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Documentation on current developments in production and applications of a β-(1-3)-D glucan (Curdlan)

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ABSTRACT

Curdlan is an exclusive bacterial homopolysaccharide of D-glucose units composed entirely of β(1-3) glycosidic linkages. It is an economically important secondary metabolite having diverse applications. A brief review on the process and conditions required for curdlan production and the need for utilization of available cheap raw materials are highlighted in this paper.

Keywords: Curdlan, production, cheap substrates, applications

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INTRODUCTION

Polysaccharides derived from various microorganisms are of prime importance for usage in several industries. Microbial polysaccharides can be classified into three groups based on their cellular location: (i) cytosolic which provide carbon and energy source for the cell (ii) cell wall polysaccharides like peptidoglycan (iii) exuded polysaccharides commonly referred as Exopolysaccharides (EPS) [1]. Curdlan, an EPS produced during post-stationary phase of microbial growth, was discovered by Professor Harada and his co-workers. The name curdlan arose from its ability to "curdle" upon heating in an aqueous solution [2]. It was approved for commercial use in 1996 with the trade name of PUREGLUCAN®. Curdlan is insoluble in water and alcohols and soluble in alkali solutions and DMSO [3]. One of the unique properties of this polymer is that it is capable of forming irreversible and thermostable "high set" gel once after heating to 80°C. These properties have led to the importance of the polymer in different areas of research and industry. Curdlan is currently used as an aid in formulation, thickening or texture modification of variety of foods such as ice-creams, sauces and noodles [4] and as heavy metal remover and concrete additive.

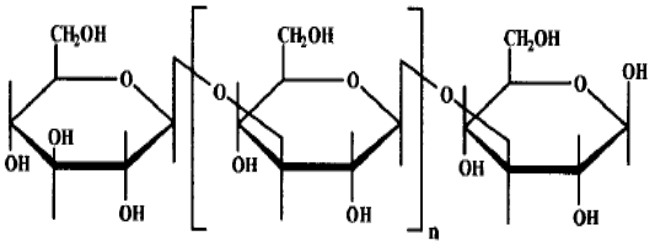

Figure No.1 Structure of curdlan [25]

Metabolic Pathway of Curdlan Biosynthesis

Curdlan biosynthesis is carried out in three stages (a) substrate uptake, (b) metabolism and (c) polymerization [5]. Entry of substrate (glucose) into the cell's cytoplasm takes place by means of active transport. Consequently, the substrate is utilized for the synthesis of primary metabolites and precursors for EPS synthesis. First step involves the phosphorylation of glucose to form glucose-6-phosphate by hexokinase-1 enzyme at the expense of ATP.

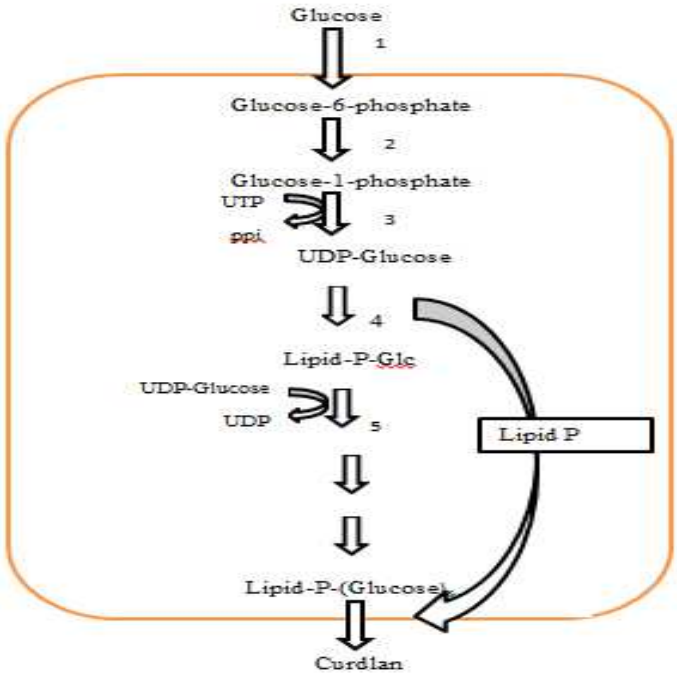

Figure No.2 Biosynthetic Pathway for Curdlan Production [25]

Table-1 Summary of curdlan production by different microorganisms

Microorganism	Substrate	Mode of fermentation	Yield Y_{PS} (g/g)	Concentration of curdlan (g/L)	Concentration of biomass (g/L)	Production rate (g/L h)	Ref
Agrobacterium sp. ATCC 31750	Sucrose	Batch	0.88	24	2.75	_	[6]
Agrobacterium sp.	Sucrose	Batch	0.1	2.42	12.2	0.84	[7]
Alcaligenes faecalis ATCC 31749	Glucose	Batch	-	28.16	9.58	_	[8]
Alcaligenes faecalis ATCC 31749	Glucose	Fed Batch	0.6	72	11.9	_	[9]
Agrobacterium sp. ATCC 31749	Glucose	Batch	0.22	16.35	4.3	0.67	[9]
Agrobacterium sp.	Sugarcane molasses	Fed batch	0.35	42	_		[10]
Agrobacterium sp.	Sucrose	Fed batch	0.45	60	_	0.2	[10]
*Bacillus sp.*SNC07	Glucose	Batch	0.16	3	_	_	[11]

The enzyme, phosphoglucomutase-2, catalyzes the conversion of glucose-6-phosphate into glucose-1-phosphate in the next step. Uridine-diphosphate-glucose (UDP glucose) being the precursor for curdlan biosynthesis is formed from uridine triphosphate (UTP) by the enzyme UDP-glucose phosphorylase. The Glucose moiety in the precursor was attached to a lipid molecule namely isoprenoid lipid phosphate by the enzyme transferase-4 thereby releasing UDP. This is followed by the polymerization reaction catalyzed by polymerase enzyme-5 leading to the formation of β-(1, 3) glucosidic linkages and release of the polymer into the extracellular environment after chain elongation. In

subsequent steps, UDP is converted into UTP by UDP kinase and the curdlan synthesis cycle is started overall anew [25].

Factors influencing curdlan production

Initial pH

Initial pH is considered as one of the most potential parameters governing microbial growth and product formation as they appear to be two diverse phases. Maximum growth was found to be at pH 7 whereas polymer production was high in the pH range of 4.5 – 5.5 [9]. Lee et al. 2001[13] examined the pH profile for the production of curdlan in batch mode of fermentation and obtained a steady increase in curdlan concentration from 36 g/L at constant pH to 64 g/L at pH 5.5. Further studies employing feedback optimal control showed significant curdlan production (60 g/L) at pH 5.5 [14].

Nitrogen sources

Kim et al. 2000 observed that nitrogen-limiting condition was favorable for curdlan production provided the medium containing sufficient levels of sulfate and phosphate [14]. The roles of two different nitrogen sources namely ammonium chloride and urea were determined by Jiang, 2013. It was concluded that curdlan production was maximum (28.16 g/L) when 0.30 g/NL of urea was used [8]. Saudagar and Singhal, 2004[15] studied the effect of nitrogen sources namely ammonium acetate, ammonium citrate, ammonium chloride, ammonium sulfate, potassium nitrate, and sodium nitrate on curdlan production. Based on the observations, the maximum production of curdlan (4.8 g/L) was achieved by ammonium chloride (3.5 g/L) in the production medium. Xia, 2013 [16] reported the elevated glucosyltansferase activity resulting in increased curdlan production upon addition of 0.3% tween 80.

Phosphate concentration

A constant concentration of phosphate is maintained under nitrogen-free conditions for curdlan production and hence optimal amount of phosphate must be present in the medium. Curdlan concentration of 65 g/L was obtained when the residual phosphate concentration reached 0.5 g/L [14]. A medium supplemented with 0.048 mol/L of low polyphosphate such as $(NaPO_3)_6$ resulted in 30 g/L of curdlan [17].

Carbon sources

Carbon substrates present a sole basis for growth of a culture. A variety of carbon sources like glucose, sucrose and maltose pose predominant effects on curdlan production. A defined range of the three sources from 5 to 25% was screened for obtaining maximum productivity of curdlan. The results demonstrated a yield of 4.8 g/L at 15% sucrose, 4.0 g/L at 20% glucose and 3.55 g/L at 20% maltose. In some of the studies, it was noted that curdlan concentration was reduced with increase in the level of carbon source in the production medium [15]. Lee et al. 1997 [10] reported that a maximum concentration of 60 g/L curdlan by *Agrobacterium* sp. was achieved using sucrose under limiting nitrogen concentration.

Low-cost substrates for curdlan production

Selection of medium for microbial fermentation is most sought after since it decides the overall cost of the entire process. Defined synthetic media commonly supplied for microbial growth and product formation are less preferred in industrial scale due to their excessive content of expensive nutrients. Hence researchers are focusing on high productivity of polymers using low cost agricultural as well as food wastes. Until date very few cheap substrates have been screened and utilized for production of curdlan. Lee et al.1997 [10] described the potential usage of sugarcane molasses, an agro-waste, for curdlan production in a double stage fed batch process. Concentration of polymer obtained using this waste was found to be 42 g/L after 120h. A comparative study was investigated for production of curdlan by wild and mutant strains of *Agrobacterium* sp. using condensed corn distiller solubles and also the concentration of curdlan was observed to be 5.3 g/L and 4.9 g/L by mutant and parent strains respectively [18].

Versatile Applications and future research prospective

Combination of macrophages and curdlan sulfur-trioxide- pyridine complex caused the enhanced proliferation of cytokines which are active signaling molecules of our natural defense system. From this, curdlan sulfate was proposed to be used as a new immunotherapy agent and anti-viral vaccine adjuvant for treatment of HBV infection [19]. An industrially important application of curdlan is its ability to remove heavy metals from herbs during preparation of oriental medicine when mixed with activated carbon [20].Yan Sun et al demonstrated the antibacterial effects such as suppression of growth and colony formation with the help of prepared curdlan/chitosan blends. A

conjugated complex of carboxymethylated curdlan and cholesterol synthesized by means of probe sonication was applied for encapsulation of drug epirubicin used in chemotherapy [21]. Studies on the therapeutical applications of sulfated curdlan reflected their potentiality as anti-HIV and anti-viral property of glycidol derivative [22].and anti-malarial agents [23]. Curdlan is used as stabilizer in jelly foods, bio-thickener for noodles and as immobilizing supports. This β-glucan is consumed as edible fibers. It is used as a texturizer in meat, dairy and baking industries owing to its unique properties (tasteless, odorless and colorless) and water holding capacity [24].

CONCLUSION

The distinguished properties like non-toxicity, biodegradability, permeability and thermo-stability assures curdlan and its derivatives a promising future in research and development. Therefore it is necessary to focus on the reduction in production cost of curdlan by implementation of cheap and renewable agricultural wastes as substrates on a large scale.

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