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Review of Strategies for the Industrial Production of α-amylase by *Bacillus subtilis*

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Abstract: *Bacillus subtilis can secrete industrially important proteins such as proteases and α-amylases and used on industrial scale. α-Amylase enzyme has market demands due to its applications in food, bakery, detergent industries, starch liquefaction, pre-digestion of the animal feed to enhance its quality, sizing of the fibres in textiles. The safety issues associated with the use of this bacteria for industrial applications are studied and it has been observed that the products obtained from it are having GRAS status of US Food and Drug Administration. Bacillus subtilis is considered the most widely experimental organism to conduct the genetic modification studies due to its properties which make it a suitable host for biosynthesis of the products. The genomic structure of Bacillus subtilis can be modified with the help of high quality genomic sequences. The genetic strategies for such modifications include the use of mutagenic treatments, screening of better expression systems, use of better promoters and high secretion level peptides. Another aspect of these strategies to enhance the enzyme yield includes the application of different fermentation methods and use of different substrates. Present review article summarizes some of such strategies applied for obtaining higher yields of α-amylase enzyme using Bacillus subtilis.*

Keywords: Bacillus Subtilis, α-amylase, Fermentation, Genetic Modification, Screening, Enzyme Production.

I. INTRODUCTION

Bacillus subtilis, a rod shaped Gram positive bacterium is well known for the production of many useful compounds for the mankind. These include many vitamins, proteins and antibiotics. This bacteria has become the production host for many of the biotechnological industries due to its potential of synthesis of these products which have industrial and medicinal values. *Bacillus subtilis* can secrete industrially important proteins such as proteases and α-amylases.[1] The genetic background of the Bacillus subtilis is studied thoroughly and it has been observed that it has higher secretion levels of the proteins and therefore it is possible to use this microorganism on the large industrial scale.[2] The safety issues associated with the use of this bacteria for industrial applications are also studied and it has been observed that the products obtained from it are having GRAS status of US Food and Drug Administration. It is possible to use this microorganism to synthesize some enzymes like amino peptidase, amylase and proteases in excess amounts which is the sole expectation on the industrial scale.[3] Now a days *Bacillus subtilis* is considered the most widely experimental organism to conduct the genetic modification studies due to its properties which make it a suitable host for biosynthesis of the products. Enzymes are the extracellular products which are obtained through the fermentation process in the industries. Production of such extracellular enzymes by *Bacillus subtilis* occurs at the stage of sporulation.[4] α-Amylase enzyme has market demands due to its applications in textile, food, bakery and detergent industries. α-Amylase enzyme has wide applications in starch liquefaction, pre-digestion of the animal feed to enhance its quality, sizing of the fibres in textiles. Detergent industry is a newer avenue for this enzyme. It is now possible to modify the genomic structure of *Bacillus subtilis* with the help of high quality genomic sequences and specific protocols for such modifications. These genetic strategies include the use of mutagenic treatments, screening of better expression systems, use of better promoters and high secretion level peptides. Another aspect of these strategies includes the application of different fermentation methods.

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Production of α-amylase enzyme at industrial scale is achieved through various fermentation techniques like submerged fermentation and solid state fermentation. Out of these, the solid state fermentation is most preferred method due to the advantages like lower cost, simplicity of the process and better yield.[5] This fermentation method involves the cultivation of the selected species of microorganism on surface of the solid media, providing the essential moisture levels. Most of the enzymes produced on the industrial scale are produced by submerged fermentation processes. Generally the solid state fermentation methods are applied for the fungi, but these are proven to be beneficial with Bacillus genera especially for the production of enzyme α-amylase. The advantages of applying the solid state fermentation method include the more purified and concentrated product yield. The higher yields in solid state fermentation process facilitate the downstream processing and make the purification processes easy.

There are many studies aimed at the development of models for metabolites production in *Bacillus subtilis*. One such model proposed by Shane et al. is based on the glucose repression method. Shene et al. [4] proposed a simple unstructured model for microbial growth and metabolite production with glucose repression.[6] Dawes and Thornley proposed a segregated model in which the population of cells of Bacillus was divided into three groups containing different sporangial stages.[7] A continuous culture method was applied by these researchers to get the higher yields. Fordyce and Rawlings further extended this model of segregation by introducing the concept of two compartments for the consumption of substrate.[8] Biological wastes especially the agricultural wastes contain the required growth nutrients such as sugars and proteins for the *Bacillus subtilis*. Banana wastes are ideal in this respect and therefore the strains of *Bacillus subtilis* isolated from the banana waste can produce the α-amylase at higher levels. Efforts have been made in this respect to isolate, identify and purify the strains of *Bacillus subtilis* from banana wastes and apply various cultivation methods to get the maximum yield of the enzyme. Solid state fermentation strategies can be used to get the maximum yields of the enzyme employing such wastes from agriculture. A tropical tuber crop like Cassava has high content of starch. It can also be used as substrate for the production of α-amylase through solid state fermentation method. Cassava is used in agro-industry for the production of starch flour. In this process large amounts of wastes in the form of peels and fibrous residue are generated. This waste has nearly 85% moisture content. Presently such wastes are discarded off and dumped in the landfills. Such wastes can also be utilized as substrate for the production of α-amylase. Wheat bran and rice husk bran can also be used as substrates in solid state fermentation process and the proper cultivation parameters can be identified.

This review article discusses the different strategies that have been applied in enhancement of production of the α-amylase, an industrially important bio-product by *Bacillus subtilis*. The aspects of the enhanced industrial production of this enzymes discussed here include the genetic modification of *Bacillus subtilis*, use of different substrates and cultivation methods of the species for the maximum yield.

II. Problem Statement

There are many industrial applications of the α-amylase enzyme. This enzymes brings out the conversion of complex carbohydrate like starch into simple carbohydrates like glucose and maltose. There are many starchy grains which are used in industries to make the value added products. Wheat, rice, maize, millets etc. contain the starch as the chief constituent. These grain crops are cultivated worldwide and very high grain productions are possible due to advancements in the field of agriculture today. It is possible to use these grains for the making of many value added products such as starch powder, flour, syrups. But the actual value addition is achieved when these starchy substrates are digested with the help of enzymes and converted into the simpler sugars like glucose, maltose, fructose etc. Therefore there is always a great demand for the enzymes like α-amylase which act on the starchy substrates. Commercially available α-amylase enzymes are produced by fermentation technology with the use of specific microorganisms as the source of enzymes. Substrates used for growth of these microorganisms are varied.

The task of present review article is to check for different strategies which can be applied to make the currently used methods of production of αamylase enzymes by using *Bacillus subtilis.*

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III. Review of Literature

Enzymes are the biocatalysts that bring out the conversion of the substrate into the desired products. These enzymes can be obtained from the biological sources such as plants, animals and microorganisms. Many of the enzymes obtained from these sources are found to possess the industrial applicability. Therefore the search for the novel enzyme producing living organisms is continuously done. Microorganisms are being used widely for the synthesis of many biologically active and industrially applicable enzymes. The substrate for the amylase is starch. It hydrolyses the starch. The discovery of this enzymes was done by Kirchhoff in 1811.[9] The nomenclature of the enzyme was done by Kuhn in 1925. It was named as α-amylase as the hydrolysis products are in the form of alpha configuration. The pure crystalline structure of α-amylase is available.

3.1 Sources of the Substrates

Alpha amylase (E.C 3.2.1.1) catalyses the hydrolysis of a-D-(1,4) glycosidic linkages in starch components and related carbohydrates. Ramachandran et al (2007)[10] demonstrated that the utilization of agro-industrial residues, including oil cakes as the substrate for the fermentation has growing interests as they are inexpensive energy rich resources and also eliminate large-scale accumulation of the biomass.

Species of Bacillus need nutrient media which are rich in the carbohydrate content. These bacteria grow well and synthesise the more amount of extracellular enzymes when grown on the rich sources of starch and other required nutrients. Generally peels of fruits and vegetables contain these nutrients in excess. Goyal et al (2005)[11] have shown experimentally that the enzyme production is higher if the carbon source used are starch and glycerol. It has also been shown that if yeast extract is used along with 1% lactose and starch, the yield of extracellular enzyme like α-amylase is increased. Some of the agricultural wastes are best suited to use as substrates for enzyme production. Sivaramakrishnan (2006)[12] suggested the agricultural wastes like rice bran, wheat bran, millets, corn, orange waste etc.

3.2 Methods of Cultivation

Michaels, A. S. preferred the use of continuous fermentation process to obtain better yields and this can be achieved by using immobilised cells on solid supports or using membrane bound cells.[13] Another method used in this regard is aqueous two phase system in which living cells would recirculate in a column and product obtained is collected at the top of upper phase.[14] Pandey et al (2005)[15] emphasised on solid state fermentation which is proven to be the better option to obtain comparatively more yields.

For the industrial production of alpha amylase, it is expected that the cost involvement should be lesser. In this respect, solid state fermentation proves to be comparatively better than the submerged fermentation. From ancient times, amylase was produced on the large scale by submerged fermentation, but the yield and product recovery are affected in this method. Haddaoui (1999)[16] studied the application of of different synthetic nutrient media in solid state fermentation methods to obtain more yields. Tanyildizi & Elibol (2005)[17] stated that there is need of proper optimization and manipulation of growth media so as to obtain the maximum yields to meet the industrial demand.

3.3 Strategies Applied for the Maximum Yields

Baysal, Z. et al.(2003)[18] used a synthetic solid state growth media for the fermentation. *Bacillus subtilis* was grown on this media containing starch as a carbon source and yeast extract as nitrogen source. A 120 hours incubation was done and the product was isolated in the supernatant after centrifugation. Enzyme assay were then done. The parameters which can affect the enzyme production were studied and optimization of these parameters was done. It was observed in this study that the yields are more at 48 hours incubation in solid state fermentation. It has been also shown that 30% of moisture level is best suited for the maximum yields. If levels of moisture are more than this, the yields are low. At 30% moisture level, the initial yields are less due to congestion of the media and non-availability of the nutrients. In this study it was also observed that the concentration of inoculum have no considerable effect on the yield of enzyme.

Huang, H. et al.(2003)[19] introduced a segregated model for the alpha amylase production using *Bacillus subtilis*. The concept is based on the stages in the growth cycle of the bacterium such as vegetative cells, sporangium stage and spore

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stage. When the cells are deprived of the nutrients, the vegetative cells immediately shifts to the sporangia form. It has been observed that the production of hydrolytic enzymes like amylase occurs at the initial stages of germination of sporangia. The sporangia are resistant to most of the adverse environmental conditions. This segregation approach can be effectively applied in the solid state fermentation procedures to obtain the desirable product in more quantities.

Konsoula, Z.et al. (2006)[20] applied the immobilized cell culture technique for the enhancement of the enzyme production by *Bacillus subtilis*. They used the calcium alginate gel capsules for this. Sterile conditions were maintained while immobilization of the cells was being done. Bacterial cells were entrapped into the capsules of calcium alginate. Synthetic growth media was used for the cultivation in which capsules of the immobilized cells were inoculated in it. Fermentation was done for 48 hours and the product was isolated by precipitation method. The enzyme assay was performed to access the yield. In this study the yield of the product was assessed with immobilizaed and free cells of *Bacillus subtilis* and it was found that the more yield can be obtained by immobilized cells as the continuous fermentation can be achieved.

Krishna, C., & Chandrasekaran, M.(1996)[21] estimated that the banana fruit stalk can be used as substrate for this type of fermentation as it contains the required nutrients in optimum quantities. The strains of *Bacillus subtilis* isolated from the banana wastes have more yields of the enzyme. They have applied the solid state fermentation method using banana fruit stalk. Growth medium used by them consisted of the mineral salt medium suggested by Ramesh and Lonsane (1989). All the other parameters of the growth were optimised and the yields were studied. After the completion of the fermentation, the product assay was performed. Meddha and Chandra(1980) method for enzyme activity assay was done. It was observed in this study that the optimum moisture level of the substrate for maximum yield was 70%. Maximum amylase activity is observed when inoculum concentration is 10%. Enzyme yield was higher with the incubation time of 24 hours and after that the yield was reduced. .

Ma, Y., Shen, W., Chen, X., Liu, L., Zhou, Z., Xu, F., & Yang, H. (2016)[22] used the mutagenesis approach for *Bacillus subtilis* strain to enhance the alpha amylase production. They have used ARTP mutagenesis for this. It has been observed that the mutant strains possess the 1.34 fold more activity of enzyme production as compared to the wild type strain. This study clearly indicated that the ARTP mutagenesis facilitates the secretion of more quantities of the extracellular enzyme. Eventhough there are chances that the wild type bacterial cells can have some sort of damage due to the mutagenic treatment, but the selection procedures can be applied in more effective means to get the high yielding cells.

Ploss, T. N. et al.(2016)[23] used the introduction of stronger promoter sequences for the biosysmthesis of the extracellular protein-enzyme by the *Bacillus subtilis* cells. They have applied plasmid induced genetic transformation technique for this. It was observed that this change in primer has significant effect on the synthesis of the enzyme by the cells.

Rajagopalan, G., & Krishnan, C. (2008)[24] used the wastes from agriculture for the production of alpha amylase by *Bacillus subtilis* KCC103 strain. This strain showed the absence of repression by glucose when sugarcane wastes were used as substrate. In this study, instead of starch, sugarcane waste was used and cost of the process was reduced. Catabolite derepression was responsible for this strain to utilize the nutrients other than the starch. The yields were also more with the lesser incubation time.

IV. Further Scope of the Study

The study implies that all the tools and techniques used to increase the product yield in mentioned studies have expected outcomes that is the more yield is obtained. At the same time, it can be seen that the cost-effective procedures and materials can also be developed for the production of alpha amylase enzyme which is having a considerable market value. Therefore researches can be extended on the *Bacillus subtilis* species and other low cost substrate options can be screened. Also it is possible to develop and screen for the novel mutant strains of this bacteria having more enzyme production abilities. The research can also be extended to application of other suitable growth factors which will assist and enhance the production. Other species of the Bacillus can also be screened for the enzyme production.

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V. CONCLUSION

The review of the research on the production of α-amylase enzyme by *Bacillus subtilis* for the industrial usage has revealed many strategies that have been applied to enhance the more and maximum yields at low cost and lesser time. There are many approaches applied in this regard. The applications of this hydrolytic enzyme are well studied and there is a constant demand for this enzyme in the commercial markets. There are the standardised procedures being used in industries for the conversion of complex carbohydrates into the simpler ones. The financial aspects of the production of this enzymes have to be taken into consideration while screening and developing the novel methods of production of amylase at industrial scale.

In the research papers reviewed various tools and techniques were applied with respect to the producer microorganism, the substrate used for the growth and the various growth parameters. Some techniques involved the use of low cost agricultural wastes to minimize the production cost, others involved the use of genetically modified strains of *Bacillus subtilis* for better yields. Some growth factors as time of incubation, inoculum concentration, temperature, medium supplementation were also studied for the increasing yield of the product. Most of the techniques used have proved to be significantly effective in obtaining the more yields.

REFERENCES

[1]. Westers, L., Westers, H., & Quax, W. J. (2004). Bacillus subtilis as cell factory for pharmaceutical proteins: a biotechnological approach to optimize the host organism. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1694*(1-3), 299-310.

[2]. Guan, C., Cui, W., Cheng, J., Zhou, L., Liu, Z., & Zhou, Z. (2016). Development of an efficient autoinducible expression system by promoter engineering in Bacillus subtilis. *Microbial Cell Factories*, *15*(1), 1-12.

[3]. Jaouadi, N. Z., Jaouadi, B., Aghajari, N., & Bejar, S. (2012). The overexpression of the SAPB of Bacillus pumilus CBS and mutated sapB-L31I/T33S/N99Y alkaline proteases in Bacillus subtilis DB430: new attractive properties for the mutant enzyme. *Bioresource technology*, *105*, 142-151.

[4]. Mountain, A. (1989). Gene expression systems for Bacillus subtilis. In *Bacillus* (pp. 73-114). Springer, Boston, MA.

[5]. Mulimani VH, Ramalingam Patil GN. Amylase production by solid state fermentation: a new practical approach to biotechnology courses. Biochem Edu 2000; 28:161/3.

[6]. Shene, C., Andrews, B. A., & Asenjo, J. A. (1999). Fedbatch fermentations of Bacillus subtilis ToC46 (pPFF1) for the synthesis of a recombinant β-1, 3-glucanase: experimental study and modelling. *Enzyme and microbial technology*, *24*(5-6), 247-254.

[7]. Dawes, I. W., & Thornley, J. H. M. (1970). Sporulation in Bacillus subtilis. Theoretical and experimental studies in continuous culture systems. *Microbiology*, *62*(1), 49-66.

[8]. Fordyce, A. P., & Rawlings, J. B. (1996). Segregated fermentation model for growth and differentiation of Bacillus licheniformis. *AIChE journal*, *42*(11), 3241-3252.

[9]. Tiwari, Sp and Srivastava, R and Singh, Cs and Shukla, Kartikeya and Singh, Rk and Singh, Pushpendra and Singh, Ravindra and Singh, Nl and Sharma, Rajesh,(2015), pages (1886-1901),Amylases: an overview with special reference to alpha amylase, volume4, Journal of Global Biosciences

[10]. Ramachandran, S., Patel, A. K., Nampoothiri, K. M., Chandran, S., Szakacs, G., Soccol, C. R., & Pandey, A. (2004). Alpha amylase from a fungal culture grown on oil cakes and its properties. *Brazilian archives of biology and technology*, *47*, 309-317.

[11]. Goyal, N., Gupta, J. K., & Soni, S. K. (2005). A novel raw starch digesting thermostable α-amylase from Bacillus sp. I-3 and its use in the direct hydrolysis of raw potato starch. *Enzyme and Microbial Technology*, *37*(7), 723-734.

[12]. Sivaramakrishnan, S., Gangadharan, D., Nampoothiri, K. M., Soccol, C. R., & Pandey, A. (2006). a-Amylases from microbial sources–an overview on recent developments. *Food Technol Biotechnol*, *44*(2), 173-184.

IJARSCT
ISSN (Online) 2581-9429
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[13]. Andersson, E., Johansson, A. C., & Hahn-Hägerdal, B. (1985). α-Amylase production in aqueous two-phase systems with Bacillus subtilis. *Enzyme and microbial technology*, *7*(7), 333-338.

[14]. Mosbach, K., Birnbaum, S., Hardy, K., Davies, J., & Bülow, L. (1983). Formation of proinsulin by immobilized Bacillus subtilis. *Nature*, *302*(5908), 543-545.

[15]. Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T., Singh, D., & Mohan, R. (2000). Advances in microbial amylases. *Biotechnology and applied biochemistry*, *31*(2), 135-152.

[16]. Haddaoui, E; Chambert,R ; Petit-Glatron,M.F; Lindy,O; ; Sarvas,M Bacillus subtilis kamylase: The rate limiting step of secretion is growth phase-independent, FEMS Microbiology. Lett. 173 (1999) 127–131.

[17]. Tanyildizi, M.S.; Ozer, D; Elibol, M., Optimization of α-amylase production by Bacillus sp. using response surface methodology, Process Biochemistry. 40 (2005) 2291–2296

[18]. Baysal, Z., Uyar, F., & Aytekin, C. (2003). Solid state fermentation for production of α-amylase by a thermotolerant Bacillus subtilis from hot-spring water. *Process Biochemistry*, *38*(12), 1665-1668.

[19]. Huang, H., Ridgway, D., Gu, T., & Moo-Young, M. (2003). A segregated model for heterologous amylase production by Bacillus subtilis. *Enzyme and Microbial Technology*, *32*(3-4), 407-413.

[20]. Konsoula, Z., & Liakopoulou-Kyriakides, M. (2006). Thermostable α-amylase production by Bacillus subtilis entrapped in calcium alginate gel capsules. *Enzyme and Microbial Technology*, *39*(4), 690-696.

[21]. Krishna, C., & Chandrasekaran, M. (1996). Banana waste as substrate for α-amylase production by Bacillus subtilis (CBTK 106) under solid-state fermentation. *Applied Microbiology and Biotechnology*, *46*(2), 106-111.

[22]. Ma, Y., Shen, W., Chen, X., Liu, L., Zhou, Z., Xu, F., & Yang, H. (2016). Significantly enhancing recombinant alkaline amylase production in Bacillus subtilis by integration of a novel mutagenesis-screening strategy with systems-level fermentation optimization. *Journal of biological engineering*, *10*(1), 1-11.

[23]. Ploss, T. N., Reilman, E., Monteferrante, C. G., Denham, E. L., Piersma, S., Lingner, A., & van Dijl, J. M. (2016). Homogeneity and heterogeneity in amylase production by Bacillus subtilis under different growth conditions. *Microbial cell factories*, *15*(1), 1-16.

[24]. Rajagopalan, G., & Krishnan, C. (2008). α-Amylase production from catabolite derepressed Bacillus subtilis KCC103 utilizing sugarcane bagasse hydrolysate. *Bioresource technology*, *99*(8), 3044-3050.