African Journal of Food Science and Technology (ISSN: 2141-5455) Vol. 4(3) pp. 57-63, March, 2013
Available Online http://www.interesjournals.org/AJFST

Full Length Research Paper

Pullulan: Production and usage in food ındustry

Pınar Oğuzhan and Filiz Yangılar

Ardahan University, The Faculty of Engineering, Food Engineering Department,
Ardahan, Turkey

Accepted March 12,213

Pullulan is one of such polymers synthesized by the yeast-like fungus *Aureobasidium pullulans*. Pullulan is a linear α-D-glucan built of maltotriose subunits, connected by (1-6)-α-D-glucosidic linkages. Pullulan is an important exopolysaccharide having applications in several industrial sectors like pharmaceutical, food and cosmetic industries. In addition to recently pullulan is also being investigated for its biomedical applications in various aspects like targeted drug and gene delivery, tissue engineering, wound healing and in diagnostic imaging using quantum dots. Pullulan is being used extensively in the food industry as a food ingredient for over 20 years in Japan, and has Generally Regarded As Safe (GRAS) status in the USA. Pullulan, which is generally materialized with microbial origin in food the production has widely usage as a coating agent in food formulations and packaging industry owing to its numerous properties. Despite the large number of uses, some of the problems associated with fermentative production of pullulan are (i) the formation of a melanin pigment; (ii) the inhibitory effects caused by high sugar concentrations in the medium; and (iii) the high cost associated with pullulan precipitation and recovery. In this review, general properties of pullulan, pullulan production, usage of pullulan in food industry were examined.

Keywords: Pullulan, Food industry, *Aureobasidium pullulan.*

INTRODUCTİON

Microbially produced polysaccharides have properties that are very useful in various industrial applications (Choudhury et al. 2011). A new fungal exopolysaccharides (EPSs) with intestering industrial properties are well known (Singh et al. 2008). Pullulan is an extracellular and neutral microbial polysaccharide produced by *Aureobasidium pullulans* in starch and sugar cultures (Cheng et al., 2010; Karim et al., 2009; Wu et al., 2012; Xiao et al., 2012 a,b). *A. pullulans* have five different cell morphologies like swollen blastospores, yeast-like cells, mycelia, chiamydospores and yound blastospores (Ronen et al., 2002; Sugumaran et al., 2013). *A. pullulans* is a black yeast or yeast-like fungus widely spread in all ecological niches, e.g., forest soils, fresh and sea water, plant, and animal tissues, etc. (Leathers, 2003; Wu et al., 2012). It is a linear mixed linkage α-D-glucan consisting mainly of maltotriose repeating units interconnected by α-(1→6) linkages. The regular alternation of α-(1→4) and α-(1→6) bonds results in structural flexibility and enhanced solubility (Bouveng et al., 1963; Catley, 1970; Chi and Zao, 2003; Gniewosz and Duszkiewicz-Reinhard, 2008; Goksungur et al., 2011; Jakovlijevic et al., 2001; Jiang et al., 2010, 2011; Leathers, 1993; McIntyre and Vogel, 1993; Roukas, 1998; Saha and Zeikus, 1989; Taguchi et al., 1973; Wallenfels et al., 1961; Wu et al., 2009a). Pullulan and its derivatives have numerous potential for food, pharmaceutical and other industrial applications (Chi and Zao, 2003; Deshpande et al., 1992; Gaur et al., 2010; Hannigan, 1984; Leathers 2003; Seviour et al., 1992; Shingel, 2004; Singh et al., 2008; Singh et al., 2010; Sutherland, 1998; Yuen, 1974). Pullulan can be used as coating and packaging material, sizing agent for paper, starch replacer in low-calorie food formulations, in cosmetics, lotions, shampoos and industrial applications (Deshpande et al., 1992; Leathers, 2003; Wu et al. 2009a). At present, the pullulan preparation process differs according to the intended application. For use as industrial adhesives, dispersants and coagulants, the fermentation broth is simply concentrated, dried and pulverized. For use in food applications, the culture is decolorized using activated charcoal, concentrated, dried and pulverized. As for pharmaceutical uses, the fermentation broth is purified by alcohol fractionation or

---

*Corresponding Author E-mail: pinaroguzhan@ardahan.edu.tr

membrane filtration, decolorized using activated charcoal followed by desalination, concentration, drying and pulverization (Thibault and LeDuy, 1999; Wu et al., 2009b). Pullulan is being used extensively in the food industry as a food ingredient for over 20 years in Japan, and has Generally Regarded As Safe (GRAS) status in the USA (Choudhury et al., 2012; USFDA, 2002). Pullulan which was earlier considered as an indigestible polymer was shown to be slowly digestible and found application as a low-calorie food additive providing bulk and texture (Choudhury et al., 2012; Wolf 2005). Recently pullulan is also being investigated for its biomedical applications in various aspects like targeted drug and gene delivery, tissue engineering, wound healing and in diagnostic imaging using quantum dots (Choudhury et al., 2012; Rekha and Sharma, 2007). Despite the large number of uses, some of the problems associated with fermentative production of pullulan are (i) the formation of a melanin pigment; (ii) the inhibitory effects caused by high sugar concentrations in the medium; and (iii) the high cost associated with pullulan precipitation and recovery (Choudhury et al., 2011; Youssef et al., 1999). Pullulan is widely used in high performance liquid chromatography (HPLC) columns and in size exclusion chromatography as a molecular mass standard (Buliga and Brant, 1987; Sugumaran et al., 2013). The main parameters which are important for the synthesis of pullulan, carbon source, nitrogen concentration, production environment pH, oxygen concentration and temperature. *A. pullulans* many sugar as a carbon source used by the cell growth and polysaccharide production (Ürküt, 2007). Another important factor is nitrogen in producing pullulan. Nitrogen source, usually ammonium ion (NH^+_4), plays a significant role in pullulan production. The depletion of nitrogen is regarded as a signal for exopolysaccharide formation of *A. pullulans* fermentation (Bulmer et al., 1987; Cheng et al., 2011; Gibbs and Seviour, 1996). Alternative nitrogen sources like urea, ammonium salts, etc. have been used with limited success for pullulan production (West and Reed-Hamer, 1991, 1994). The cost of pullulan production currently is relatively high and therefore, it is prudent to search for inexpensive carbon and nitrogen sources, which are nutritionally rich enough to support the growth of the microorganism as well as the production of pullullan (Wu et al., 2009a). There are various reports on the production of pullulan from different sources such as sweet potato (Wu et al., 2009a), soybean pomace (Seo et al., 2004), potato starch waste (Barnett et al., 1999), coconut by-products (Thirumavalavan et al., 2009), deproteinized whey (Roukas, 1999a), agro-industrial wastes such as grape skin pulp extract, starch waste, olive oil waste effluents and beet molasses (Israilides et al., 1994), brewery wastes (Roukas, 1999b), beet molasses (Roukas, 1998), jaggery which is a concentrated sugar cane juice (Vijayendra et al., 2001), carob pod (Roukas and Biliaderis, 1995) and Jerusalem artichoke (Goksungur et al., 2011; Shin et al., 1989). However, in those cases, the yield was not significantly high and hence the economics of the process was also not much favorable. Therefore, it is required to evaluate low cost substrates to develop a cost effective process. Agri-industrial residues are used as feed stock for production of different chemicals and biochemicals like ethanol, citric acid, lactic acid due to their easy availability of large quantity, low cost and high nutrient content (Leathers, 2003). Hence, it may also be possible to develop a cost-effective process for production of pullulan by using agri-industrial residues (Sharma et al., 2012).

Ürküt et al. (2007), reported that the production of pullulan from synetic medium by *A. pullulans* P56 immobilized in Ca-alginate beads was investigated using batch and repeated batch fermentation system. The highest pullulan concentration (19.52±0.37 g dm-3) was obtained with 2.0-2.4 mm beads prepared from 2% sodium alginate solution. Pullulan production was mainly accomplished by immobilized fungal cells since leaked cells in the fermentation medium comprised 17.4% of the total fungal population at the end of fermentation. The pullulan proportion was 84.5% of the total polysaccharide in the fermentation medium. Response surface methodology was used to investigate the effects of three fermentation parameters (initial pH, agitation speed and incubation time) on the concentration of pullulan. Results of the statistical analysis showed that the fit of the model was good in all cases. The maximum pullulan concentration of 21.07±0.48 g dm-3 was obtained at the optimum concentrations of process variables (pH 7.31, agitation speed 191.5 rpm, incubation time 101.2 h). The gel beads produced pullulan under the optimized conditions for six consecutive batch fermentations without marked activity loss and deformation.

West (2011), reported that the production of the exopolysaccharide pullulan using entrapped cells of the fungus *A. pullulans* ATCC 42023 was investigated relative to carbon source. Fungal cells grown on glucose or sucrose as a carbon source were entrapped in calcium alginate beads and found to be capable of synthesizing the polysaccharide for two production cycles. Using 2.5% glucose or sucrose as a carbon source, productivity was 18.3 or 21.9 mg polysaccharide/g cells×h, respectively after the initial production cycle and decreased to 9.6 or 8.5 mg polysaccharide/g cells×h, respectively, after the second production cycle. Independent of carbon source, the entrapped fungal cells exhibited a higher yield during the initial cycle than the second production cycle while the entrapped ATCC 42023 cells elaborated a polysaccharide with a higher pullulan content during the second production cycle compared to the initial production cycle.

Israilides et al. (1998), reported that ethanol-precipitated substances after fermentation of various agro-industrial wastes by *A. pullulans* were examined for their pullulan content. Grape skin pulp extract, starch waste, olive oil waste effluents and molasses served as substrates for the fermentation. A glucose-based defined medium was used for comparison purposes. Samples were analysed by an enzyme-coupled assay method and by high-performance anion-exchange chromatography with pulsed amperometric detection after enzymic hydrolysis with pullulanase. Fermentation of grape skin pulp extract gave 22.3 g l-1 ethanol precipitate, which was relatively pure pullulan (97.4% w/w) as assessed by the coupled-enzyme assay. Hydrolysed starch gave only 12.9 g l-1 ethanol precipitate, which increased to 30.8 g l−1 when the medium was supplemented with NH4NO3 and K2HPO4; this again was relatively pure pullulan (88.6% w/w). Molasses and olive oil wastes produced heterogeneous ethanol-precipitated substances containing small amounts of pullulan, even when supplemented with nitrogen and phosphate. Overall, grape skin pulp should be considered as the best substrate for pullulan production. Starch waste requires several hydrolyis steps to provide a usable carbon source, which reduces its economic attraction as an industrial process.

Wu et al. (2009a), reported that a strain *A. pullulans* AP329, was used for the production of pullulan by employing hydrolysed sweet potato as cultivation media. Hydrolysis with a-amylase alone resulted in the lowest yields of pullulan. In contrast continuous hydrolysis with pullulanase and the b-amylase in sweet potato itself gave higher yields, but prolonged hydrolysis with amyloglucosidase decreased the yield. The maximum pullulan yield (29.43 g/l) was achieved at the dextrose equivalent value of 45 and pH of 5.5 for 96 h. As a substitute of sucrose, hydrolysed sweet potato was found to be hopeful and the yield of pullulan was higher than that of glucose and sucrose. The molecular weight of pullulan obtained from hydrolysed sweet potato media was much higher than that of sucrose and glucose media. Results of this work indicated that sweet potato was a promising substrate for the economical production of pullulan.

Goksungur et al. (2011), reported that the production of pullulan from hydrolysed potato starch waste by *A. pullulans* P56 wasinvestigated. The liquefaction of potato starch was done by Ca-alginate immobilized amyloglucosidase and pullulanase enzymes in a packed bed bioreactor. Various organic nitrogen sources were tested and none of the nitrogen sources gave pullulan concentrations as high as that obtained with yeast extract. Response surface methodology was used to investigate the effects of three factors (incubation time, initial substrate concentration and initial pH) on the concentration of pullulan in batch cultures of *A. pullulans*. No previous work has used statistical analysis on the optimization of process parameters in pullulan production from hydrolysed potato starch waste. Maximum pullulan concentration of 19.2 g/l was obtained at the optimum levels of process variables (incubation time 111.8 h, initial substrate concentration 79.4 g/l, initial pH 7.26). The optimization led to a 20% increase in pullulan concentration.

Features and Structure of Pullulan

Pullulanase (EC 3.2.1.41, pullulan 6-glucanohydrolase) is a debranching enzyme which hydrolyses the a-1,6-glucosidic linkages in pullulan and other amylaceous polysaccharides, and belong to a family of 13 glycosyl hydrolases, also called the a-amylase family (Janecek et al., 1997; Matzke et al., 2000; Singh et al., 2010). Pullulanases are widely distributed among animals, plants, fungi and bacteria. Detailed enzymatic mechanisms of substrate degradation and the resulting final products are different in each case (Doman´-Pytka and Bardowski, 2004; Singh et al., 2010). The average molecular weight of pullulan is the range from $5,3x10^{3}$-$2x10^{6}$ depending on the strain used and the culture medium (Ürküt, 2007). Pullulan produces a high viscosity solution at a relatively low concentration and can be used for oxygenimpermeable films and fibers, thickening or extending agents, or adhesive or encapsulating agents (Ma et al., 2012; Mcneil and Kristiansen, 1990; Singh et al., 2008). Despite being a α-D-glucan, pullulan is resistant to α-D-amylolysis and may be used in low-calorie food formulation. The chemical formula of pullulan is $(C_6H_{10}O_5).H_2O$ (Gaur et al., 2010).

Pullulan is produced by *A. pullulans* and some other microorganisms in Table 1.Bauer (1938) made early observations on extracellular polymer formation by *A. pullulans,* and Bernier (1958) isolated and began to characterize the polysaccharide. Bender et al. (1959) studied the novel glucan and named it "pullulan." During the 1960s, the basic structure of pullulan was resolved (Bouveng et al., 1962, 1963; Sowa et al., 1963; Ueda et al., 1963; Wallenfels et al., 1961, 1965). Bender and Wallenfels (1961) discovered the enzyme pullanase, which specifically hydrolyzes the α(1~6) linkages in pullulan and converts the polysaccharide almost quantitatively to maltotriose (Leathers, 2003). On this basis pullulan is commonly viewed as an α(1~6) linked polymer of maltotriose subunits (Figure 1). (Yatmaz and Turhan, 2012). Pullulan undergo enzymatic hydrolysis by both (1-6)-α-D-and (1-4)--D-pullulanases. The (1-6)-α-D-pullulanases cleave the (1-6)-α-D-glucopyranosidic linkages. Complete hydrolysis of pullulan using (1-6)-α-D-pullulanase yields maltotriose as major product along with traces of maltotetraose. The (1-4)-α-D-pullulanases act on (1-4)-α-D-glucosidic linkages at their reducing ends adjacent to (1-6)-α-D linkages. Complete hydrolysis of pullulan with (1-4)-α-D-pullulanase gives isopanose as the main product. Products of enzymatic pullulan degrad-

Table 1. Microbial sources of Pullulan

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

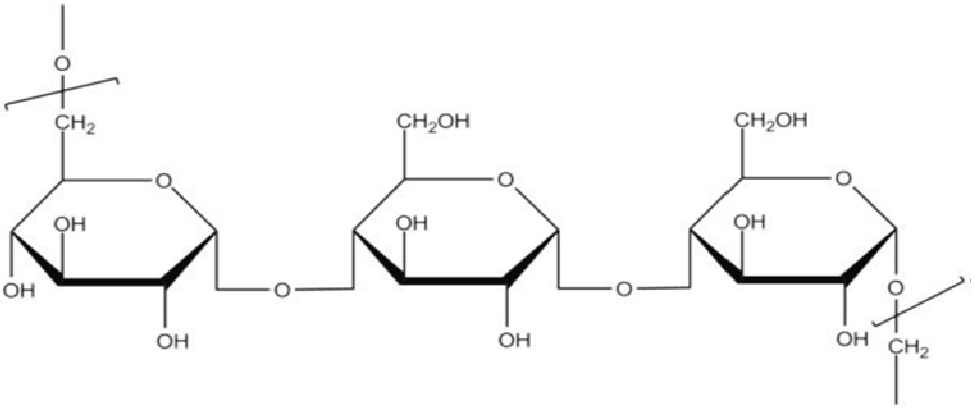

Figure 1. Chemical structure of pullulan

ation are used in food and pharmaceutical industry (Singh et al., 2010).

Pullulan's solubility can be controlled or provided with reactive groups by chemical derivatization. Due to its excellent properties, pullulan is used as a low-calorie ingredient in foods, gelling agent, coating and packaging material for food and drugs, binder for fertilizers and as an oxidation-prevention agent for tablets. Other applications include contact lenses manufacturing, biodegradable foil, plywood, water solubility enhancer and for enhanced oil recovery (Israilides et al., 1998; Leathers, 2003; Schuster et al., 1993). It 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 2. (Karim et al., 2009; Singh et al., 2008).

Utilization of Pullulan in Food Industry

Pullulan can be used effectively edible films owing to both food ingredient and film-forming ability thanks to its features (Yatmaz and Turhan, 2012). Pullulan can be used as coating of food and packaging material and starch replacer in low-calorie food formulations in food ındustry. Also can be used as spice and flavoring in seasoning agent for the microencapsulated (Israilides, 1994, 1998; Ürküt 2007). Pullulan is widely used in soups, sauces and beverages thanks to intensifier properties (Yatmaz and Turhan, 2012). It can also be used to stabilize the quality and texture of mayonnaise (Singh et al., 2008). Pullulan can be used as a denture adhesive, a binder and stabilizer in food pastes, and to adhere nuts to cookies (Leathers, 2003). Pullulan can be employed as a binder for tobacco (Miyaka, 1979), seed coatings and plant fertilizers (Matsunaga et al., 1977, 1978; Singh et al., 2008). Pullulan, can be used

Table 2. Main quality parameters of pullulan

Parameter	Specification
Appearance	White or yellowish-white powder
Water solubility (25°C)	Easily soluble
Specific optical activity $[\alpha]$ D_2O (1% in water)	Min. $+160^o$
Polypeptidies (%)	Max. 0.5
pH of solutions	5-7
Mineral residue-ash (sulphated, %)	Max.3
Moisture (loss on drying, %)	Max.6
Molecular weight (range, kDa)	100-250

appropriately in the production of low-calorie (diet) foods due to its take place in GRAS list and the slow digestion (Yatmaz and Turhan, 2012). Underivatized films are readily dissolved in water, thus having property to melt in the mouth as edible food coatings (Conca and Yang, 1993; Singh et al., 2008). 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 and De Mulder-Johnston, 1997; Singh et al., 2008). 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 (Leathers, 2003; Singh et al., 2008; Yamaguchi and Sunamoto, 1991).

Singh et al. (2010), reported that maltotriose syrup can be produced by enzymatic hydrolysis of the polysaccharide ‘pullulan’ using the debranching enzyme, pullulanase. This syrup possess many excellent properties as low freezing point depression, mild sweetness, keeps in moisture, prevention of retrogradation of starch in foodstuffs, less color formation compared with maltose syrups, glucose syrups or sucrose, good heat stability, low solution pullulan obtained was used for preparing maltotriose syrup using pullulanse. These properties are useful in food industries (Zoebelein and Böllert, 2001).

CONCLUSİON

Pullulan is a unique polysaccharide with a multitude of demonstrated practical applications. Pullulan has widely usage owing to its unique properties. 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. Technical improvements in pullulan production, such as engineering innovations or improved production strains, could reduce the cost of production.

REFERENCES

Barnett C, Smith A, Scanlon B, Israilides CJ (1999). Pullulan production by *Aureobasidium pullulans* growing on hydrolysed potato starch waste. Carbohydr Polymer. 38:203-209.

Bauer R (1938). Physiology of Dematium pullulans de Bary. Zentralbl Bacteriol Parasitenkd Infektionskr Hyg Abt 2, 98:133-167.

Bender H, Lehmann J, Wallenfels K (1959). Pullulan, an extracellular glucan from Pullularia pullulans. Biochem Biophys Acta. 36:309-316.

Bender H, Wallenfels K (1961). Investigations on pullulan. II. Specific degradation by means of a bacterial enzyme. Biochem Z. 334: 79-95.

Bernier B (1958). The production of polysaccharides by fungi active in the decomposition of wood and forest litter. Can J Microbiol. 4:195-204.

Bouveng HO, Kiessling H, Lindberg B, McKay J (1962). Polysaccharides elaborated by *Pullularia pullulans.* 1. The neutral glucan synthesised from sucrose solutions. Acta Chern Scand. 16:615-622.

Bouveng HO, Kiessling H, Lindlberg B, McKay J (1963). Polysaccharides elaborated by Pullularia pullulans. Acta Chem Scand. 17:797-800.

Buliga GS, Brant DA (1987). Temperature and molecular weight dependence of the unperturbed dimensions of aqueous pullulan. Int J Biol Macromolec. 9:71-76.

Bulmer MA, Catley BJ, Kelly PJ (1987). The effect of ammonium ions and pH on the elaboration of the fungal extracellular polysaccharide, pullulan, by *Aureobasidium pullulans.* Appl. Microbiol. Biotechnol. 25(4):362-365.

Catley BJ (1970). Pullulan: a relationship between molecular weight and fine structure. FEBS Lett. 10: 190-193.

Cheng KC, Demirci A, Catchmark JM, Puri VM (2010). Modeling of pullulan fermentation by using a color variant strain of *Aureobasidium pullulans.* J. Food Eng. 98:353-359.

Cheng KC, Demirci A, Catchmark JM, Puri VM (2011). Effects of initial ammonium ion concentration on pullulan production by *Aureobasidium pullulans* and its modeling. J. Food Eng. 103:115-122.

Chi Z, Zhao S (2003). Optimization of medium and cultivation conditions for pullulan production by a new pullulan-producing yeast strain. Enzym Microb Tech. 33:206-211.

Choudhury AR, Saluj P, Prasa GS (2011). Pullulan production by an osmotolerant *Aureobasidium pullulans* RBF-4A3 isolated from flowers of *Caesulia axillaris.* Carbohydr Polymer. 83:1547-1552.

Choudhury AR, Bhattacharyya MS, Prasad GS (2012). Application of response surface methodology to understand the interaction of media components during pullulan production by *Aureobasidium pullulans* RBF-4A3. Biocatalysts Agr Biotechnol. 1:232-237.

Conca KR, Yang TCS (1993). Edible food barrier coatings. In C. Ching, D. L. Kaplan, EL, Thomas (Eds.), Biodegradable polymers and packaging. Lancaster: Technomic; 357-369.

Cooke WB (1959). An ecological life history of *Aureobasidium pullulans* (de Bary) Arnaud. Mycopathologia. 12: 1-45.

Corsaro MM, De Castro C, Evidente A, Lanzetta R, Molinaro A, Parilli M, Sparapano L (1998). Phytotoxic extracellular polysaccharide fractions from *Cryphonectria parasitica* (Murr) Barr strains. Carbohydr Polymer. 37:167-172.

Delben F, Forabosco A, Guerrini M, Liut G, Torri G (2006). Pullulans produced by strains of *Cryphonectria parasitica*-II. Nuclear magnetic resonance evidence. Carbohydr Polymer. 63:545-554.

Deshpande MS, Rale VB, Lynch JM (1992). *Aureobasidium pullulans* in applied microbiology: A status report. Enzym Microb Tech. 14:514-527.

Doman´-Pytka M, Bardowski J (2004). Pullulan degrading enzymes of bacterial origin. Crit Rev Microbiol. 30:107-121.

Fraser CG, Jennings HJ (1971). A glucan from *Tremella mesenterica* NRRL-Y6158. Can J Chem. 49:1804-1807.

Forabosco A, Bruno G, Sparapano L, Liut G, Marino D, Delben F (2006). Pullulans produced by strains of *Cryphonectria parasitica*-I. Production and characterization of the exopolysaccharides. Carbohydr Polymer. 63:535-544.

Gaur R, Singh R, Gupta M, Gaur MK (2010). *Aureobasidium pullulans*, an economically important polymorphic yeast with special reference to pullulan. Afr. J. Biotechol. 9 (47):7989-7997.

Gibbs PA, Seviour RJ (1996). Does the agitation rate and/or oxygen saturation influence exopolysaccharide production by *Aureobasidium pullulans* in batch? Appl. Biochem. Biotechnol. 46 (5-6): 503-510.

Gniewosz M, Duszkiewicz-Reinhard W (2008). Comparative studies on pullulan synthesis, melanin synthesis and morphology of white mutant *Aureobasidium pullulans* B-1 and parent strain A.p.-3. Carbohydr Polymer. 72:431-438.

Goksungur Y, Uzunoğulları P, Dağbağlı S (2011). Optimization of pullulan production from hydrolysed potato starch waste by response surface methodology. Carbohydr Polymer. 83:1330-1337.

Hannigan K (1984). Edible plastic. Food Eng. 56: 98-99.

Israilides C, Scanlon B, Smith A, Harding SE, Jumel K (1994). Characterization of pullulans produced from agro-industrial wastes. Carbohydr Polymer. 25(3):203-209.

Israilides CJ, Smith A, Harthill JE, Barnett C, Bambalov G, Scanlon B (1998). Pullulan content of the ethanol precipitate from fermented agro-industrial wastes. Appl. Microbiol. Biotechnol, 49(5):613-617.

Jakovljevic D, Vrvic MM, Radulovic M, Hranissavljevic-Jakovljevic M (2001). Fine structural analysis of the fungal polysaccharide pullulan elaborated by *Aureobasidium pullulans,* CH-1 strain. J. Serb Chem. Soc. 66 (6):377-383.

Janecek S, Svensson B, Henrissat B (1997). Domain evolution in the a-amylase family. J. Mol. Evol. 45: 322-331.

Jiang L (2010). Optimization of fermentation conditions for pullulan production by *Aureobasidium pullulan* using response surface methodology. Carbohydr Polymer. 79:414-417.

Jiang L, Wu S, Kim JM (2011). Effect of different nitrogen sources on activities of UDPG-pyrophosphorylase involved in pullulan synthesis and pullulan production by *Aureobasidium pullulans*. Carbohydr Polymer. 86:1085-1088.

Karim MR, Lee HW, Kim R, Ji BC, Cho JW, SonWT, Oh W, Yeuma JH (2009). Preparation and characterization of electrospun pullulan/montmorillonite nanofiber mats in aqueous solution. Carbohydr Polymer. 78:336-342.

Krochta JM, De Mulder-Johnston C (1997). Edible and biodegradable polymer films: Challenges and opportunities. Food Technol. 51:61-74.

Leathers TD (1993). Substrate regulation and specificity of amylases from *Aureobasidium strain* NRRL Y-12, 974. FEMS Microbiol Lett. 110:217-221.

Leathers TD (2003). Biotechnological production and applications of pullulan. Appl. Microbiol. Biotechnol. 62:468-473.

Ma J, Jiang G, Yao S, Jin H, Wang C (2012). Studies on the optimal culture conditions of *Aureobasidium pullulans* to produce exopolysaccharides. J Biomed Sci Eng. 5:203-207.

Matsunaga H, Fujimura S, Namioka H, Tsuji K, Watanabe M (1977). Fertilizer composition. US Patent Office, Pat. No. 4 045 204.

Matsunaga H, Tsuji K,Watanabe M (1978). Coated seed containing pullulan-based resin used as binder. US Patent Office, Pat. No. 4 067 141.

Matzke J, Herrmann A, Schneider E, Bakker EP (2000). Gene cloning, nucleotide sequence and biochemical properties of a cytoplasmic cyclomaltodextrinase (neopullulanase) from *Alicyclobacillus acidocaldarius,* reclassification of a group of enzymes. FEMS Microbiol Lett. 183: 55-61.

McIntyre DD, Vogel HJ (1993). Structural studies of pullulan by nuclear magnetic resonance spectroscopy. Starch. 45:406-410.

McNeil B, Kristiansen B (1990). Temperature effects on polysaccharide formation by *Aureobasidium pullulans* in stirred tanks. Enzyme Microb Technol. 12: 521-526.

Miyaka T (1979). Shaped matters of tobaccos and process for preparing the same. Canadian Patent Office, Pat. No. 1 049 245.

Oliva EM, Cirelli AF, De Lederkremer RM (1986). Characterization of a pullulan *in Cyttaria darwinii*. Carbohydr Res. 158: 262-267.

Reis RA, Tischer CA, Gorrin PAJ, Iacomini M (2002). A new pullulan and a branched (123)-(126)-linked b-glucan from the lichenised ascomycete *Teloschistes flavicans*. FEMS Microbiol Lett. 210: 1-5.

Rekha MR, Sharma CP (2007). Pullulan as a promising biomaterial for biomedical applications: a perspective. TrendsBiomater. Artif. Organs 20:116-121.

Ronen M, Guterman H, Shabtai Y (2002). Monitoring and control of pullulan production using vision sensor. J. Biochem. Biophys. Meth. 3:243-249.

Roukas T, Biliaderis CG (1995). Evaluation of carob pod as a substrate for pullulan production by *Aureobasidium pullulans.* Appl. Biochem. Biotechnol. 55:27-44.

Roukas T (1998). Pretreatment of beet molasses to increase pullulan production. Process Biochem. 33:805-810.

Roukas T (1999a). Pullulan production from deproteinized whey by *Aureobasidium pullulans.* J Ind Microbiol Biotechnol. 22: 617-621.

Roukas T (1999b). Pullulan production from brewery wastes by *Aureobasidium pullulans.* World J Ind Microbiol Biotechnol. 15: 447-450.

Saha BC, Zeikus JG (1989). Novel highly thermostable pullulanase from thermophiles. Trends in Biotech. 7: 234-239.

Seo HP, Son CW, Chung CH, Jung D, Kim SK, Gross RA (2004). Production of high molecular weight pullulan by *Aureobasidium pullulans* HP 2001 with soybean pomace as a nitrogen source. Bioresource Technol. 95: 293-299.

Seviour RJ, Stasinopouslos SJ, Auer DPF, Gibbs PA (1992). Production of pullulan and other exopolysaccharides by filamentous fungi. Crit Rev Biotechnol. 12: 279-298.

Schuster R, Wenzig E, Mersmann A (1993). Production of the fungal exopolysaccharide pullulan by batch-wise and continuous fermentation. Appl Microbiol Biotechnol. 39:155-158.

Sharma N, Prasad GS, Choudhury AR (2012). Utilization of corn steep liquor for biosynthesis of pullulan, an important exopolysaccharide. Carbohydr Polymer (In press).

Shin YC, Kim YH, Lee HS, Cho SJ, Byun SM (1989). Production of exopolysaccharide pullulan from inulin by a mixed culture of *Aureobasidium pullulans* and *Kluyveromyces fragilis*. Biotechnol Bioeng. 33: 129-133.

Shingel KI (2004). Current knowledge on biosynthesis, biological activity, and chemical modification of the exopolysaccharide, pullulan. Carbohydr Polymer. 339:447-460.

Singh RS, Saini GK, Kennedy JF (2008). Pullulan: Microbial sources, production and applications. Carbohydr Polymer. 73:515-531.

Singh RS, Saini GK, Kennedy JF (2010). Maltotriose syrup preparation from pullulan using pullulanase. Carbohydr Polymer. 80:401-407.

Sowa W, Blackwood AC, Adams GA (1963). Neutral extracellular glucan of *Pullularia pullulans* (de Bary) Berkhout. Can J Chern. 41:2314-2319.

Sugumaran KR, Gowthami E, Swathi B, Elakkiya S, Srivastava SN, Ravikumar R, Gowdhaman D, Ponnusami V (2013). Production of pullulan by *Aureobasidium pullulans* from Asian palm kernel: A novel substrate. Carbohydr Polymer. 92:697-703.

Sutherland LW (1998). Novel and established application of microbial polysaccharide. Trends. Biotechnol. 16:41-46.

Taguchi R, Kikuchi M, Sakano Y, Sakuma M, Kobayashi T (1973). Structural uniformity of pullulan produced by several strains of *Pullularia pullulans*. Agr Biol Chem. 37(7): 1583-1588.

Thibault J, LeDuy A (1999). Pullulan, microbial production methods. In MC. Flickringer and SW, Drew (Eds.). Encyclopedia of bioprocess technology, fermentation, biocatalysis and bioseperation 4:2232-2247). New York: Wiley.

Thirumavalavan K, Manikkadan TR, Dhanasekar R (2009). Pullulan production from coconut by-products by *Aureobasidium pullulans.* Afr J Biotechnol. 8: 254-258.

Ueda S, Fujita K, Komatsu K, Nakashima Z (1963). Polysaccharide produced by the genus *Pullularia.* I. Production of polysaccharide by growing cells. Appl Microbiol. 11: 211-215.

USFDA (2002). Agency Response Letter: GRAS Notice No. GRN 000099 [Pullulan]. US Food and Drug Administration (US FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety; College Park, Maryland.

Ürküt Z (2007). Optimization of pullulan production using-Ca-alginate Immobilized *Aureobasidium pullulans* P56. Ege University Institute of Science. Master Thesis, pp 125, Bornova-İzmir, Turkey.

Ürküt Z, Dagbaglı S, Goksungur Y (2007). Optimization of pullulan production using-Ca-alginate Immobilized *Aureobasidium pullulans* by response surface methodology. J Chem Tech Biotechnol. 82 (9): 837-846.

Vijayendra SVN, Bansal D, Prasad MS, Nand K (2001). Jaggery: A novel substrate for pullulan production by *Aureobasidium pullulans* CFR-77. Process Biochem. 37: 359-364.

Waksman N, De Lederkremer RM, Cerezo AS (1977). The structure of an a-D-glucan from *Cyttaria harioti* Fischer. Carbohydr Res. 59:505-515.

Wallenfels K, Bender H, Keilich G, Bechtler G (1961). On pullulan, the glucan of the slime coat of *Pullularia pullulans*. Angew Chem. 73:245-246.

Wallenfels K, Keilich G, Bechtler G, Freudenberger D (1965). Investigations on pullulan. IV. Resolution of structural problems using physical, chemical and enzymatic methods. Biochem Z. 341: 433-450.

West TP, Reed-Hamer B (1991). Ability of *Aureobasidium pullulans* to syn-thesize pullulan upon selected sources of carbon and nitrogen. Microbios. 67: 117-124.

West TP, Reed-Hamer B (1994). Elevated polysaccharide production by mutants of the fungus Aureobasidium pullulans. FEMS Microbiology Letters. 124:167-172.

West TM (2011). Effect of carbon source on polysaccharide production by alginate-entrapped *Aureobasidium pullulans* ATTC 42023 cells. J Basic Microbiol. 51 (6): 673-677.

Wu S, Jin Z, Tong Q, Chen H (2009a). Sweet potato: A novel substrate for pullulan production by *Aureobasidium pullulans.* Carbohydr Polymer. 76:645-649.

Wu S, Jin Z, Kim JM, Tong Q, Chen H (2009b). Downstream processing of pullulan from fermentation broth. Carbohydr Polymer. 77:750-753.

Wu S, Chen J, Pan S (2012). Optimization of fermentation conditions for the production of pullulan by a new strain of *Aureobasidium pullulans* isolated from sea mud and its characterization. Carbohydr Polymer. 87:1696-1700.

Wolf BW (2005). Use of pullulan as a slowly digested carbonhydrate. United States patent no. 6916796.

Xiao Q, Lim LT, Tong Q (2012a). Properties of pullulan-based blend films as affected by alginate content and relative humidity. Carbohydr Polymer. 87:227-234.

Xiao Q, Tong Q, Lim LT (2012b). Pullulan-sodium alginate based edible films: Rheological properties of film forming solutions. Carbohydr Polymer. 87:1689-1695.

Yamaguchi S, Sunamoto J (1991). Fatty emulsion stabilized by a polysaccharide derivative. US Patent Office, Pat; 4:997, 819.

Yatmaz E, Turhan İ (2012). Pullulan production by fermentation and usage in food industry. Food. 37(2): 95-102.

Youssef F, Roukas T, Biliaderis CG (1999). Pullulan production by a nonpigmented strain of *Aureobasidium pullulans* using batch and fed batch culture. Process Biochem. 34: 355-366.

Yuen S (1974). Pullulan and its applications. Process Biochem. 22:7-9.

Zoebelein H, Böllert V (2001). Dictionary of renewable resources. USA: Wiley-VCH.