Isolation and study of native rhizobiums from the soil of agricultural areas of Bonaft-Dize village, Taft-yazd

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Rhizobia are gram-negative soil bacteria that form symbiotic relationships with legumes. The roots of these plants are able to absorb and fix atmospheric nitrogen, and this fixed nitrogen is made available to other plants. Rhizobia are able to store poly-β-hydroxybutyrate (PHB) in their cells. This substance is a precursor for the production of bioplastics. The aim of this research was to isolate and study indigenous rhizobia from agricultural soils and investigate their symbiotic relationship with host plants. The rhizobia were isolated from nodules on the root hairs of legumes. The nodules were washed with sterile distilled water and 0.1 % mercuric chloride to remove all bacteria on their surface. The nodules were then crushed with sterile forceps and inoculated into a special rhizobia culture medium called yeast extract mannitol agar (YEM) and incubated at room temperature for one week. To observe the morphology of the rhizobia, one of the nodules was placed on a microscope slide and crushed. A smear of the exudate was prepared and stained with Grame staining. After cultivation, a Sudan black staining was performed to observe PHB. Rhizobia have a mucoid colony morphology due to the production of an exopolysaccharide capsule. Simultaneously with the release of rhizobia into the root hair cells, these cells divide and enlarge, and the nodules become clearly visible. The rhizobial cells differentiate into bacteroids, which have a letter-like shape but revert to a rod-like shape after cultivation. Optimal growth took place at 25-30 °C and a pH value of 6-7. In the Sudan black staining, the PHB fat granules were visible as black areas with a transparent centre, while the bacteria were red. With further studies on the biodiversity of indigenous Rhizobium bacteria, they can be used to enrich agricultural soils and produce natural plastics.

Keywords: Rhizobia, symbiosis, leguminous plants, nitrogen fixation, PHB, bioplastics