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Detection and Molecular Identification of Bacteria Forming Polyhydroxyalkanoate and Polyhydroxybutyrate Isolated from Soil in Saudi Arabia

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Abstract : Soil samples were collected from five different regions in the Kingdom of Saudi Arabia. Microbiological methods included dilution methods and pour plates to isolate and purify bacteria soil. The ability of isolates to develop biopolymer was investigated on petri dishes containing elements and substance concentrations stimulating developing biopolymer. Fluorescent stains, Nile red and Nile blue were used to stain the bacterial cells developing biopolymers. In addition, Sudan black was used to detect biopolymers in bacterial cells. The isolates which developed biopolymers were identified based on their gene sequence of 1 6sRNA and their ability to grow and synthesize PHAs on mineral medium supplemented with 1% dates molasses as the only carbon source under nitrogen limitation. During the study 293 bacterial isolates were isolated and detected. Through the initial survey on the petri dishes, 84 isolates showed the ability to develop biopolymers. These bacterial colonies developed a pink color due to accumulation of the biopolymers in the cells. Twenty-three isolates were able to grow on dates molasses, three strains of which showed the ability to accumulate biopolymers. These strains included Bacillus sp., Ralstonia sp. and Microbacterium sp. They were detected by Nile blue A stain with fluorescence microscopy (OLYMPUS IX 51). Among the isolated strains Ralstonia sp. was selected after its ability to grow on molasses dates in the presence of a limited nitrogen source was detected. The optimum conditions for formation of biopolymers by isolated strains were investigated. Conditions studied included, best incubation duration (2 days), temperature (30°C) and pH (7-8). The maximum PHB production was raised by 1% (v1v) when using concentrations of dates molasses 1, 2, 3, 4 and 5% in MSM. The best inoculated with 1% old inoculum (1= OD). The ideal extraction method of PHA and PHB proved to be 0.4% sodium hypochlorite solution, producing a quantity of polymer 98.79% of the cell's dry weight. The maximum PHB production was 1.79 g/L recorded by Ralstonia sp. after 48 h, while it was 1.40 g/L produced by R.eutropha ATCC 17697 after 48 h.

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