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Studies on isolation, characterization and thermal adaptation of probiotic *Bacillus subtilis* SK09

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Health promoting microorganisms such as Probiotics are recently been used as food additive and therapeutic supplement in order to enhance prophylaxis and digestion. A Probiotic which can be given as a supplement to lactose intolerance people to enhance their digestion of dairy products would be of a good economic and therapeutic value. Various bacterial strains such as *Lactobacilli* which can ferment lactose and their viability in the stomach are greatly affected by its inability to form spore and endure adverse environment of the digestive tract. Moreover, probiotic bacteria are sensitive to thermal stress created by the gut environment and hence it is crucial to assess the thermal adaptation characteristics of a probiotic organism.

Here we report the isolation and characterization of a spore forming Probiotic *Bacillus* species, with the ability to ferment lactose, from dairy effluent. Biochemical test and 16S rRNA sequencing were done in order to establish the species and was found to be *Bacillus subtilis* strain *SK09* with a unique ability to ferment lactose. This novel isolate was subjected to varying temperatures growth conditions and checked for its thermal adaptation. Experiments were carried out with cultures grown in Lactose peptone medium at varied temperature range from 25°C to 40°C. Periodical sampling were collected to ascertain the Biomass concentration and growth parameters such as Specific growth rate (μ), Doubling time (t_d) and Yield coefficient ($Y_{x/s}$) with reference to biomass formation. After 24h of growth period the extracellular secretions present in the supernatant were analyzed for amylase and β-galactosidase activity as well the net protein content.

The strain showed a maximum growth rate at 35°C and stress related adaptation for a thermal shock of 40°C by inducing 30kDa 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 tha t the repression of many proteins occurred at 40°C compared with the profile at 35°C, indicating that not only induction but also repression controls were set up at 40°C. 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. Lactose hydrolyzing enzyme β-galactosidase (116kDa) produced at varying temperature was also quantified for its activity. The role that these proteins play in helping lactose fermentation is yet to be elucidated.