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A Review on production and purification of Polysialic acid (PSA): A Potential Drug delivery material.

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Abstract: Polysialic acid is new potential biopolymer which is used to control the drug delivery in biomedical applications. Polysialic acid (PSA) is a capsular polysaccharide, which is isolated from some bacterial cells by aerobic fermentation. This review article presents an overview of cultivation parameters and state of art advances in PSA production. Bioreactors such as batch and fed batch used for PSA production are presented. Yield of PSA from cells and substrate in batch and fed batch mode are studied and compared. Studies on the effect of parameters such as nutrients requirement and pH for improving PSA production are getting attention. Downstream steps required for PSA purification are also briefly discussed.
Keywords: Polysialic acid, Production of Polysialic acid and Purification.

INTRODUCTION

Polysialic acid called as colominic acid, is a linear polymer of sialic acid with degree of polymerization between 8 and 200 residues attached by α-2, 8 and / or α-2, 9 glycosidic bonds[1,2]. PSA was first identified in *E.coli* K235 in 1957[3]. Bacteria such as *Neisseria meningitides*, *E.coli*, *Haemophillus ducreyi* and *Pasteurella haemolytica* can produce capsular PSA[4,5]. Angata and varki explained that PSA is not available in plant sources and arthropods, and some kinds of bacterial cells[6]. Due to its outermost location, PSA plays a vital role in biological processes such as embryogenesis, neural cell growth, differentiation, cell-cell mediating and membrane transport[7]. In *E.Coli* nine genes are interfere with this biosynthesis pathway from glucose to sialic acid and PSA. All these genes and corresponding enzyme systems in biosynthesis pathway have been studied recently[8]. Purified PSA having α-2, 8 glycosidic linkage is a poor immunogen in human beings and mammals. As a result, PSA could not accelerate the synthesis of antibodies needed for phagocytic removal of organism[9]. According to this, polysialisation of protein based drugs have superior biological activity than PEGlylation with poly Ethelyne Glycol (PEG), which is not a biodegradable material and often actuate anti-PEG antibodies which would lower the residence time of the conjugate in circulating blood[10]. PSA plays an important role in biomedical engineering and tissue engineering due to its physico chemical property as well as physiological function. PSA is also used as a drug control and scoffold material in biomedical field[9, 10, 11, 12]. Polysialic acid based drugs have been used for curing several diseases like H5N1 bird-flu and neuropathic

diseases and treatment for inflammations and brain tumors[13, 14].

Production of PSA had been investigated with different bacterial strain in submerged fermentation[15, 16, 17, 18, 19, 20]. PSA biosynthesis in bacterial cell is governed by temperature as well as pH and aeration requirements[17]. Due to its limitated production, the cost of polysialic acid was more than 200 US dollars per gram[21].

ROLE OF NUTRIENTS IN POLYSIALIC ACID (PSA) PRODUCTION

CARBON AND NITROGEN SOURCE

Nutritional requirements for PSA production was optimized with *E.coli* K1. As a result, yield of PSA has been improved[19]. Xylose and l-proline combination in defined medium was chosen for PSA production[17]. PSA was obtained from *E.coli* K1 using carbon source such as glucose, xylose, sorbitol , mannose and nitrogen source such as ammonium sulfate, l-proline, glutamate on the yield of PSA production[18]. Cell concentration was improved by glucose as a carbon source, but PSA production was inhibited because of acetate formation. High concentration of PSA was obtained when sorbitol as a carbon source[19, 20]. PSA production was performed by E.coli K1 from glucose and ammonium sulphate by Chen et al.,[22].

Sorbitol was used as a main carbon source for PSA production. Honda, H et al., 1997 has concluded that sorbitol was a good carbon source, and that PSA would not be obtained from glucose, although cells were growing well[23]. In the bio synthesis pathway of polysialic acid in *E.coli*, UDP-GlcNAc derived from sorbitol is the precursor PSA synthesis and cell wall synthesis[24]. As a result, when sorbitol was maintained as high level in batch mode, there was enough UDP-GlcNAc synthesized which enhance the cell wall synthesis, thereby increasing the yield of PSA in batch culture. *Neonatal meningitis* was isolated from infected urinary tract, produced PSA using sorbitol as a carbon source. Osmotic pressure on cells was induced by sorbitol, which accelerates PSA synthesis[25]. Sorbitol as a carbon source plays an important role in PSA production using *E.coli* K 235. Low concentration of sorbitol (< 20 g/l) favours only for bacterial cell growth however PSA concentration was lowerd. On the other hand, higher concentration of sorbitol (> 40g/l) accelerated PSA production rate but inhibited bacterial cell growth in batch fermentation. Sorbitol concentration in the range of 20-40 g/l was chosen and PSA yield was increased by 20% [26].

Nitrogen is an important source for cell growth and PSA production. Ammonium chloride can be used as a nitrogen source for PSA production, which is rapidly utilised by biochemical reaction than sorbitol. The production of cell as well as PSA can be improved further as long as sufficient carbon and nitrogen sources are present in the medium[20].

PHOSPHATE

Effect of Phosphate concentration on cell growth and PSA production rate was studied by Jian-Rong Wu et al., Cell growth rate was improved by high concentration of phosphate solution which acts as a buffer solution, where as PSA production was maximum when phosphate concentration was decreased to 2.5 g/l with controlled pH value of 6.4 [27]. High concentration of phosphate interferes with PSA purification. More PSA yield of 1.9 g/l was found at which 0.3 g/l ammonia supplied by casamino acid[23].

pH AND TEMPERATURE

pH is a important factor for PSA production. Optimum pH for polysialic acid production was about 6.4 for *E.coli*[17]. The effect of pH on PSA production was studied by Zhan et al., 2002. Low concentration of PSA was produced when pH value was lower than 5 since partial activity lose of some enzymes involved in biosynthesis of PSA. When pH of the culture medium was maintained at pH 6.4 during the stationary phase, production of PSA was extended[20]. Rodriguez-Aparicio, L.B et al., examined that PSA production was firmly temperature regulated[17].

FERMENTATION METHODS

BATCH FERMENTATION

Poly sialic acid (PSA) production was investigated by Zhan et al., 2002 from sorbitol using *E.coli* K 235. The result demonstrated that maximum cell concentration as well as PSA production was achieved by batch cultivation at 6.4 pH and yield of PSA was found to be 0.264g/g[20]. Large scale production of PSA was investigated in 10lt bioreactor with *E.coli* K1in batch mode by Rode et al., 2008. The results suggested that no PSA was obtained in the medium containing glucose +glutamate combination, xylose + ammonium sulphate, sorbitol +ammonium sulphate and mannose + ammonium sulphate combination. 0.28g/l concentration of PSA was obtained by glucose and ammonium sulfate combination in defined medium where as more yield of PSA was obtained in xylose and l-proline combination containing defined medium. However, glucose and ammonium sulphate combination in defined medium have been screened due to its cost effect. Yield of PSA with glucose was found to be 0.015 g/g in batch mode. Acetate

formation was a crucial factor for PSA production. Acetate Carbon yield was obtained as13.7% in batch mode[18]. PSA production was investigated in batch mode using sorbitol from *E.coli* CCTCC M208088 by Jin-Long Liu et al., 2010. The results demonstrated that 0.201 g/g yield of PSA was obtained [28]. PSA production was investigated in batch mode using sorbitol as a carbon source from *E.coli* K235 under constant pH 6.4 at 37ºC and yield of PSA was obtained as 0.352 by Jin-Long Liu et al., 2010. Enhanced PSA production was investigated using sorbitol from *E.coli* CCTCC M208088 in 15 l stainless steel reactor at 37ºC [26]. High concentration of PSA production (40g/l) was achieved under optimum sorbitol concentration [27]. PSA production from Escherichia coliK1 was investigated in 2 lt working volume bioreactor at 37ºC and yield of PSA from glucose was found to be 0.026 g/g. During the batch mode, cells were grown at a high specific growth rate of 0.7 hr^{-1} and at 6.5 h, acetate formation was developed in the system [22]. Acetate formation is the critical factor in batch mode for PSA production and which is minimised by fed batch fermentation[18, 22, 26].

FED BATCH FERMENTATION

In order to avoid acetate formation and improve the PSA yield, PSA production was carried out in fed batch mode. PSA concentration was highly enhanced by continuous feeding of sorbitol and ammonium chloride under constant pH 6.4. The result suggested that yield of PSA from cells was obtained as 0.264 g/g in fed batch condition and that was 102% higher than that obtained in batch mode[20]. High concentration of PSA was obtained by continuous supply of glucose at low concentration by Rode et al., 2008. The result demonstrated that yield of PSA with respect to glucose was found to be 0.056 g/g fed batch mode which was high value than in batch mode and acetate carbon yield was reduced from 13.7% in batch mode to 4.6% in fed batch mode which was a crucial factor for PSA production[18]. PSA production was investigated in various fed batch mode such as pulse, constant and exponential in order to improve the production of PSA[26]. Cell concentration was improved in constant, exponential and pulse fed batch mode. However yield of PSA from cells decreased when compared to batch culture. Based on these findings new scheme was developed by Jin-Long Liu et al., 2010. Effect of sorbitol concentration in the range from 10 g/l to 80 g/l on yield of PSA per cells and maximum cell growth rate was studied. Yield of PSA was increased by 20% than batch mode when sorbitol concentration was maintained in the range of 20-40 g/l [26]. In order to achieve high biomass as well as PSA synthesis from *E.coli* CCTCC M208088, sorbitol was supplied to the fermentation system after the batch mode with ammonium sulphate for proper pH control. Maximum biomass and PSA production rate were obtained under controlled pH by the scheme of ammonim feed water with sorbitol, which were 55.2% and 67.2% higher than batch and continuous supply of sorbitol without maintaining pH [28]. High yield of PSA was obtained from *E.coli* CCTCC M208088 in fed batch mode by Jian-Rong Wu et al., 2010. Yield of PSA was not affected by low initial concentration of phosphate in basal medium supplied. PSA production was more than 5.2 g/l by ammonia feed water supplied with fed in medium, since pH was controlled [27]. Chen et al., 2011 conformed that PSA production kinetics was growth related product. High yield of PSA was developed by fed batch mode under constant growth rate of cells and constant glucose concentration in the medium. The result demonstrated that yield of PSA from glucose was improved in fed batch mode because of reduction in acetate formation in the system [22].

DOWNSTREAM PROCESSING

Purification of polysialic acid is an important step since the cost of polysialic acid is based upon the downstream steps involved. Researchers have proposed several steps and methods for purification of polysialic acid but each method of seperation is based on the ionic charge of the polysialic acid[18, 28]. It has been found that affinity between ionic surfactants and oppositely charged polyssachride are stronger in aqueous solution which results in precipitation of polysaccharide [29, 30]. Since polysialic acid was anionic in nature because of high content of carboxyl group present in it, relatively cationic compounds such as cetyl pyridinium chloride(CPC), cetavlon were used for precipitating the polysialic acid[18, 28]. Several precipitating reagents like ethanol, cetavlon, NH_4SO_4, poly ethylene glycol(PEG) and acetone were tried for recovery of polysialic acid[18]. More than 85% recovery was achieved by cetavlon treatment. Low concentration of polysialic acid was obtained by acetone precipitation. However precipitation of polysialic acid using ammonium sulphate and PEG did not show promising results[18]. For better separation of polysialic acid, the following arrangement was used by B.Rode et al., 2008: aceton→cetavlon→ethanol. Cetyl pyridinium chloride (CPC) was used to separate polysialic acid from fermentation medium[28]. Factors like ratio of cetyl pyridium chloride (CPC) and PSA (g/g), temperature, aqueous solution of NaCl on recovery of PSA were investigated. Supply of 3g CPC/g PSA, 6-7pH, less than 0.1M NaCl and less than 40ºC temperature were favourable condition for the recovery of polysialic acid[28]. Precipitation of polysialic acid was achieved by CPC and ethanol treatment. New arrangement i.e, ethanol precipitation→ filtration→

CPC treatment→ ethanol treatment was developed for better recover of polysialic acid from fermentation medium by Jin-Long Liu et al., 2010. The generalised procedure of recovery of PSA was proposed by Jin-Long Liu et al., 2010 and B.Rode et al., 2008. 90% of polymer is precipitated by cetavlon and 98% of purification is achieved using CPC [18, 28].

The precipitated polymer was usually resolved by using water and further purification was achieved by using chromatography techniques. Size exclusion chromatography (sephadryl s-300) column was tried to improve further purification of polysialic acid[18].

Table 1: Comparison of Polysialic acid (PSA)Yield and production rate in batch and fed batch fermentation

Strain name	Carbon source	Mode of Fermentation	Yield of PSA from substrate Y_{PS}(g/g)	Yield of PSA from cells Y_{PX}(g/g)	Concentration of PSA(g/l)	Production rate of PSA(g/l hr)
E.coli K 235[20]	Sorbitol	Batch	-	0.264	1.187	0.0334
E.coli K 235[20]	Sorbitol	Fed Batch	-	Increased by 102% than batch mode	2.606	-
E.coli K1[18]	Glucose	Batch	0.020	-	-	-
E.coli K1[18]	Glucose	Fed batch at end of 13.2 h	0.056	-	-	-
E.coli CCTCC M208088[28]	Sorbitol	Batch without pH control	-	0.201±0.0045	0.0274±0.0013	1.535±0.073
E.coli CCTCC M208088[28]	Sorbitol	Fed batch – feedin of ammonia for pH control	-	0.216±0.0058	0.0642±0.0033	2.566±0.13
		Fed batch-feeding of ammonia for pH control and sorbitol addition	-	0.334±0.0067	0.197±0.0083	5.505±0.232

E.coli K235[26]	Sorbitol	Batch	-	0.352±0.0013	4.499±0.072	-
E.coli K235[26]	Sorbitol	Pulse Fed batch Constant fed batch Exponential fed batch		0.267±0.0012 0.264±0.0009 0.218±0.0006	4.405±0.097 4.574±0.065 4.148±0.064	-
E.coli CCTCC M208088[27]	Sorbitol	Batch	-	-	3.4	-
E.coli CCTCC M208088[27]	Sorbitol	Fed batch-ammonia feed water supplied with fed batch medium under constant pH	-	-	5.2	-
E.coli K1[22]	Glucose	Batch	0.026	-	-	-
E.coli K1[22]	Glucose	Fed batch with constant specific growth rate. Fed batch with constant glucose concentration	0.035 0.043	- -	- -	- -

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