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# Production of certain extracellular enzymes by some bacteria and amplification of cellulase gene from *Bacillus* species

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Bacteria have received attention, due to their ability to produce extracellular enzymes beneficial in various industries. In the present study, extracellular enzyme production by two thermophilic bacteria (*Meiothermus ruber, Tepidimonas ignava*) and eight other bacterial isolates (*Bacillus thuringiensis, Bacillus amyloliquefaciens, Bacillus pumilus, Bacillus aryabhattai, Pseudomonas stutzeri, Pseudomonas aeruginosa, Sphingomonas* sp., *Burkholderia lata*) was investigated. Extracellular amylase, protease, pectinase and cellulase production was studied *in vitro* in media containing starch, skimmed milk, citric pectin and carboxymethylcellulose respectively, at 28 °C, 35 °C, 45 °C and 55 °C. Hydrolyzing Capacity Index (HCI) at day seven was calculated to identify the isolates, which hydrolyzed a substrate with minimal colony formation. Such isolates would have a higher potential in industrial applications. HCI values were analyzed using one-way ANOVA and Tukey's multiple comparison tests. All isolates, except thermophilic *M. ruber*, produced at least one extracellular enzyme within 1-3 days. *T. ignava*, *B. thuringiensis* and *P. aeruginosa* produced amylases. All isolates except *B. aryabhattai* and *M. ruber* produced proteases. *B. thuringiensis, Sphingomonas* sp., *B. amyloliquefaciens* and *P. stutzeri* produced cellulases. Pectinases were produced only by *B. lata*. Thermophilic *T. ignava* produced amylases and proteases at 28 ºC and 35 ºC but did not produce any enzyme at 55 °C, the temperature of the Maha Oya hot springs from which it was isolated. *B. amyloliquefaciens, P. stutzeri*, *P. aeruginosa*, *B. pumilus*, *Sphingomonas* sp. and *B. lata* produced proteases, which were stable at higher temperatures; 45 °C and 55 °C. It was the only enzyme to be produced at those temperatures. According to the HCI values, *B. thuringiensis* and *P. stutzeri* were the most efficient degraders of starch and cellulose, respectively. *P. stutzeri, Sphingomonas* sp. and *B. lata* were the best protein degraders. A gene coding for glycoside hydrolase (a cellulase) was amplified from bacteria by PCR using primers designed for *Bacillus licheniformis* ATCC 14580. Although expected amplicon size was ~1683 bp, amplicons of apporiximately 500 bp, 600 bp and 1000 bp were generated from cellulase producing *B. thuringiensis.* According to the information available in NCBI, *B. thuringiensis* has glycoside hydrolase gene of 738 bp suggesting that those amplicons could also be some glycoside hydrolase genes of different lengths. This should be confirmed by DNA sequencing. PCR product was generated by the same primers for *B. aryabhattai* as well, although it did not produce cellulases *in vitro*. It could be due to non-expression of the particular gene at the experimental conditions used in this study. These *Bacillus* species are perceived as sources of purified cellulases and the particular genes would be useful also in transformation of other organisms for industrial purposes.

**Keywords:** Bacteria, Extracellular enzymes, Cellulases, Glycoside hydrolase gene