# ABSTRACT

The purpose of the study was to isolate local organisms capable of producing pullulanase enzyme. A starch debranching enzyme named pullulanase was extracted from cultural filtrates of *Bacillus* species. Sampling was done from different locations including various food processing industries, dumping sites, canal sites as well as from gardens. 29 soil samples were collected and from these 29 samples, 34 bacterial strains were isolated and characterized morphologically and biochemically. On the basis of morphological and biochemical characterizations four bacterial iaolates were selected and were named as GCU-Bs-1.1, GCU-Bs-9.1a, GCU-Bs-20.1 and GCU-Bs-20.1. On the basis of 16S rRNA sequencing, it was revealed that GCU-Bs-1.1 showed 95 % homology with *Bacillus subtilis* whereas GCU-Bs-9.1a, GCU-Bs-20.1 and GCU-Bs-21.2 showed 94% homology with *Bacillus subtilis.* Four different media were used for fermentation studies, out of which medium number 4 was very effective for maximum enzyme production. GCU-Bs-1.1, GCU-Bs-9.1a, GCU-Bs-20.1 and GCU-Bs-21.2 showed 9.0 U/ml, 8.5 U/ml, 8.0 U/ml and 9.5 U/ml enzyme activities in M-4 respectively. All of four bacterial isolates showed maximum enzyme production at pH 6 and temperature 37°C. An inoculums size of 2 % and inoculum of 18 hours old age was resulted in maximum enzyme production. At 48 hours of incubation maximum production of enzyme was observed by all four bacterial isolates. GCU-Bs-1.1, GCU-Bs-20.1 and GCU-Bs-21.2 showed maximum enzyme production in 50ml of productive medium whereas GCU-Bs-9.1a showed maximum production at 25ml volume. Above 50 ml production was reduced due to lack of oxygen as *Bacillus subtilis* is an aerobic bacterium and increase in volume caused anaerobic conditions. Different carbon sources like dextrin, fructose, glucose, maltose and sucrose were used for fermentation by these four selected bacterial isolates. Out of these 5 carbon sources, dextrin and fructose were found to be best for maximum enzyme production. Enzyme was most active at 65°C when incubated at different temperatures. Optimum pH for enzyme activity was 6. Effect of metal ions was detected on pullulanase activity and it was found that in the presence of $Ca^{2+}$ and $Mn^{2+}$ ions its activity was enhanced but in the presence of $Cu^{2+}$, $Fe^{3+}$ and $Co^{2+}$ ions its activity was totally blocked by all bacterial isolates.