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Screening of potential bioremediation enzymes from hot spring bacteria using conventional plate assays and liquid chromatography - Tandem mass spectrometry (Lc-Ms/Ms)

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Abstract

The search for an eco-friendly, non-toxic, economical and efficient means of cleaning water through bioremediation is not only more favourable but critical to maintaining water quality globally especially in water-scarce countries. Thermophilic bacteria including Bacillus species are an important source of novel enzymes for biotechnology applications. In this study, 56 bacterial isolates which were cultured from five hot springs in South Africa were identified predominantly as Bacillus sp. or Bacillus-related spp by 16S rDNA gene sequencing. These isolates were screened for potentially useful enzymes for water bioremediation. Using conventional agar plate assays, 56% (n = 43), 68% (n = 38) and 16% (n = 31) were positive for amylase, protease and bromothymol blue decolorisation respectively. In liquid starch culture, three amylase-positive isolates differentially degraded starch by 34% (isolate 20S) to 98% (isolate 9T). Phenol degradation revealed that five out of thirty reduced phenol up to 42% by colorimetric assay. A thermophilic strain of Anoxybacillus rupiensis 19S (optimal growth temperature of 50 °C), which degraded starch, protein and phenol, was selected for further analysis by tandem LC-MS/MS. This newer technique identified potential enzymes for water bioremediation relating to pollutants from the food industry (amylase, proteases), polyaromatic hydrocarbons and dye pollutants (catalase peroxidase, superoxide dismutase, azoreductase, quinone oxidoreductase), antibiotic residues (ribonucleases), solubilisation of phosphates (inorganic pyrophosphatase) and reduction of chromate and lead. In addition, potential enzymes for biomonitoring of environmental pollutants were also identified. Specifically, dehydrogenases were found to decrease as the level of inorganic heavy metals and petroleum increased in soil samples. This study concludes that bacteria found in South African hot springs are a potential source of novel enzymes with tandem LC-MS/MS revealing substantially more information compared with conventional assays, which can be used for various applications of water bioremediation.