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Temperature Adaptation Study on Probiotic *Bacillus subtilis SK09* Based on its Extracellular Proteins

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Abstract: The assessment of the thermal adaptation characteristics of a probiotic organism is crucial since the probiotic bacteria are sensitive to thermal stress created by the gut environment. Probiotic *Bacillus subtilis SK09* was subjected to varying temperatures growth conditions and checked for its thermal adaptation. Experiments were carried out with cultures grown in Nutrient broth at three different temperatures namely 25, 35 and 40ºC. After 24 h the extracellular secretions present in the cell free supernatant were analyzed for amylase and β-galactosidase activity as well the net protein content. *Bacillus subtilis SK09* showed a prominent growth and protein profile in 35ºC with maximum enzyme activity and total protein production compared to other two conditions. At 40°C the strain exhibited a 30 kDa protein which might have been induced due to thermal stress.

Key words: Amylase, β- galactosidase, thermal adaptation, thermal stress, probiotic

INTRODUCTION

The use of human friendly microorganism such as probiotics for the consumption as food additive and a therapeutic is increasing rapidly (Conway *et al*., 1987; FAO/WHO, 2002; Donkor *et al*., 2007; Fuller, 1994). Probiotics are live microorganisms which when administrated in adequate amounts confer to health benefit or nutrition of the host by improving the intestinal microbial balance (Fuller, 1994). Various bacterial strains which can ferment lactose are used as probiotic supplement for lactose intolerance people. The majority of commercially available probiotic formulations contain *Lactobacilli* sp. as they are more tolerant and more stable in food products. But *Lactobacilli* sp. viability in the stomach is greatly affected by its inability to form spore and endure the acidic environment of the stomach (Crittenden *et al*., 1996; Ma´ire Begley *et al*., 2006). To be most effective in improving lactose utilization in lactose mal- absorbers, it is desirable that the cultures maintain both viability and β - galactosidase activity during their colonization in gut (Lorca and Valdez, 1998). Therefore spore forming probiotic such as *Bacillus subtilis* with lactose fermenting ability can be choice of probiotic organism for the use in lactose intolerance people.

The *Bacillus subtilis SK09* was isolated previously by us from primary clarifier of dairy effluent, this strain has the ability to ferment lactose as well sporulate under stress environment (Sreekumar and Soundarajan, 2010a). The quality of successful probiotic organisms is determined by its extracellular secretions such as enzymes, byproducts and antibiotics (Kirjavainen *et al*., 2001). These secretions are highly temperature sensitive (Jones *et al*., 1987). Here we discuss the temperature adaptation of *Bacillus subtilis SK09* at three different temperatures, which correspond to varying internal body temperatures (Revutskii, 1959). Under these conditions, the synthesis of specific sets of proteins, might be induced or enhanced. The role that these proteins play in helping lactose fermentation is yet to be elucidated. Amylase (51 kDa) is one of the key enzymes produced by *B. subtilis* (Celal and Çirakolu, 2001) for starch hydrolysis, which was taken as the criteria to check the variation in amylase activity in the extracellular secretions at varying temperatures. Lactose hydrolyzing enzyme β - galactosidase (116 kDa) produced at varying temperature was also quantified for its activity.

MATERIALS AND METHODS

The study was conducted during January - March 2010 at Department of Biotechnology, St. Joseph's College of Engineering, Old Mamallapuram Road, Chennai-600119, India.

Microorganism and culture conditions: The experimental strain *Bacillus subtilis SK09* was isolated previously from primary clarifier of dairy effluent and characterized by us (Sreekumar and Soundarajan, 2010b). The cultures were maintained in Nutrient agar slants (M001, HiMedia, India) at 4ºC. 24 h prior to the day experiment the cultures were thawed to room temperature. Nutrient broth as Primary inoculums was prepared in aliquots of 10ml and sterilized in an autoclave at 121ºC and 15psi; these were inoculated with one loopful of

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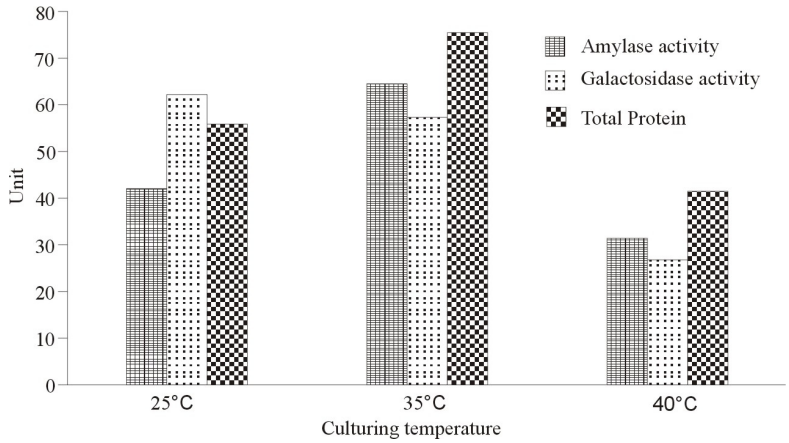

Fig. 1: Enzyme activity profile of amylase, β-galactosidase and total proteins of *Bacillus subtilis SK09* cells grown at 25, 35 and at 40ºC for 24 h

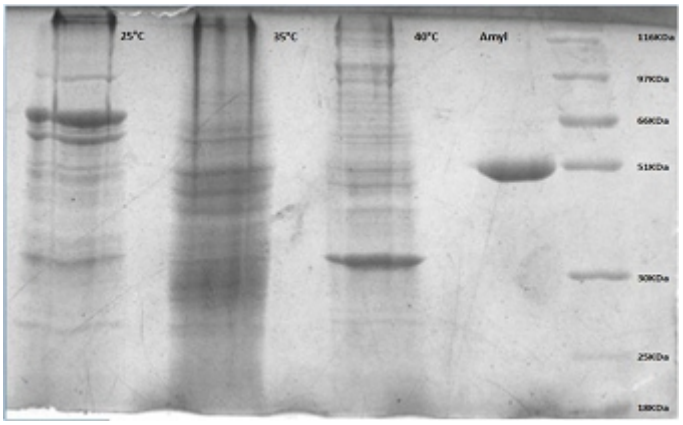

Fig. 2: SDS-PAGE profile of total proteins of *Bacillus subtilis SK09* cells grown at 25, 35 and at 40ºC for 24 h

experimental organism from nutrient agar slants. The cultures were grown overnight at 37ºC at 150 rpm.

Temperature stress: Triplicates of 100 mL Nutrient broth was prepared in a 500 mL Erlenmeyer flask with sterile distilled water and adjusted to pH 7.0, then sterilized in an autoclave at 121ºC and 15psi. The broth was cooled in a laminar air chamber and inoculated with 5% v/v of primary inoculum. Each of these flasks was placed in a rotary shaker maintained at 25, 35 and 40ºC for 24 h and 150 rpm. After 24 h the microbial cultures were centrifuged in sterile 50 mL round bottomed centrifuge tubes at 10000 rpm for 15 min and 4ºC. The cell free supernatant was collected in sterile 100 mL polystyrenes storage vials and stored at 4ºC for future use.

Amylase activity: The amylase activity of extracellular secretions was quantified by analysis starch hydrolysis. 10 mL of supernatant from each batch was added to 90 mL of 1% starch solution. The reaction mixture was continuously stirred using a magnetic stirrer for 1 h (Tanyildizi *et al*., 2005). 1 mL of reaction mixture diluted 1:10 times with Deionized water was assayed for starch hydrolysis using glucose oxidase peroxidase enzymatic method (GOD/POD kit, Medox, India) (Anjan, 2007). The OD was observed at 540 nm and the absorbance was compared with 1 mg/mL glucose standard to estimate the extent of starch hydrolysis.

Beta galactosidase activity: Beta galactosidase activity was estimated using 2-Nitrophenyl-β-D-galacto pyranoside (ONPG- sigma, India) as previously reported (Lederberg, 1950). 1 mL of collected supernatant was added to 3 mL of 5% ONPG in pH 7.0-0.05M phosphate buffer. The reaction was stopped 15 min with 1 mL of 1N Sodium carbonate (Na_2CO_3) solution. The reaction mixture was then analyzed for its optical density in UV-Visible double beam spectrophotometer (Systronics 2201, India) at 420 nm. The readings were compared with standard O-Nitrophenol (ONP) curve.

Total protein analysis: The total protein content in the supernatant at each of temperatures was quantified using the method described by (Bradford, 1976). Bovine Serum Albumin (BSA) was used as the standard protein. All dilutions were done from the 0.1 mg/mL stock solution.

SDS-PAGE analysis: The supernatant was used for protein analysis by SDS-PAGE (Laemmli, 1970), using molecular weight markers in a range of 10 to 150 kDa (Medox, India). Polyacrylamide gels were silver stained for protein detection (Oakley *et al*., 1980).

RESULTS AND DISCUSSION

The graph at Fig. 1 shows the effect of varying temperature conditions on Amylase activity, Galactosidase activity and total protein content. It is clearly seen that the temperature has a very definitive impact on all the above said characteristics. At 25 and 35ºC the amylase activity is considerably more than compared to that of 40ºC. The amylase activity is maximum at 35ºC which corresponds to more production of amylase as seen in the SDS PAGE at 51 kDa (Fig. 2).

The β - galactosidase activity is seen more at 25ºC than at other two temperatures. The total protein was found to be maximum at 35ºC and also seen prominently in SDS-PAGE with more number of bands. At 40ºC the culture showed very less protein secretion which can be attributed to thermal stress. The 40ºC lane in the gel shows a prominent band at 30 kDa, which may be a stress adaptation protein.

CONCLUSION

The *Bacillus subtilis SK09* strain showed good protein profile at 35ºC which is an optimum temperature for a better probiotic activity. The strain also showed stress related adaptation for a thermal stress of 40ºC by inducing 30 kDa protein, this fact would suggest that *B. subtilis SK09* is able to increase the synthesis of a number of thermal responsive proteins to cope with increasing temperatures. At the same time, it was observed that the repression of many proteins occurred at 40ºC compared with the profile at 35ºC, indicating that not only induction but also repression controls were set up at 40ºC. Therefore it is proved that *Bacillus subtilis SK09* strain is capable enough to survive and adapt to the internal gut temperatures and express its probiotic activity.

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REFERENCES

Anjan, B., 2007. Development of a rapid and inexpensive plasma glucose estimation by two-point kinetic method based on glucose oxidase-peroxidase enzymes. Ind. J. Clin. Biochem., 22(1): 156-160.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Celal, Ü. and Ç. Çirakolu, 2001. α-Amylase production by *Bacillus subtilis* and *B. amyloliquefaciens* in different PEG solutions. World J. Microbiol. Biotechnol., 17(1): 93-94.

Conway, P.L., S.L. Gorbach and B.R. Goldin, 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J. Dairy Sci., 70: 1-12.

Crittenden, R.G. and M.J. Playne, 1996. Production, properties and applications of food-grade oligosaccharides. Trend. Food Sci. Technol., 7: 353-361.

Donkor, O.N., S.L.I. Nilmini, A.P. Stolic, T. Vasiljevic and N.P. Shah, 2007. Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. Int. Dairy J., 17(6): 657-665.

Revutskii, E.L., 1959. Temperature changes in the human gastrointestinal tract and its rhythmic activity when empty. Bull. Exp. Biol. Med., 47(5): 29-32.

FAO/WHO, 2002. Expert Consultation: Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Cordoba, Argentina. Retrieved from: http://www.who.int/foodsafety/publications/fs_management/probiotics/en/.

Fuller, R., 1994. Probiotics: An overview. In: Gibson, S.A.W. (Ed.), Human Health: The Contribution of Microorganisms. Springer-Verlag, New York, NY, pp: 63-73.

Lorca, G.L. and G.F. de Valdez, 1998. Temperature adaptation and cryotolerance in *Lactobacillus acidophilus*. Biotechnol. Lett., 20(9): 847-849.

Jones, P.G., R.A. Van Bogelen and F.C. Neidhardt, 1987. Induction of proteins in response to low temperature in *Escherichia coli*. J. Bacteriol., 169: 2092-2095.

Kirjavainen, P.V., E. Apostolou, T. Arvola, S.J. Salminen, G.R. Gibson and E. Isolauri, 2001. Characterizing the composition of intestinal microflora as a prospective treatment target in infant allergic disease. FEMS Immunol. Med. Microbiol., 32(1): 1- 7.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Lederberg, J., 1950. The beta-d-galactosidase of *Escherichia Coli*, *strain K-12*. J. Bacteriol., 60: 381-392.

Tanyildizi, M.S., D. Özer and M. Elibol, 2005. Optimization of α-amylase production by *Bacillus* sp. using response surface methodology. Process Biochem., 40(7): 2291-2296.

Ma´ire, B., C. Hill and C.G.M. Gahan, 2006. Bile salt hydrolase activity in probiotics. Appl. Environ. Microbiol., 72(3): 1729-1738.

Oakley, B.R., D.R. Kirsch and N.R. Morris, 1980. Experimentally improved reliability of ultrasensitive silver staining of protein in polyacrylamide gels, Anal. Biochem., 105: 361-363.

Sreekumar, G. and K. Soundarajan, 2010a. *Bacillus subtilis strain SK09*, 16s Ribosomal RNA Gene Sequence. Retrieved from: http://www.ncbi.nlm.nih.gov/nuccore/297251625.

Sreekumar. G. and K. Soundarajan, 2010b. Isolation and characterization of probiotic *Bacillus subtilis SK09* from dairy effluent. Ind. J. Sci. Technol., 3(8): 863-866.