Abstract

Isolation of Cellulolytic Bacteria from Soil and Identification by 16S rRNA Gene Sequencing and Characterization of Cellulase

Sazzad Khan[1], Faria Mahjabeen[1], M. Mahboob Hossain[1], Trosporsha Tasnim Khan[1*]

[1] Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, 66 Mohakhali, Dhaka-1212, Bangladesh.

Over the past few years, a noteworthy interest has been observed regarding the production of microbial enzymes, especially for the industrial applications. While, among many of the industrial used enzymes, microbial cellulases have been playing a major role in industrial applications as well as constitute a building block of a major group of the industrial enzymes. Despite attracting the attention of many researchers, it has tremendous industrial applications including textile industry, pulp, and paper industry, laundry and detergent industry, food, and animal feed industry. The research study aims to isolate, identify as well as screen bacteria with high cellulase activity from the soil sample. A total of 68 isolates were obtained from soil collected from a Dairy Farm, located in Dhaka division, Bangladesh. The following isolates were screened through clear zone ratio on CMC agar plate with Gram's Iodine. Out of 68 isolates, 31 isolates expressed better zone of hydrolysis and finally two of them (isolate 47 and 66) with maximum clear zone ratios were further evaluated by secondary screening for enzyme production. Between the two isolates, isolate 66 was selected as most efficient enzyme producer and so far had an extracellular crude enzyme activity of 0.167 U/ml and specific activity of 0.333 U/mg respectively. The potential isolate 66 was further presumptively identified through a series of biochemical tests, colony characteristics and cultural characteristics, and found that it belongs to the genus Bacillus. Molecular analysis using 16S rRNA gene of the isolate indicated it to be Bacillus subtilis. It was found to be a gram-positive, rod-shaped and spore-forming bacterium and was facultative anaerobe. The optimum pH and temperature for the activity of the crude enzyme were found to be 5 and 65°C respectively. However, further characterization of the enzyme, as well as the producer organism, is needed for the better understanding of its ability as a potential industrial cellulase producer.

Keywords: Bacillus subtilis, Cellulase, 16S rRNA