PULLULANASE: A POTENTIAL ENZYME FOR INDUSTRIAL APPLICATION

Ramdas Malakar, Dr. Archana Tiwari and **S.N.Malviya***

School of Biotechnology, University Institute of Technology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal (M.P.). India

Corresponding author*: malviyasatyam@yahoo.com

This article is available online at www.ssjournals.com

ABSTRACT

Pullulanase is one of the most important enzymes in starch processing. This enzyme is used on a large scale in glucose and maltose syrup industries. In recant times, the agricultural wastes rich in polysaccharides can be processed to useful from the sucrose and glucose instant of dumping. Pullulanase is a very potent enzyme for degradation of starch to glucose or maltose. Pullulanase hydrolyses α-1, 6-glycosidic linkage of branched chain and α-1, 4-glycosidic linkage and α-1, 6-glycosidic linkage of polysaccharides. It is also known as de-branching enzyme. Pullulanase has been used in some industries like glucose and maltose syrup production, baking and cyclodextrin production recently. In future pullulanase enzyme can be widely used in industries if improve we the stability and activity of this enzyme upon searching novel extremophile microorganism. The number of applications will increase manifolds and with the availability of thermostable enzyme a number of new possibilities for industrial processes shall emerge.

KEY WORDS: Pullulanase enzyme, Extremophile microorganism, starch hydrolysis.

INTRODUCTION

Pullulanase (pullulan α- glucano hydrolase; EC 3.2.1.41) is an extracellular carbohydrase which was first discovered by Bender and Wallenfels in 1961 from mesophilic organism *Klebsiella pneumoniae* (formerly known as *Aerobactor aerogenes* or *Klebsiella aerogenes*). Pullulanase are also called de-branching enzyme which hydrolyze the extracellular yeast, polysaccharide and pullulan. To cause an essentially quantitative conversion of pullulan into the trisaccharides, maltotriose, pullulan a linear α-glucan elaborated by *Pullularia pullulan*. It consists of repeating units of α-maltotriose joined "head to tail" by 1, 6 bonds. Pullulanase specifically attack on α-1, 6-glycosidic linkage and it also attacks on α-1, 4-glycosidic linkage with other residues, these properties have made pullulanase a useful agent in structural studies of oligosaccharide and polysaccharide. (Ling et al., 2009) (Knish 1979), (Hantai 1969). (Drummond et al., 1969), (Saha et al., 1988) and (Enlvoldsen 1977).

A number of thermo stable pullulanase with dual specificities have been investigated. Pullulanase of thermophilic

microorganisms, attack on both α-1, 4-glycosidic linkage and α-1, 6-glycosidic linkage in Amylopectin and malto-oligosaccharide (Koch et al., 1997). Extremophilic microorganisms which grow at 60-80^0 C are called as thermophilic microorganisms (Rudiger et al., 1995). There are three kinds of bond that are cleaved by enzymes called pullulanase (EC. 3.2.1.41) isopullulanase (EC.3.2.1.57) and neopullulanase (EC.3.2.1.35). The pullulanase can be sub divided into two types pullulanase type-I and pullulanase type-II. Pullulanase type-I hydrolyses only α-1, 6-glycocidic linkage but pullulanase type –II specifically attack on α-1,4-glycocidic linkage and α-1,6-glycocidic linkage (Kriegshauser et al., 2000).

Thermostable enzymes are required for a number of industrial processes as in the saccharification of starch. The main amylolytic enzymes used in the starch industry are α-amylase, β-amylase, glucoamylase and pullulanase. Glucoamylase and pullulanase have improved thermostability as compared with the enzyme in use for the saccharification process (Madi et al., 1987). *Chlostridium thermohydrosulfuricum* is an anaerobic thermophilic bacteria that ferments starch to ethanol and have been investigated for starch degradation by the strain E-39 and reported a pullulanase and a glucoamylase activity from cell extract of this strain (Melashiemi 1988). An enzyme preparation from broad beans that hydrolyses the α-1, 6 branch linkages of amylopectin, α-amylopectin, β-limit dextrin and amylopectin α-limit dextrin was discovered by Hobson Whelan and peat and termed r-enzyme subsequently Macwilliam and Harris described the fractionation of bean and malted barley extracts on aluminium such that separate 1, 6 bond hydrolyses were found (Drummond 1970).

Pullulanase is distributed in plants and bacteria. The enzyme in plants, readily cleaves the α-1, 6 glycosidic linkages in pullulan β-limit dextrin and α-limit dextrin while debranching amylopectin to a lesser extent than polyglucans. Pullulanase activity was detected in the endosperm of rice seed (Amasaki 2008). The enzyme is produced by several bacteria and is also present in higher plant (Enlvoldsen 1977).The highly thermostable pullulanase type-II enzyme is the most interesting from industrial point of view. Clearly these enzymes could facilitate a major change in the current strategy for starch processing (Ni hin 2002).

Pullulanase activity is determined using enzyme digests (1.0ml) containing pullulan (5.0 mg) and acetate buffer (pH 5.0 final concentration 100 ml) incubated at 37^{0}C Reducing sugars liberated are determined using the nelson adaptation of Somagyls copper reduction method. Calibrated glucose one unit pullulanase is the amount which liberates one micromole of reducing sugar per minute under these conditions (Marshal 1973). The enzyme industries and starch processors continue to search for better enzyme to improve standard processes and Pullulanase created new products (Douglas 1999). In present time debranching enzyme for example pullulanase of *Klebsiella pneumoniae* and *Bacillus* species have gained increasing interest as they possess good activity and stability at high temperatures and these are factors required for an ideal amylolytic enzyme, therefore

thermophilic organisms are often first choice (Melashiemi et al., 1987).

SOURCES OF PULLULANASE

In the last decade, a number of hyperthermophilic archaea, have been isolated which are able to grow around the high temperatures. Some organisms with highest growth temperature are members of the genera *Thermotoga maritima* and *Pyrococcus* etc. described in the table no. 1 (Haki et al., 2003).

BIOCHEMISTRY OF ENZYME PULLULANASE

Pullulanase three dimensional structure is show in fig.1.and Pullulanase could attack Pullulan by either of two modes of action shown in fig.2 these are (a) an exo-action in which hydrolysis is restricted to the α-1,6 glycosidic linkage nearest to the terminal non reducing end, or reducing end with the stepwise release of maltotriose as the only low-molecular weight product of the reaction (b) an endo action in which initial hydrolysis can occur at internal as well as external α-1,6 glycosidic linkage, with the intermediate production of hexa- nona and larger oligosaccharides, in addition to maltotriose (Drummond et al.,1969).

PROPERTIES OF PULLULANASE

The optimum pH for the activity of pullulanase was observed at pH 6.0 and temperature 55^0C without Ca^{2+}. Maximum activity was observed at pH 6.5, although growth of *Bacillus cerus* FDTA 13 was observed at pH 4.5-7.5, 70% activity was shown between 5.5 - 8 pH. Various data have been shown (Rudiger et al., 1995).

FACTORS AFFECTING THE PULLULANASE ENZYME

pH

pH is the most important physical parameter that effect growth as well as secretion of extracellular enzyme. pH optimum for growth and enzyme production by *G. thermoleovorans* were pH 7.0, respectively. Microorganism is known to produce enzyme optimally at pH optimal for their growth.(Noorwez et al., 2006). Pullulanase possess activity and stability on long ranges of pH 2-10. But the optimum the Pullulanase enzyme activity is shown in pH range 5.5 - 6.5, From *Bacillus cerus* H1.5. Above pH 7 the activity of pullulanase is greatly reduced (Ling et al. 2009). Maximum activity was observed at pH 6.5, although growth of *Bacillus cerus* FDTA 13 was observed 70% activity was shown between pH 5.5 – 8. the pH optimumwas determinedto be 6.0 (Rudiger et al., 1995). The optimum activity was determined at pH 6 (Nair et al., 2006).

Temperature

We know that every 10^0C rise in temperature, increases the enzyme activity upto 2-3 times. The optimum activity of the enzyme is within the temperature range of 55^0C -100^0C. Most thermophilic microorganisms show highest activity between temperature range 55^0C-80^0C and some hyperthermophilic microorganisms show highest activity produced at 80^0C – 115^0C (Ling et al., 2009).. The optimum temperature of the purified enzyme is 100^0C and a rapid decreases in pullulanase activity was observed above

this point (Rudiger et al., 1995).Temperature is most important parameter that affect growth as well as secretion of extracellular enzyme. Temperature optimum for growth and enzyme production by *G. thermoleovorans* is 70^0C, respectively. (Noorwez et al., 2006).

Time

Time is a main factor affecting Pullulanase enzyme activity which is reduces the time of substrate conversion. The shelf life of the enzyme is 2h at 50^0C temperature and pH 6.0 in *Bacillus cereus*. (Ling et al., 2009).

Metal ions

The Pullulanase activity is strongly inhibited by $Ni^{2+,}$ Co^{2+}, Mg^{2+}, Cu^{2+}, and Zn^{2+} at 0.2 mM concentration. But Ca^{2+} is strong stimulator which increases the activity of Pullulanase enzyme around 179%. Strong inhibitor of Pullulanase enzyme activity is Cu^{2+}. Metal ions are main factor affecting Pullulanase enzyme activity, in presence of Ca^{2+} highest activity is shown by microorganism (Ling et al., 2009).

Polyols

The effect of polyols on the enzyme activity is strongly increased in the presence of sucrose and highly decreased in the presence of glycine. The highest improvement in relative stability of 172.67 was obtained with sucrose. The enzyme was almost denatured with the presence of glycine (Ling et al., 2009).

Substrate concentration

Increasing the concentration of substrate the activity is increased in the presence of Pullulan 100% of activity is determined. Incubation of pullulanase from isolate ven 5 pullulan, glycogen, starch, amylase, amylopectin, dextrin or a mixture of oligosaccharide 0.5% wt/vol. each in 20mM sodium phosphate buffer pH 6.0 at 85^0C for different time stand that maximal relative activity was obtained with pullulan 100% and that 41% activity was obtained with a mixture of oligosaccharides (Koch et al., 1997).

Carbon source

A carbon source is the most affective factor for pullulanase producing microorganism. In presence of soluble starch the production of pullulanase enzyme was increased as compared to other carbon source. Maximum production of pullulanase was observed with soluble starch, it is therefore a good source of carbon for production of pullulanase enzyme. Glucose, amylase and glycogen severely repress pullulanase production by approximately 70-80%. Except pullulan all other carbon source gives maximum pullulanase activity in the 12h fermentation (Nair et al., 2006).

Nitrogen source

Nitrogen source is one of the most common factors which affect the pullulanase enzyme production. Yeast extract is more efficient source of nitrogen for pullulanase production. Peptone does not increase pullulanase production in the medium. Pullulanase activity seemed to be considerably induced by yeast extract since the activity with yeast extract as sole source of

nitrogen paralleled that of the medium with combination of tryptone and yeast extract (Nair et al., 2006).

Minerals

Minerals are very necessary for the production of enzyme, minerals are required in very low quantity for the microorganisms, it is used by microorganism in the form of trace elements. It can be seen that only K_2HPO_4 enhanced the pullulanase production and it was also observed that in the medium with $MgSo_4$ pullulanase activity was stable until the 48h as compared the control. (Nair et al., 2006).

Agitation

Agitation is a main factor for the production of enzyme. In the production of enzyme proper mixing of nutrient is very necessary for the proper growth of microorganism. proper mixing of nutrient and oxygen supply determine the optimal production of extracellular enzyme. The agitation rate is 200 per minute (Noorwez et al., 2006).

INDUSTRIAL APPLICATIONS OF PULLULANASE ENZYME

Starch Processing Industry

Pullulanase are used in sugar syrup industries to complete the hydrolysis of starch initiated by α-amylases. The combined application of pullulanase with other amylolytic enzymes increase the quality of sugar syrups. When starch is treated with amylase and pullulanase simultaneously this consequently increases the efficiency of a saccharification reaction. This method has an advantage of generating higher yields of a desired end product from starch. Thermostable and acidophilus pullulanase from *Bacillus stearothermophilus* is useful here. (Nair et al., 2006), (Michaelis et al., 1985), (Saha et al., 1988). (Silva 2005).

Pullulanase enzyme preparation intended for use as a processing aid in the food industries, in particular pullulanase is useful in the debranching of corn starch in the production of certain corn sweeteners. (Modderman 1995). (Haki et al., 2003), (Bertoldo 2002).

Baking Industry

The baking industry is a large consumer of starch and starch-modifying enzymes. Staling effect is the major problem in the baking industry. The staling effect includes increase of crumb firmness, loss of crispness of the crust, decrease in moisture content of the crumb and loss of bread flavor, leading to the deterioration of quality (Vander et al., 2002). Although this problem can be overcome using chemical treatment, enzymatic treatment is more preferred because consumers nowadays demand for products without chemicals and higher acceptances by the consumers for enzymes, which are produced from natural ingredients, is seen. Pullulanase play an important role in the enzymatic anti-staling treatment (Noorwez et al., 2006), (Bertoldo 2002).

Branched Cyclodextrin (CDs) Production

There is a very interesting and highly economical valued application of pullulanase in branched cyclodextrins (CDs) production. CDs and branched CDs, such as maltosyl-CDs and glucosyl-CDs are homogeneous cyclic

oligosaccharides, which are composed of only glucose units (Kitahata et al., 2000). Thus, CDs and branched CDs have been widely used for stabilizing labile materials, masking odors, and solubilizing insoluble or poorly soluble drugs (Bertoldo 2002), (Tanimoto et al., 2005), (Okada et al., 1988).

Glycogen and amylopectin-debranching enzyme such as pullulanase and isoamylase can be used as an effective additive in dish washing and laundry detergent as reported by for alkaline endo glucanase from some strain of *Bacillus* the properties of which also fulfill the essential requirements for enzyme to be used for such purposes (Ara 1993).

CONCLUSION

We have concluded the recent time investigations of Extremophile and thermophilic bacteria which produces pullulanase enzyme. Isolated and started conversions of starch to glucose or maltose under conditions appropriate for industrial application. In the industrial production some conditions are required such as pH, temperature, stability, chemical catalyst, rate of production of conversion reaction by enzyme catalyst. Substrate specificity, rate of production of enzyme, growth rate of microorganism and quantity of enzyme are some factors which are challenge for a future work of industrial application. In future application of pullulanase enzyme can increase the production increase the range of pH, stability on higher temperature, shelf life time of enzyme with the help of optimization of different parameters.

REFERENCES

1. Nair, S. U., Singhal, R. S., and kamat, M. Y., (2006). Enhanced Production of Thermostable Pullulanase Type-I Using *Bacillus Cereus* FDTA and Its Mutant, Food technol. Biotechnol, Vol. no.44 (2), pp. 275-282.
2. Koch, R., Canganella, F., Hippe, H., Jahnke, K. D., and Antranikian, G., (1997). Purification and Properties of a Thermostable Pullulanase from a Newly Isolated Thermophilic Anaerobic Bacterium *Fervidobacterium Pennavorans* Ven5, Applied and Environmental, Vol. no. 63, pp. 1088-1094.
3. Rudiger, A., Jorgensen, P. L., and Antranikian, G., (1995). Isolation and Characterization of a Heat-stable Pullulanase from the Hyperthermophilic Archeon *Pyrococcus woesei* after cloning and expression of Its Gene in *Escherichia coli;* Applied and Environmental microbiology, Vol. no. 61, pp. 567-575.
4. Michaelis, S., Chapon, C., Enfert, C.D., Pugsley, A. P., and Schwartz M., (1985). Characterization and Expression of the Structural Gene for Pullulanase, a Maltose-Inducible Secreted Protein of *Klebsiella pneumoniae*, Journal of Bacteriology, Vol. no.164, pp. 633-638.
5. Melasniemi H., (1987). Effect of Carbon Source on Production of Thermostable α-Amylase Pullulanase and α-Glucosidase by *Clastridium thermohydrosulfuricum,* Journal of general microbiology, Vol. no. 133, pp. 883-890.
6. Ling, H. S., Ling, T.C., Rosfarizan and Ariff, A. B., (2009). Characterization of Pullulanase type-

II from *Bacillus cereus* H1.5, American journal of Biochemistry and Biotechnology, Vol. no. 5, pp.170-179.
7. Melasniemi, H., (1987).Characterization of α-amylase and Pullulanase Activities of *Clostridium thermoydrosulfuricum,* Biochem. Journal, Vol. no. 246, pp. 193-197.
8. Saha, B. C., Mathupala, S. P., and Zeikus J. G., (1988). Purification and Characterization of a Highly Thermostable Novel Pullulanase from *Clostridium thermohydrosulfuricum,* Biochem Journal, Vol. no. 252, pp. 343-348.
9. Noorwez, S. M., Ezhilvannan, M. and Satyanarayana, T. (2006). Production of a High Maltose-Forming Hyperthermostable and Ca^{2+} Independent Amylopullulanase by an Extreme Thermophil *Geobacillus thermoleovorans* in submerged fermentation, Indian Journal of Biotechnology, Vol. no. 5, pp. 337-345.
10. Kriegshauser, G. Liebl, W. (2000). Pullulanase from the Hyperthermophilic Bacterium *Thermotoga maritime*, Purification by β-Cyclodextrin Affinity Chromatography, Journal of Chromatography, Vol. no. 737, pp. 245-251.
11. Drummond, G. S., Smith, E. E., and Whelan, W. J., (1969). Mechanism of Action of Pullulanase, FEBS LETTERS, Vol. no. 5, pp. 85-88.
12. Madi, E., Antranikian, G., Ohmiya, K., and Gottschalk, G., (1987). Thermostable Amylolytic Enzymes from a new *Clostridium* Isolate, Applied and Environmental Microbiology, Vol. no. 53, pp. 1661-1667.
13. Pugsley, A. P., and Kornacker, M. G., (1991).Secretion of the Cell Surface Lipoprotein Pullulanase in *Escherichia coli,* Journal of Biological Chemistry, Vol. no. 266, pp.13640-13645.
14. Melasniemi, H., (1988). Purification and some Properties of the Extracellular α-amylase Pullulanase Produced by *Clostridium thermohydrosulfuricum,* Biochem. Journal, Vol. no. 250, pp. 813-818.
15. Ara, K., Saeki, K., and Ito, S., (1993). Purification and Characterization of an Alkaline Isoamylase from an Alkalophilic strain of Bacillus; Journal of general microbiology, Vol. no. 139, pp. 781-786.
16. Demirjian, D. C., Moris-Varas, F., and Cassidy, C. S., (2001). Enzyme from Extremophiles, Current Opinion in Chemical Biology, Vol. no. 5, pp. 144-151.
17. Drummnd, G. S., Smith, E. E., and Whelan, W. J., (1970).On the Specificity of Starch Debranching Enzymes, FEBS LETTERS, Vol. no. 9, pp.136-140.
18. Brandt, C. J., Catley, B. J., and Awad, W. M., JR (1976). Extra-Cellular and Protease-Released Pullulanase, Journal of Bacteriology, Vol. no. 125, pp. 501-508.
19. Modderman, J. P., and Fol H. H., (1995). Safety Evaluation of Pullulanase Enzyme Preparation Derived from Bacillus Licheniformis Containig the Pullulanase from *Bacillus deramificans*, Regulatory Toxicology and Pharmacology, Vol. no. 21, pp 375-381.
20. Haki, G.D., Rakshit, S.K., (2003). Developments in Industrially Important Thermostable Enzyme, Bioresource Technology, Vol. no. 89, pp. 17-34.

21. Yamasaki, Y., Nakashima, S., and Konno, H., (2008). Pullulanase from Rice Endosperm, Acta Biochimica Polonica, Vol. no.55, pp. 507-510.
22. Chang-Pi-Hin, F., Erra-Pujada, M., Dauchez, M., Duchiron, P., and ODonohue, M. J., (2002). Expression and Characterization of the Catalytic Domain of An Archaealm Family 57 Pullulanase-Type II, Biologia, Bratislava, Vol. no. 57, pp.155-162.
23. Tripathi, P., Leggio, L. L., Mansfeld, J., Ulbrich-Hofmann, R., Kayastha, A. M., (2007). α-Amylase from Mung Beans (Vigna radiata) – Correlation of Biochemical Properties and Tertiary Structure by Homology Modelling, Phytochemistry, www.science direct.com, Vol. no. 68, pp. 1623-1631.
24. Enevoldsen, B. S., Reimann, L., and Hansen, N. l., (1977). Biospecific Affinity Chromatography of Pullulanase, FEBS LETTERS, Vol. no. 79, pp. 121-124.
25. Gomes, J., and Steiner, W., (2004). The Biocatalytic Potential of Extremozymes, Food Technology and Biotechnology, Vol. no. 42, pp. 223-235.
26. Bello-Perez, L. A., Sanchez-Hemandez, L., Moreno-Damian, E., and Toro-Vazquez, J. F., (2002). Laboratory Scale Production of Maltodextrins and Glucose Syrup from Banana Starch, Food Technology, Vol. no. 53, pp. 44-48.
27. Silva, T. M., Attili-Angelis, Carvalho, A. F. A., Silva, R. D., Boscolo, M., and Gomes, E., (2005). Production of Saccharogenic and Dextrinogenic Amylases by Rhizomucor Pusillus A 13.36, Journal of Microbiology, Vol. no. 43, pp. 561-568.
28. Marshall, J. J., (1973). Inhibition of Pullulanase by Schardinger Dexxtrins; FEBS LETTERS, Vol. no. 37, pp. 269-273.
29. Satyanarayana, T., Raghukumar, C., and Shivaji, S., (2005). Extremophilic Microbes: Diversity and Perspectives, Current science, Vol. no. 89, pp. 78-90.
30. Burg B. V. D., (2003). Extremophiles as a Source for Novel Enzymes, Current Opinion in Microbiology, Vol. no. 6, pp. 213-218.
31. Jamal, P., Alam, M. Z., and Salleh, N. U., (2008). Media Optimization for Bioproteins Production from Cheaper Carbon Source, Journal of Engineering Science and Technology, Vol. no.3, pp. 124-130,
32. Sivaramakrishnan, S., Gangadharan, D., Nampoothiri, K. M., Soccol, C. R., and Pandey, A., (2006). α-Amylases from Microbial Sources-An Overview on Recent Developments, Food Technol. Biotechnology, Vol. no.44, pp. 173-184.
33. Borovsky, D., Smith, E.E., and Whelan, W.J., (1975). Temperature-Dependence of the Action of Q-Enzyme and the Nature of the Substrate for Q-Enzyme, FEBS LETTERS, Vol. no. 54, pp. 201-205.
34. Santi, I., Pezzicoli, A., Bosello, M., Berty, F., Mariani, M., TELford, J. L., Grondi, G., M. Soriani, G., (2008). Functional Characterization of a Newly Identified Group B Streptococcus Pullulanase Eliciting Antibodies Able to Prevent Alpha-Glucans Degradation, PLOS ONE www.plosone.org, Vol. no. 3, pp. 1-13.
35. Saha, B. C., and Zeikus, J. G., (1990). Characterization of Thermostable Cyclodextrinase from *Clostridium thermohydrosulfuricum* 39E, Applied

and Environmental Microbiology, Vol. no. 56, pp. 2941-2943.
36. Kubo, A., Fujita, N., Harada, K., Matsuda, T., Satoh, H., and Nakamura, Y., (1999). The Starch-Dbranching Enzymes Isoamylase and Pullulanase are Both Involved in Amylopectin Biosynthesis in Rice Endosperm, Plant physiology, Vol. no. 121, pp. 399-409.
37. Megazyme International (2008). Assay of Pullulanase Using Red Pullulan. pp. 136-140
38. Crabb, W. D., and Shetty, J. K., (1999). Commodity Scale Production of Sugars from Starch, Current Opinion in Microbiology, Vol. no. 2, pp. 252-256.
39. Urlaub, H., and Wober, G., (1975). Identification of Isoamylase A Glycogen-Debranching Enzyme From *Bacillus amyloliquefaciens,* FEBS LETTERS Vol. no.57. pp.1-4.
40. Bongaerts, R. J. M., Heinz, H.P., Ulrichadding, and Zysk, G., (2000). Antigenicity, Expression and Molecular Characterization of Surface-Located Pullulanase of *Streptococcus Pneumoniae*, Infection and Immunity, Vol. no.68 (12), pp. 7141-7143.
41. Bertoldo, C., and Antranikian, G., (2002). Starch-Hydrolyzing Enzymes from Thermophilic Archaea and Bacteria, Current Opinion in Chemical Biology, Vol. no. 6, pp. 151-160.
42. Stankovic, I., (2005). Pullulan; Chemical and Technical Assesment, JECFA Vol. 65, pp. 1-8.
43. Fielder, M., Pirt, S.J., Tarpey, I., Wilson, C., Cunningham, P., Ettelaie, C., Binder, A., Bansal, S., and Ebringer, A., (1995). Moleucular Mimicry and Ankylosing Spondylitis: Possible Role of a Novel Sequence in Pullulanase of *Klebsiella pneumoniae*, FEBS LETTER, Vol. no. 369, pp.243-248.

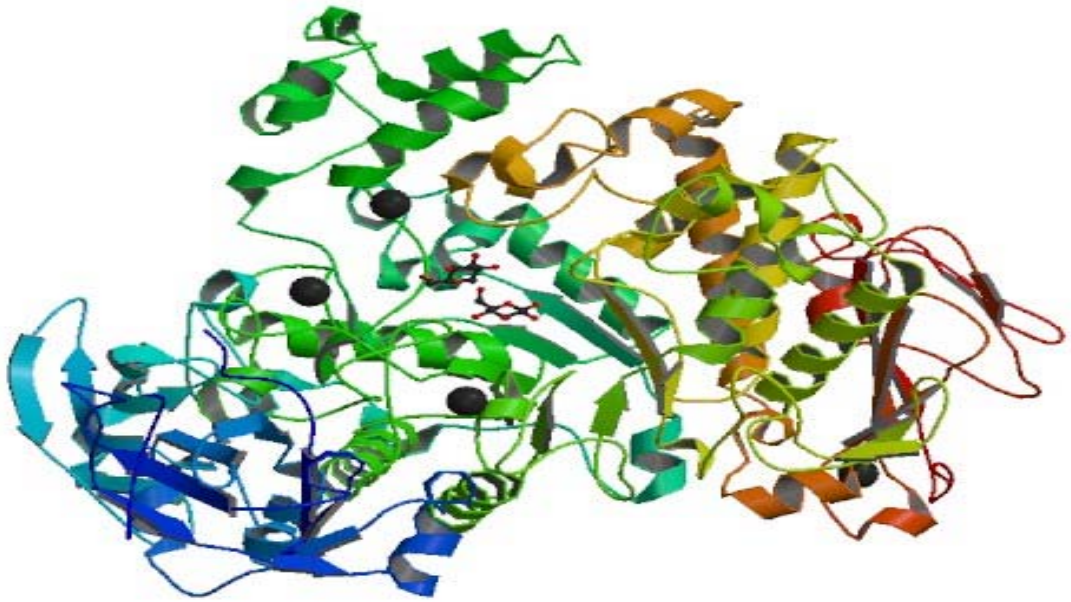

Fig.1 Biological assembly 1(500 pixels)

Crystal Structure Analysis of *Klebsiella pneumoniae* Pullulanase complexed with isomaltose. Assumed biological Molecule (Protein data bank www.rcsb.org/pdb/images/2fh8)

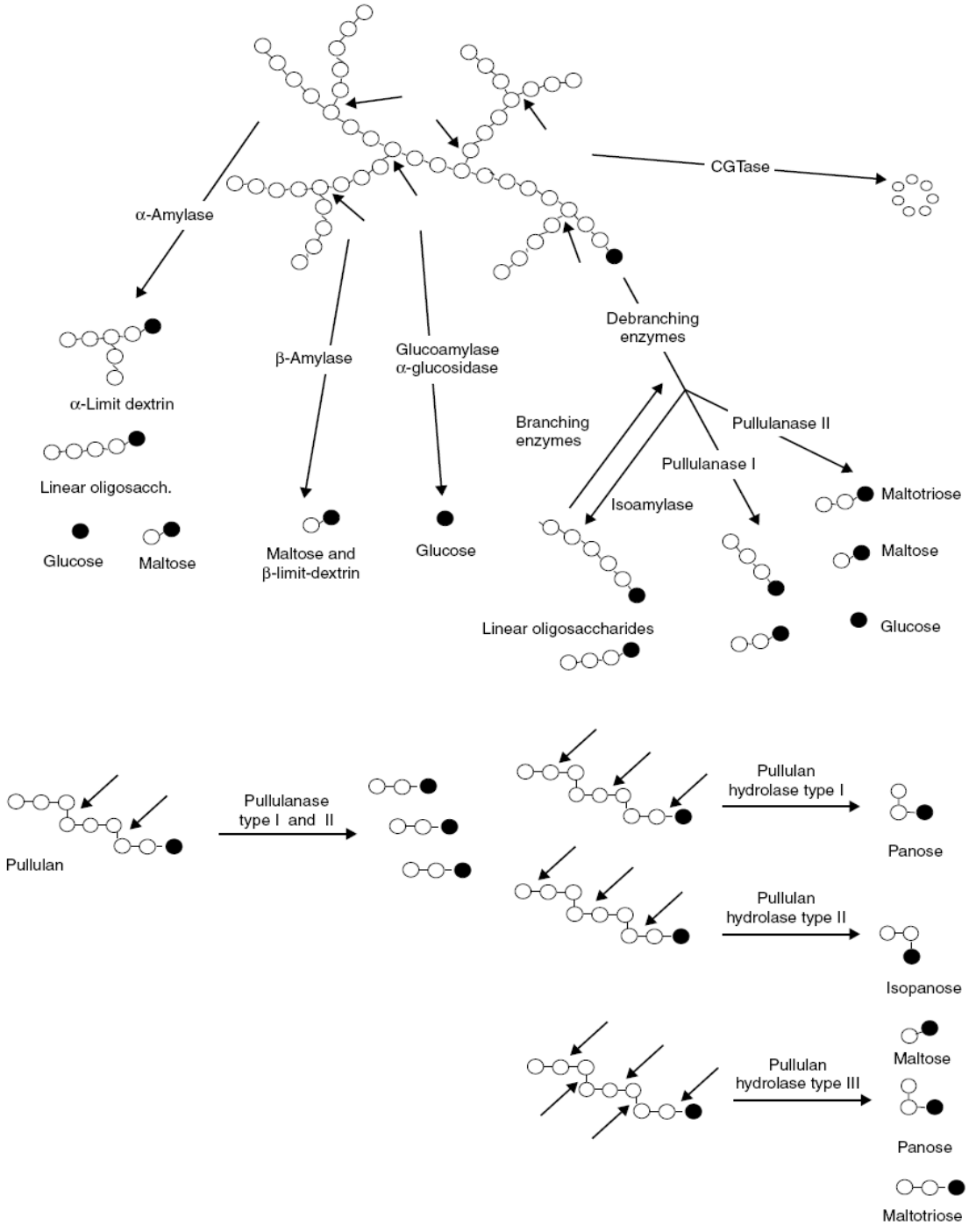

Fig.2 The action patterns of starch-hydrolyzing enzymes (Bertoldo and Antaranikian, 2002)