

| Microorganisms | Reference(s) |
|---|---|
| *Aureobasidium pullulans* | Bauer (1938); Cooke (1959); Leathers (2003) |
| *Tremella mesenterica* | Fraser and Jennings (1971) |
| *Cytaria harioti* | Waksman et al. (1977); Oliva et al. (1986) |
| *Cytaria darwinii* | Waksman et al. (1977); Oliva et al. (1986) |
| *Cryphonectria parasitica* | Corsaro et al. (1998); Forabosco et al. (2006); Delben et al. (2006) |
| *Teloschistes flavicans* | Reis et al. (2002) |
| *Rhodototula bacarum* | Chi and Zhao (2003) |

## 5. Upstream processing

Microbially produced polysaccharides have properties which are extremely useful in different industrial applications. During the fermentation process/upstream processing of EPS production, the characteristics of the liquid media change drastically. At the beginning, the liquid has a Newtonian behavior with viscosity close to that of pure water but the formation of EPSs results in a rapid increase in the apparent viscosity and a change to non-Newtonian rheology. Since the microbial polysaccharide production process is aerobic, the supply of the liquid media with oxygen during the fermentation is of great importance. Most of the frequently cited work on pullulan is concerned with the control of pullulan synthesis by culture conditions and the relationship between pullulan production and cell morphology. The published accounts of pullulan production are confusing as well as contradictory, which may be due to the reason that multiple factors interact in the regulation of pullulan biosynthesis. Extensive attention has been paid to the microbial sources for pullulan production (Guterman & Shabtai, 1996), pullulan production with respect to the nutrients (Deshpande, Rale, & Lynch, 1992; LeDuy & Boa, 1983; LeDuy, Varmoff, & Chagraoui, 1983), fermentation conditions as pH (Lacroix, LeDuy, Noel, & Choplin, 1985; Ono, Yasuda, & Ueda, 1977), temperature (McNeil & Kristiansen, 1990), minerals as $Zn^{+2}$, $Fe^{+2}$, $Mn^{+2}$, $Ca^{+2}$ and $Cu^{+2}$ (Reeslev & Jensen, 1995) and process technology for commercial pullulan manufacture (Thibault & LeDuy, 1999). The considerable obstacles faced during the pullulan production include high viscosity of the fermentation broth and melanin pigmentation. At the completion of fermentation, the resulting broth consists of microbial cells and cellular debris, residual media components from culture medium and extracellular metabolites produced and excreted during the fermentation.

The comparative studies reveal that *A. pullulans* strains differ considerably with respect to growth, pullulan yield

and cell morphology (Cernakova, Kockova-Kratochvilova, Suty, Zemek, & Kuniak, 1980; Gadd & Cooper, 1984; Kockova-Kratochvilova, Cernakova, & Slavikova, 1980; Kremnicky et al., 1996; Leathers et al., 1988; Park, 1982; Silman, Bryan, & Leathers, 1990; Ueda et al., 1963). The specific isolates of *A. pullulans* require specific optimal conditions for pullulan production. The sugars such as sucrose, glucose, fructose, maltose, starch, or malto oligosaccharides support pullulan production by *A. pullulans* (Badr-Eldin, El-Tayeb, El-Masry, Mohamad, & Abd El-Rahman, 1994; Behrens & Lohse, 1977; Bender & Wallenfels, 1961; Catley, 1971b; Imshenetskii et al., 1981b; Imshenetskii, Kondrat'eva, Dvadtsamova, & Vorontsova, 1985; Leathers et al., 1988; Ueda et al., 1963; West & Reed-Hamer, 1991). The sugars such as xylose, arabinose, mannose, galactose, rhamnose and lactose though used less frequently also support pullulan production but with reduced yields (Bouveng et al., 1962; Bouveng et al., 1963; Imshenetskii et al., 1981b; LeDuy et al., 1983; Ueda et al., 1963). Glycerol can also contribute to pullulan synthesis but only in the presence of the inducer glucose (Catley, 1971b). The induction of pullulan synthesis is inhibited by cycloheximide, indicating that new protein synthesis is required. Pullulan synthesis is also inducible by glucose, fructose or saccharides, which can be broken down to these sugars (Catley, 1972).

Often it has been reported that there is correlation between *A. pullulans* morphology and pullulan production, although the correlations cause is not yet clear. It has been reported that yeast-like forms of *A. pullulans* are the primary producers of pullulan (Catley, 1980; Heald & Kristiansen, 1985). Pullulan is produced in the late exponential and early stationary phase of cultures primarily by yeast-like cells (Catley, 1971a; Catley, 1973; Ono et al., 1977). It has also been reported that the rate of pullulan synthesis is independent of pH (Heald & Kristiansen, 1985). Pullulan has been reported to be a secondary metabolite produced by yeast-like cells during ammonium limitation (Bulmer et al., 1987). In comparison, a 50% mixture of yeast-like and hyphal cells favour pullulan production under chemostat conditions. Pullulan production by batch cultures is related to the formation of yeast-like cells (McNeil, Kristiansen, & Seviour, 1989; Reeslev & Jensen, 1995; Reeslev, Jorgensen, & Jorgensen, 1993; Reeslev, Nielsen, Olsen, Jensen, & Jacoson, 1991). It has also been reported that bioreactor design and nitrogen sources affect pullulan production by *A. pullulans*, but its morphology has no affect (Gibbs & Seviour, 1992). Ammonium and complex nitrogen sources have been found to be superior to nitrate for pullulan production from *A. pullulans* (Reed-Hamer & West, 1994; West & Reed-Hamer, 1991). Lee and Yoo (1993) obtained optimal yields of pullulan at an initial pH of 6.0 using *A. pullulans*. It has been reported that pH 4.5 is optimal for pullulan production, though yeast-like growth is favored at pH 6.5 (Madi, McNeil, & Harvey, 1996). Pullulan formation by *A. pullulans* is associated mainly with swollen cells and chlamydospores (Andrew, Harris, Spear, Lau, & Nordheim, 1994; Simon et al., 1995; Simon et al., 1993).

Optimal temperature for pullulan production vary slightly in the range of 24–30 °C, on strain-specific basis (Imshenetskii, Kondrat'eva, & Smut'ko, 1981a; McNeil & Kristiansen, 1990; Tsujisaka & Mitsuhashi, 1993; West & Reed-Hamer, 1993; Zajic, 1967). Vitamins and mineral salts have also been reported to influence the pullulan synthesis. Bender and coworkers reported that thiamine increases pullulan yields (Bender et al., 1959). Biotin, ferric chloride, manganese chloride and zinc chloride are found to enhance pullulan formation by *A. pullulans* (West & Reed-Hamer, 1992; West & Strohfus, 1997a). In contrast, inhibitory effects of $Fe^{3+}$ and $Zn^{2+}$ on the development of both yeast-like cells and polysaccharide have also been reported (Reeslev & Jensen, 1995; Reeslev et al., 1993).

Several methods and conditions for pullulan production have been reported (Kato & Nomura, 1976; Kato & Nomura, 1977; Kato & Shiosaka, 1975b; Kondrat'eva & Lobacheva, 1990; McNeil & Harvey, 1993; Murofushi, Nagura, & Moriya, 1998; Sugimoto, 1978; Thorne, Pollock, & Armentrout, 1993; Thorne et al., 2000). *A. pullulans* has been cultivated batch-wise on media comprising starch hydrolysates of dextrose equivalent 40–50 at 10–15% concentration for commercial production (Tsujisaka & Mitsuhashi, 1993). The medium also contained peptone, phosphate and basal salts, and the initial culture pH was 6.5. Optimal pullulan yield was obtained within about 100 h at 30 °C.

A number of complex carbon sources have been reported for pullulan production, including beet molasses (Roukas, 1998; Roukas & Liakopoulou-Kriakides, 1999), carob pod (Roukas & Biliaderis, 1995), cornmeal hydrolysates (Imshenetskii et al., 1985), corn syrup (West & Reed-Hamer, 1991; West & Reed-Hamer, 1993), fuel ethanol fermentation stillage (Leathers & Gupta, 1994), grape skin pulp (Israilides et al., 1998), olive oil and sucrose (Youssef, Biliaderis, & Roukas, 1998), peat hydrolysate (Boa & LeDuy, 1984; Boa & LeDuy, 1987), hydrolyzed potato starch (Barnett, Smith, Scanlon, & Israilides, 1999), spent grain liquor (Roukas, 1999) and spent sulfite liquor (Zajic, Ho, & Kosaric, 1979). Mixed-culture techniques have been employed for the utilization of lactose (LeDuy et al., 1983) and inulin (Shin, Kim, Lee, Cho, & Byun, 1989). Vijayendra and coworkers isolated *A. pullulans* CFR-77 and used it for the production of pullulan employing jaggery (unrefined sugar that is made from sugarcane juice) as a carbon source (Vijayendra, Bansal, Prasad, & Nand, 2001). Jaggery supported good growth of *A. pullulans* and the pullulan produced was pigment free and more highly viscous as compared with derived from sucrose. Pullulan production from beet molasses, via pretreatment with sulfuric acid and activated carbon has been reported (Lazaridou, Roukas, Biliaderis, & Vaikousi, 2002). The production of pullulan by *A. pullulans* can be enhanced by yeast extract or soybean pomace as a nitrogen source (Seo et al., 2004). Various carbon sources (20 g/l) namely,

glucose, gluconic acid, glucosamine, fructose, maltose, dextrin, and cellulose have been screened for the production of pullulan. In this study, glucose, sucrose, and dextrin have been reported to be the better carbon sources for pullulan production. Optimal conditions like the effect of composition of feed solution, dilution rate, and concentration of sucrose in feed solution for the continuous production of pullulan were also determined in a bioreactor (Seo et al., 2006).

The production of pullulan in batch (Vijayendra et al., 2001) and fed-batch fermentations (Moscovici et al., 1996; Shin, Kim, Lee, Cho, & Byun, 1987) has been reported. Continuous fermentation has also been carried out for pullulan production (McNeil et al., 1989; Reeslev et al., 1997; Schuster, Wenzig, & Mersmann, 1993). Pullulan production using immobilized *A. pullulans* cells have been studied by a number of workers (Mulchandani, Luong, & LeDuy, 1989; Ürküt, Dağbağli, & Göksungur, 2007; West, 2000; West & Strohfus, 1997b; West & Strohfus, 1998; West & Strohfus, 1996a, 1996b). Ürküt et al. (2007) has reported the production of pullulan from synthetic medium by *A. pullulans* P56 immobilized in Ca-alginate beads using batch and repeated batch fermentation systems. The results suggested that the immobilization of *A. pullulans* cells in Ca-alginate gel beads is suitable for batch and repeated batch production of pullulan.

Use of a reciprocating plate bioreactor for pullulan fermentations have been demonstrated by Audet (Audet, Lounes, & Thibault, 1996).

Recently, Response surface methodology (RSM) which is a collection of statistical techniques for designing experiments, building models, evaluating the effect of factors and searching optimum conditions for desirable responses is used. The classical method involving variation of one factor at a time, does not allow evaluation of the combined effects of all the factors involved in a particular process and is a time-consuming methodology. Above mentioned restrictions can be overcome by the use of RSM, which identify and quantify the various interactions among the factors involved (Göksungur, Dağbağli, Uçan, & Güvenç, 2005; Li, Bai, Cai, & Ouyang, 2002). RSM is applied extensively for optimizing the cultural medium conditions and other parameters in the bioprocesses. Many researchers have worked on optimizing medium and process parameters for pullulan production from *A. pullulans*. Göksungur et al. (2005) described the optimization of pullulan production by RSM in a stirred tank reactor using free cells of *A. pullulans* P56. They investigated the sugar concentration, agitation, and aeration at three different levels using response surface methodology. Lin, Zhang, and Thibault (2007) investigated six factors i.e. strain, carbon source, nitrogen source, nitrogen concentration, aeration and initial pH for their effects on pullulan production by *A. pullulans* using 2-level fractional factorial design at shake flask level. Simultaneous optimization of concentration and molecular weight of exopolysaccharide was carried out. The effect of major factors together with the two-factor interactions was observed and empirical models were used for optimization of levels of six factors. Ürküt et al. (2007) worked on optimization of pullulan production using immobilized *A. pullulans* by response surface methodology in batch and repeated batch fermentation systems. RSM was used to investigate the effect of three parameters i.e initial pH, agitation speed and incubation time on pullulan concentration. Ca-alginate beads were used consecutively for six runs without any marked loss of activity in repeated batch fermentations.

## 6. Downstream processing

Downstream processing is required to obtain pure biopolymer from the fermentation broth and it comprises of cell harvesting from culture broth after cultivation, removal of melanin pigments produced during fermentation and precipitating the polymer with a suitable solvent. Melanin is one of the major obstacle in pullulan production and it is responsible for dark green to black color of the broth. Seihr (1981) reported intracellular as well as extracellular synthesis of melanin by the pentaketide pathway during the last stages of fermentation when the cell morphology changes from swollen cells to true chlamydospores. Thus an appropriate downstream processing of the fermentation broth is required to alleviate the pigmentation problem in case of melanin producing strains. Generally from strains producing melanin free pullulan, recovery and purification is accomplished with one precipitation step using a suitable organic solvent. The organic solvent usually used is from the group of alcohols, esters and ethers with three or more carbon atoms. The studies and evaluations on processes for separation and purification of pullulan, led to the discovery that solvents of relatively higher molecular weight and slightly lower hydrophilicity are more suitable for such processes in comparison with lower molecular weight and higher hydrophilicity solvents as methanol, ethanol and acetone. The solvents having relatively low hydrophilicity such as propyl alcohol, isopropyl alcohol, tetrahydrofuran, dioxane etc. are capable of effecting complete precipitation of pullulan with an addition of less volume of the solvent. These solvents also display sufficient impurity removing efficacy (Kato & Nomura, 1977). Further purification of pullulan can be achieved by ultrafiltration and ion-exchange resins (Kachhawa, Bhattacharjee, & Singhal, 2003). A flow diagram showing various steps for pullulan purification is shown in Fig. 4.

Kikuchi, Taguchi, Sakanbo, and Kobayashi (1973) developed a purification procedure for separating pullulan by giving treatment to the culture media with acetyltrimethylammonium hydroxide. Pullulan purification by centrifugation, precipitation with three volumes of methanol and then by filtration has been reported (Boa & LeDuy, 1984; Boa & LeDuy, 1987). Alternatively, centrifuged broth is treated with acetone and ethanol mixture 1:1 (v/v) to precipitate the exopolysaccharide (Shin et al., 1989). Pullulan

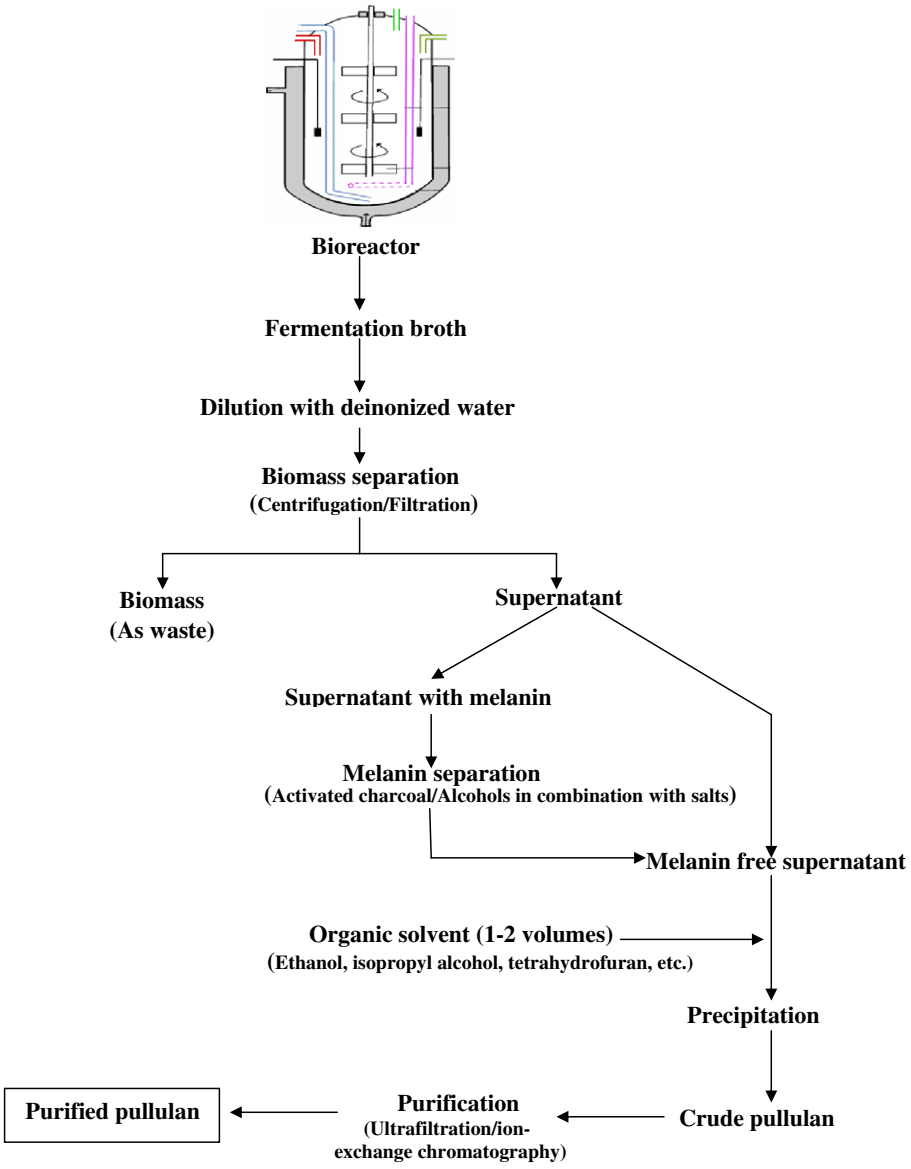


Fig. 4. Flow diagram for downstream processing of pullulan.

purification by precipitation of centrifuged broth with an equal volume of tetrahydrofuran (THF) has been carried out by Leathers (Leathers et al., 1988). Pullulan precipitation from the supernatant with an equal volume of ethanol has also been reported (Mulchandani et al., 1989). Pollock have recovered polysaccharide from the clarified broth by precipitation with IPA (Pollock, Thorne, & Armentrout, 1992). One volume of IPA was used and the precipitates were dried to a constant weight in an oven at 80 °C. Roukas and Biliaderis initially precipitated crude polysaccharide with two volumes of acetone, dissolved in distilled water and reprecipitated with absolute ethanol (Roukas & Biliaderis, 1995). Pullulan in the supernatant was recovered by addition of two volumes of ethanol at 4 °C (Barnett et al., 1999; Lazaridou et al., 2002; Youssef, Roukas, & Biliaderis, 1999). Vijayendra and coworkers purified pullulan by precipitation using two volumes of isopropyl alcohol to one volume of cell free fermentation broth followed by washing of precipitates with acetone and dried to a constant weight at 90 °C (Vijayendra et al., 2001).

Pullulan has been precipitated using two volumes of absolute ethanol from cell free broth (Kachhawa et al., 2003). The precipitates obtained were dried till a constant weight in an oven at 60 °C to give the crude pullulan. To separate melanin, demelanization by adsorption on activated charcoal or by use of solvent/solvent blends or by solvent/salt combinations has been used. Among all these treatments, a combination of ethanol/ethyl methyl ketone in 60:40 ratio has been reported to be the most effective. Cross-flow membrane technology for pullulan recovery by filtration has also been reported (Yamasaki, Lee, Tanaka, & Nakanishi, 1993a, 1993b).

## 7. Peculiar characteristics

The regular (1 → 6) linkages in pullulan are thought to impart structural flexibility and enhanced solubility (Buliga & Brant, 1987a). Recently, Angioletti (2003) has confirmed the same. This allows pullulan to mimic synthetic polymers derived from petrochemical-derived polymers in many aspects as biocompatibility, biodegradability, and both human as well as environmental compatibility. Pullulan powders are white and non-hygroscopic that dissolves readily in hot or cold water. It is non-toxic, non-mutagenic, odorless, tasteless and edible with a number-average molecular weight ($M_n$) of about 100–200 kDa and a weight-average molecular weight ($M_w$) of about 362–480 kDa (Okada et al., 1990). Values of $M_w/M_n$ have been frequently reported and lie between 2.1 and 4.1 (Petrov et al., 2002; Roukas & Montzouridpu, 2001; Wiley et al., 1993). These values are significantly lower in comparison to the other industrially important exopolysaccharides such as amylose and dextran. The difference in the biosynthetic pathways or the cell morphology-regulated mechanism for pullulan elaboration might be the possible reason for the same. It is insoluble in organic solvents except dimethylformamide and dimethylsulfoxide (Sugimoto, 1978; Tsujisaka & Mitsuhashi, 1993). Aqueous solutions of pullulan are viscous but don't form gels. The viscosity of water solutions of pullulan is proportional to the molecular weight of the pullulan. It is also stable over a broad range of pH conditions when in solution. It does not liberate any harmful gas even when burned and is spontaneously decomposed by microorganisms even when discarded as it is. Pullulan has excellent adhesive properties when dried and excellent foam retention properties when it is dissolved in water. The excellent adhesive properties of pullulan, together with its low viscosity, enables active ingredients to be effectively applied to the body. This also makes it suitable as a binder for agglomerated products, helping to improve the performance of existing manufacturing processes by providing an opportunity to create new product presentations for the consumer. Pullulan also has good oxygen barrier properties and has been developed for food-packaging applications. Detailed studies have been carried out for hydrodynamic and molecular properties of pullulan in solution (Kato et al., 1984; Kato, Okamoto, Tokuya, & Takahashi, 1982; Kawahara, Ohta, Miyamoto, & Nakamura, 1984; Nishinari et al., 1991; Nordmeier, 1993). Hydrodynamic properties depict the response of the biopolymer to the action of a solvent and this depends upon the conformational flexibility of the polymer chain.

## 8. Applications

Though, microbial biopolymers are known to possess useful physical properties, even then currently only a small number of biopolymers are produced commercially on large scale. A few fungal EPSs have been reported so far that possess appealing industrial applications. Pullulan, a water soluble biopolymer from *A. pullulans* is one of such fungal EPSs. Numerous applications of pullulan in food and pharmaceuticals manufacturing have been reviewed by Leathers (2003).

### 8.1. Food industry

Pullulan provides few calories and is treated as dietary fiber in rats and humans (Oku, Yamada, & Hosoya, 1979; Yoneyama et al., 1990). This is because of its resistance to mammalian amylases. Studies indicate that dietary pullulan functions as prebiotic, promoting the growth of beneficial bifidobacteria (Mitsuhashi, Yoneyama, & Sakai, 1990; Sugawa-Katayama, Kondou, Mandai, & Yoneyama, 1994; Yoneyama et al., 1990). Pullulan may be incorporated in solid as well as liquid food to replace starch; imparting the characteristics to food normally derived from starch as consistency, dispersibility, moisture retention, etc. Pullulan can also be used as a partial replacement for starch in pastas or baked goods. Pullulan improves the shelf life of the food as it is not a readily assimilable carbon source for bacteria, molds and fungi responsible for spoilage of food. Pullulan is also superior to starch in water retention thus retarding the spoilage of food by drying out (Hiji, 1986; Hijiya & Shiosaka, 1975b; Kato & Shiosaka, 1975a; Yuen, 1974). Yuen claimed that pullulan inhibits fungal growth in foods (Yuen, 1974). Solution properties of pullulan have been studied (Buliga & Brant, 1987b; Kato et al., 1982; Kawahara et al., 1984; Nishinari et al., 1991). Pullulan solutions resemble gum arabic having relatively low viscosity (Tsujisaka & Mitsuhashi, 1993). Pullulan can be used as low-viscosity filler in beverages and sauces. It can also be used to stabilize the quality and texture of mayonnaise. The viscosity of pullulan is not affected by heating, changes in pH and most metal ions, including sodium chloride. Adhesive properties are also exhibited by pullulan and its derivatives (Hijiya & Shiosaka, 1975a). Pullulan can be used as a binder and stabilizer in food pastes; it can also be used to adhere nuts to cookies. Pullulan can be employed as a binder for tobacco (Miyaka, 1979), seed coatings and plant fertilizers (Matsunaga, Fujimura, Namioka, Tsuji, & Watanabe, 1977a, 1978).

Pullulan films are clear and highly oxygen-impermeable with excellent mechanical properties. Pullulan films are prepared by drying a pullulan solution (usually 5–10%) on a smooth surface and it can be as thin as 5–60 μm (Yuen, 1974). Underivatized films are readily dissolved in water, thus having property to melt in the mouth as edible food coatings (Conca & Yang, 1993). The oxygen resistance of pullulan films is suitable for protection of readily oxidized fats and vitamins in food. Pullulan films can be employed as coating or packaging material of dried foods, including nuts, noodles, confectionaries, vegetables and meats (Krochta & De Mulder-Johnston, 1997). Pullulan can be used complexed with polyethylene glycol (Hijiya & Miyake, 1990) or enriched with rice protein (Shih,

1996). Pullulan can be used directly to foods as a protective glaze. Pullulan substituted with cholesterol or fatty acids can be used to stabilize fatty emulsions (Yamaguchi & Sunamoto, 1991). Wolf (2005) reported the use of pullulan as a slowly digested carbohydrate and its incorporation into food products, especially beverages and meal replacement products. Pullulan is referred as slowly digested carbohydrate as human enzymes gradually convert pullulan to glucose that results in gradual rise in blood glucose level in humans. Pullulan may be incorporated into dietetic snack foods designed for diabetics. Pullulan is also beneficial to patients who have impaired glucose tolerance.

### 8.2. Pharmaceutical industry

Pullulan and its derivatives can be used as a denture adhesive. Adhesives or pastes are prepared by dissolving or dispersing uniformly pullulan ester and/or pullulan ether in water or in a mixture of water and acetone. Adhesives and pastes containing pullulan as the main component have higher water solubility and lower moisture resistance (Hijiya & Shiosaka, 1975a). Sugar-coated pharmaceutical compositions such as tablets, pills, granules contain pullulan in the sugar layer for the purpose of preventing brownish color change of the composition with lapse of time. The solid sugar-coated preparation exhibits an enhanced impact strength and shelf life. Pullulan can also be used for pharmaceutical coatings, including sustained-release formulations. Novel preparations such as tablets, pills, granules or the like, which contain pullulan in the sugar layer serve the purpose of preventing brownish color change of the composition (Childers, Oren, & Seidler, 1991; Izutsu, Sogo, Okamoto, & Tanaka, 1987; Miyamoto, Goto, Sato, Okano, & IIjima, 1986). Oral care products have been commercialized based on pullulan films. The colorless, transparent and edible pullulan film has also attracted a great deal of interest for other uses such as a non-polluting wrapping material (Anonymous, 2001). Scott et al. (2005) reported pullulan compositions for the use in pharmaceutical products preferably for predosed formulations like soft and hard capsules. Pullulan derivatives are promising as non-toxic conjugates for vaccines (Mitsuhashi & Koyama, 1987; Yamaguchi et al., 1985). Covalent attachment between the virus and pullulan remarkably enhances the inherent producibility of immunoglobulin G and immunoglobulin M antibodies and diminishes the immunoglobulin E antibody producibility as well as sufficiently inactivating and detoxifying the virus. Pullulan can provide liposome delivery (Sunamoto et al., 1987; Takada, Yuzuriha, Katayama, Iwamoto, & Sunamoto, 1984). Sized pullulan fractions having molecular weight 30,000 to 90,000 Da can be used as a blood plasma expander in place of dextran (Igarashi, Nomura, Naito, & Yoshida, 1983; Kulicke & Heinze, 2006). There have been several attempts to develop plasma substitutes on pullulan (Seibutsu & Kenkyujo, 1983). Shingel (2004) summarized all efforts that have been made so far to understand the pharmacokinetics of intravenously applied pullulan in terms of the molar mass and concentration. Nakashio et al. (1976b) has demonstrated the use of pullulan in cosmetics, lotions and shampoos. Pullulan being non-toxic and non-irritant to human body, may be applied to any cosmetics, but is preferably used as an ingredient of cosmetic lotions, cosmetic powders, cosmetics around eyes, facial packs, shampoos, specific hair dressings (set lotions and hair lacquers), and tooth powders. Excellent transparent film-forming ability, moisture absorptivity, water solubility and tackiness are the properties making pullulan suitable for use in cosmetics. In addition, the pullulan is characteristically lower in aqueous solution viscosity than any of such high polymer for cosmetics. Leung et al. (2006) disclosed physiologically acceptable films including edible films prepared from pullulan. These edible films include pullulan and antimicrobially effective amounts of the essential oils as thymol, methyl salicylate, eucalyptol and menthol. These films are effective killers of plaque-producing gums that cause dental plaque, gingivitis and bad breath.

### 8.3. Miscellaneous industry

Pullulan can be formed into fibres resembling nylon or rayon by wet or dry spinning. Goods resembling polystyrene or polyvinyl alcohol can be formed from pullulan by compression or extrusion. The surface of molded pullulan type resin is coated with a thermosetting resin film. Molded pullulan type resins exhibit properties as transparency, toughness, gas impermeability and non-polluting (Hijiya & Shiosaka, 1974; Hijiya & Shiosaka, 1975c; Matsunaga, Tsuji, & Saito, 1976; Matsunaga, Tsuji, & Saito, 1977b; Nakashio, Tsuji, Toyota, Fujita, & Nomura, 1975b; Tsuji, Toyota, & Fujita, 1976). Pullulan is also used as an industrial flocculating agent (Zajic, 1967; Zajic & LeDuy, 1973). It can also be used in the production of paper. The invention pertains to novel paper-coating material containing pullulan which is excellent in gloss, printing gloss, adhesive strength and viscosity stability during storage. Pullulan has excellent properties as a paper-coating adhesive. The pullulan paper is high in strength and folding resistance, is tougher than a wood pulp paper. It favors ink receptivity because of its high hydrophilic nature, hence making it suitable for printing and writing (Nakashio, Sekine, Toyota, Fujita, & Domoto, 1976a; Nomura, 1976). It can improve the characteristics of paint (Nakashio, Sekine, Toyota, & Fujita, 1975a). Pullulan and its derivatives also have photographic, lithographic and electronic applications (Sano, Uemura, & Furuta, 1976; Sasago, Endo, Takeyama, & Nomura, 1988; Shimizu, Moriwaki, & Shimoma, 1983; Tsukada, Hagihara, Tsuji, Fujimoto, & Nagase, 1978; Vermeersch, Coppens, Hauquier, & Schacht, 1995). Lithography is the process of printing from specially prepared surfaces, some areas of which are capable of accepting lithographic ink, whereas other areas, when moistened with water, will not accept the ink. Pullulan and pullulan derivatives are superior to traditionally used gum Arabic solu-

tion in the protection of the surface of lithographic printing plate against oxidation and scumming as well as in the ability to enhance the hydrophilic character of metallic surface of a non-image area. Nagase, Tsuji, Fujimoto, and Masuko (1979) and Motozato, Ihara, Tomoda, and Hirayama (1986) reported the use of cross-linked pullulan beads (analogous to Sephadex ®) in gel permeation chromatography. Cross-linked pullulan is water-resistant without loss of its excellent properties such as high degrees of transparency, toughness and adhesiveness. Pullulan gels have been used for enzyme immobilization. Hydrophilic pullulan gel having a three-dimensionally reticulated structure which is obtained by the reaction between pullulan and a bifunctional compound capable of forming an ether linkage with the hydroxyl group present in glucose unit of pullulan is used as a carrier. Enzymes immobilized with pullulan gel have a high activity and good retention of activity (Hirohara, Nabeshima, Fujimoto, & Nagase, 1981). Onda and coworkers reported potential uses of cyanoethylated pullulan in electronic devices (Onda, Muto, & Suzuki, 1982). Pullulan is readily cyanoethylated by reacting it with acrylonitrile in the presence of an alkali catalyst like sodium hydroxide. The cyanoethylated pullulan possess unique properties in heat resistance, solubility in organic solvents, film-forming property, and adhesive bonding to metals. Accurately sized pullulan molecular weight species are produced commercially for their use as chromatography standards (Kawahara et al., 1984).

## 9. Conclusions

Research studies in the field of polysaccharides have revealed that pullulan is a unique polysaccharide with a variety of potential industrial and medical applications. Pullulan membranes/films are being used as coating and packaging materials for foods such as instant food seasonings, powdered tea and coffee. Pullulan-coated papers also decompose easily and do not contaminate the environment (Domań-Pytka & Bardowski, 2004). Pullulan production has been stable with its major applications in food for a number of years, but now-a-days it is also being used for formulating dietary capsules. Pullulan-based oral care products are also being commercialized. Despite of a large number of valuable applications, the major constraint prevailing on the use of pullulan is its cost, which is three times higher than the price of other polysaccharides such as dextran and xanthan. Engineering innovations or improved production strains, particularly with reduced melanin production could be beneficial to improve the economics of the production, thereby opening new avenues for pullulan utilization.

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