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International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.5, pp 2769-2773, Aug-Sept 2014

A Review on Development of Fermentative Production of Curdlan

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Abstract: Curdlan, a linear polymer with β- 1, 3 glucan is a commercial bacterial exopolysaccharide have immense application in different fields. This review focuses on the biosynthesis of curdlan and effect of nutritional factors and key operational parameters in the production of curdlan. The effect of carbon, nitrogen, phosphate sources and role of pH and oxygen supply are discussed.
Keywords: Curdlan, UDP glucose, exopolysaccharide, dissolved oxygen.

Introduction

Polysacharides are generally produced in response to environmental stresses. They are obtained from bacteria, yeast, mould and algae. Polysaccharides derived from such microorganisms may be either repeating units of single monosaccharide (homopolysaccharide) or a number of different monosaccharide (hetero polysaccharide). Most of these polysaccharides have bacterial origin and are extensively used for food and industrial applications because of its rheological and gelling properties (1). Bacterial exopolysaccharides (EPS) xanthan, curdlan and gellan were approved by Food and Drug Administration (FDA), United States (2). In this paper, we review the biosynthesis of curdlan and biotechnological aspects to improve the production of curdlan.

Curdlan is a homopolymer with linear chain of D-glucose having β-1, 3 glucosidic linkages. It is produced by certain strains of *Agrobacterium radiobacter* or *Alcaligenes faecalis* var myxogenes (3). It is an edible, biodegradable and non-toxic polymer which is insoluble in water and alcohols and soluble in sodium hydroxide and dimethylsulphoxide (DMSO). Curdlan is used in food industry to enhance thermal stability and to develop better texture. It is used as a stabilizer in many frozen and packaged foods, sauces and noodles (1,4). Curdlan also have widespread pharmaceutical application. It is used either directly or as a derivative in drug formulation and treatment against malaria, anti-acquired immunodeficiency syndrome, thrombosis, etc. It is also used as protein drug delivery vehicles and as immune stimulator (1,4). Some of its industrial applications include adsorption of heavy metals and admixture to concrete (5).

Biosynthesis of Curdlan

Curdlan biosynthesis can be divided into three stages substrate uptake, metabolisim and polymerization (6). The substrate, mainly glucose enters cell's cytoplasm by active transport. During the second stage the

substrate is catabolized to form primary metablites and precursors for EPS synthesis. Glycolysis of glucose starts with phosphorylation with hexokinase enzyme in expense of adenosine triphosphate (ATP) to form glucose-6-phosphate. Then convertion glucose-6-phosphate into glucose-1-phosphate takes place by phosphoglucomutase. Subsequently the key precursor, Uridine-diphosphate-glucose (UDP glucose) is formed by catalysis of UDP-glucose phosphorylase from uridine triphosphate (UTP). Glucosyl 1 phosphate from UDP glucose containing D glucose was attached to lipid precursor (isoprenoid lipid phosphate)releasing UDP. This initiates polymerization with β- 1, 3 glucosidic linkage and the polymer is released from the cell after chain elongation. Further UDP kinase convert UDP into UTP utilizing ATP from tricarboxylic acid cycle or glycosis and the cyclic process continues synthesizing curdlan (7).

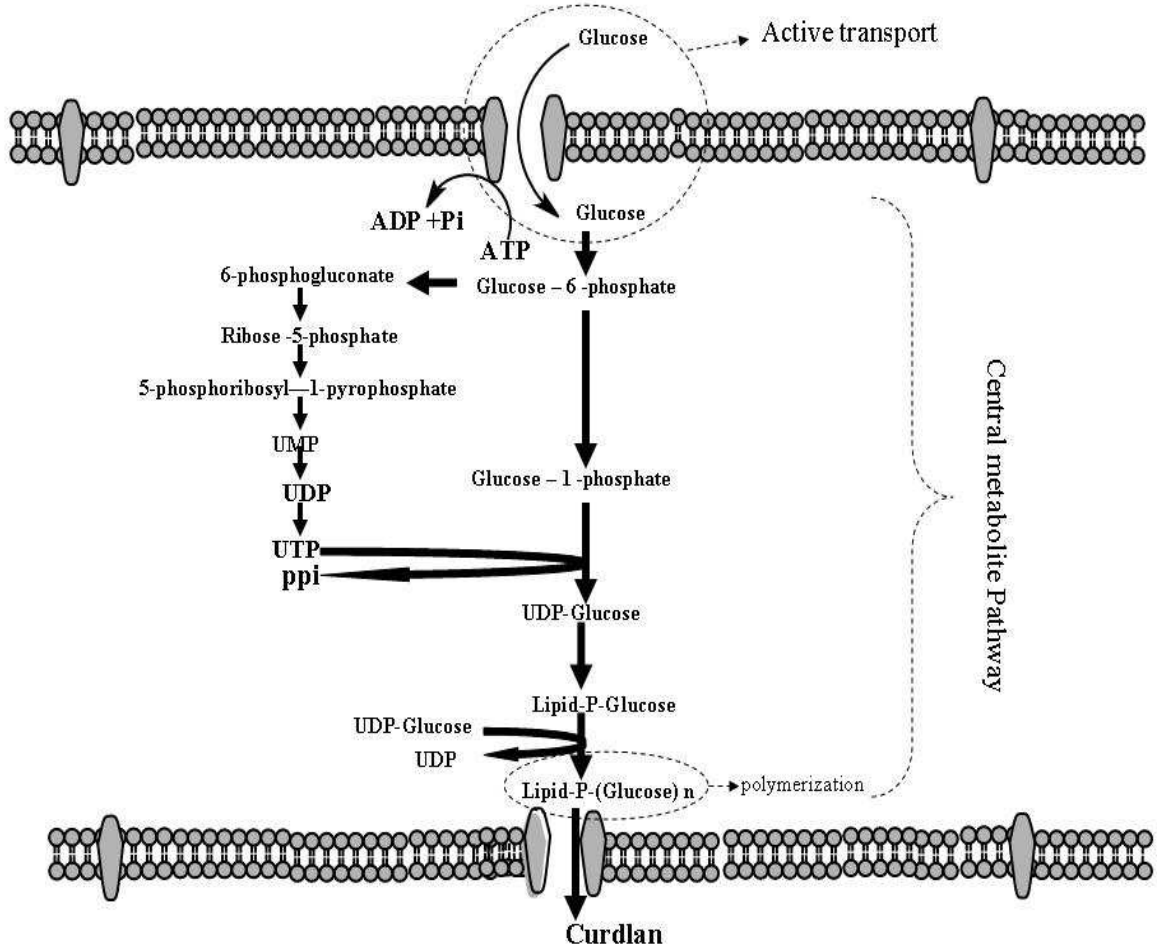

Fig.1 Biosynthestic pathway for curdlan synthesis

Fermentative Production of Curdlan

Exceptional physical properties and wide range of applications have increased the demand for curdlan in market. In order to manage the necessities different approaches were adapted both by industrial and academic research and development group to improve the production of curdlan. Curdlan is a secondary metabolite and it is produced during post-stationary phase (8). Variation in the operation parameters and nutritional factors were investigated during pre and post- stationary phases to enhance the production of curdlan both. The nutritional factors include carbon, nitrogen and phosphate sources and the operational parameters include pH, dissolved oxygen concentration and agitation speed.

Effect of Carbon Source

Carbon sources enhance the biomass development from the initial growth phase (9). Until 1997, carbon is the sole carbon source for the production of curdlan with maximum productivity of 0.2 $gL^{-1}h^{-1}$(9). But in a particular study it was reported that the production of curdlan was high when the glucose concentration is increased whereas the conversion rate was found to be less (10). Lee, I.Y. et al. (1997) investigated that Agrobacterium species gave high yield of product with sucrose and maltose as the carbon source. Sucrose

yielded the maximum productivity of curdlan at the rate of 0.5 $gL^{-1}h^{-1}$ (9). Even though maltose and sucrose are found to be excellent carbon source for the production of curdlan now researches are being carried out to produce curdlan from low cost substrates such as sugarcane or sugar beet molasses(9). Fermentation has been carried out for five days where yield of about 42g/L was obtained when molasses has been used as the carbon source. Recent reports suggest that other agricultural rich in cellulose, hemicellulose, starch and lignin were pretreated and used as substitute for production of exopolysaccharides. Hence low cost agricultural waste can be used the whole carbon source to lower the cost of the product (11).

Effect of Nitrogen Source

As discussed earlier the production of curdlan was found to be high during post stationary phase and this is associated with nitrogen depleting condition. When initial carbon source was increased, nitrogen in the media were utilized by the organism for biomass formation and during later stage of growth with nitrogen limiting condition, the yield of curdlan was found to increase (Nakanishi et al. 1992). Different nitrogen sources namely ammonium chloride, sodium nitrate, urea, and yeast extract were used for improving the production of curdlan.

Jung et al reported the production of curdlan in a mineral salt medium to maintain favorable nutrients and pH required for the maximized production of the polysaccharide. The effect of yeast extract in the mineral salt medium was analyzed and the study shows that the addition of yeast extract in to the mineral salt medium did not promote the production of curdlan even though it had a positive effect on the growth of cells. It was concluded that the presence of nitrogen containing nutrients in the yeast extract has decreased the production of curdlan which is produced mainly in nitrogen depleted conditions. In the study of the influence of ammonium chloride in the mineral salt medium, it was found that addition of ammonium chloride did not favor the production of curdlan when compared to the production carried out in the absence of ammonium chloride (10). Different species exhibits distinct production of curdlan, some species produce maximal production of curdlan during the exponential phase where rapid utilization of nutrient occurs while others produce the polysaccharide when there is a depletion of nutrients especially nitrogen source. Intercellular concentration of AMP and UMP are direct indicators of the production of curdlan. In the depleted condition of nitrogen source, the AMP and UMP levels are found to be less and the production of curdlan is enhanced (10).

Among different nitrogen sources studied, ammonium chloride was found to produce maximum yield of curdlan followed by ammonium acetate, ammonium citrate and ammonium sulphate whereas sodium nitrate and potassium nitrate had less effect on the production of curdlan(12). Recent report suggested that ammonium chloride and urea were effective in enhancing curdlan production. Jiang et al. reported that 0.3 g N L^{-1} curdlan production was achieved in 4 days with urea supplemented media and 0.25 g N L^{-1} productivity was obtained with ammonium chloride in 5 days (Jiang et al. 2013).

Effect of Phosphate

Optimal phosphate concentration is also an efficient factor for the production of curdlan. Phosphate plays a vital role in the cell growth and product formation. Farres et al., 1977 reported that phosphate concentration lowers the yield of the polysaccharide using the Klebsilla strain whereas Conti et al., 1994 proved that phosphate concentration increases the yield of the product in Pseudomonas strains. Kim et al investigated the effect of phosphate concentration in the production of curdlan, increasing the phosphate concentration from 0 to 0.1 g/L, caused a linear increase in curdlan production whereas further increase in phosphate concentration negatively affected the production process. Phosphate concentration was also found to be independent of cell density in the media. It was reported that under nitrogen depleted condition, a residual phosphate concentration is found which is essential for maximal curdlan production and in the complete absence of phosphate concentration the curdlan production was found to be low. (13). Phosphate concentration in the range of 0.1 to 0.5 g/L was considered to be residual concentration. Curdlan production can be improved by increasing the phosphate concentration According to Kim et al. curdlan production increased from 0.44 to 2.8 g/L with phosphate concentration of 0.42 to 1.68 g/L.

Effect on Oxygen Supply

As bacterial strains involved in the production of curdlan are aerobic, there is a high requirement in the dissolved oxygen concentration in the fermentation media. It was observed that when the culture volume was increased, the cell density and polymer concentration decreased which shows the necessity of oxygen transfer rate in the media. Aeration and agitation has a profound effect in increase in the production of curdlan, agitation

at 600 rpm found to favor the production of curdlan and further increase in the rpm did not produce any significant change in the production process(5).

The fermentative broth of curdlan has low viscosity and hence there is resistance to oxygen transfer from gas to liquid. Dissolved oxygen concentration played a vital role in the maximal yield of curdlan as the polymer surrounding the cell mass offer resistance to oxygen transfer from liquid to cell. It was reported that the yield varies according to the design of the fermentor (Lawford et al., 1986). Lawford and their coworkers investigated that radial flow impeller gave better yield of curdlan compared to that of the flat blade and axial flow impeller. As radial flow impeller provides high oxygen transfer rate better yield of the product can be synthesized using this impeller. Thus these results prove that high oxygen transfer rate can be obtained in low shear designed sparging devices. (14).

Effect on pH

pH is one of the most efficient factor as it induces the cell growth as well as enhances the better yield of the product. The fermentation broth of the polymer is highly viscous and this can be rectified by using the acidic pH as it is insoluble. The optimal pH for the polysaccharide production can be obtained in two phases. Lee, et al. (1999) attempted to determine the optimal pH for the synthesis of curdlan in the batch production using Agrobacterium species. The initial pH of production media was kept neutral whereas pH was altered to 5.5 during the fermentation process. Lee and coworkers obtained increased curdlan production up to 64g/L at pH 5.5. They concluded that the fermentation of curdlan require two optimal pH conditions, one for the growth of micro organism and the other for the production of curdlan (15).

Curdlan production rate is found to have a positive effect when the pH of the medium is about 6 and the glucose conversion rate is found to be about 50%.Cell growth rate also found to be high when the pH of the medium is in the range of 5 to 6 (10). In a study, it has been proved that pH is an essential factor for maximum curdlan production. When curdlan production was carried out in both regulated and unregulated pH conditions it was found that the polymer production was obtained around 4.8g/L and 2.48 g/L, for regulated and unregulated pH conditions respectively. Regulated pH conditions also enhanced the sugar utilization profile compared to the unregulated pH conditions. It was also concluded that under pH regulated condition, UDP glucose and UMP can also act as precursors for curdlan production (12). pH downshift from 7 to 5.5 can result in the increase of intra cellular concentration of key enzymes such as β-1, 3-glucan synthase, UTPglucose-1-phosphate uridylytransferase and phosphoglucosemutase that are involved in the metabolism and synthesis of curdlan. The key precursor for UDP-glucose that polymerises curdlan was also synthesised more during this pH shift (16).

Conclusion

High demand for curdlan and its derivatives initiates researchers to explore developmental methods to improve production. This demand and production cost have made curdlan a very expensive product. Various experiments have been carried out towards the improvement for curdlan production. Hence cost analysis of production using the low cost agricultural residues as the substrate can effectively bring down the cost and pave way for further research.

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