ISOLATION AND CHARACTERIZATION OF A BACTERIAL STRAIN PRODUCING AMIDASES OF THE GENUS RHODOCOCCUS

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Abstract:
To isolate active strains of microorganisms capable of transforming nitriles, 11 samples were taken from territory of «Navoiyazot» JSC (Navoiy region) and analyzed. As a result, 19 strains belonging to the bacteria of the genus Rhodococcus have been isolated into a pure culture. The concentration of acrylic acid was determined in the supernatant using high performance liquid chromatography (HPLC).

Key words:
genus Rhodococcus, liquid chromatography (HPLC), amidase activity, acrylic acid, supernatant.

Bacteria utilize nitriles using two major alternative metabolic pathways: the nitrilase and nitrile hydrate-amidase pathways:

(1) nitrilase pathway for the hydrolysis of nitriles to carboxylic acid and ammonium;
(2) The next way is to decompose by nitrile hydratase to amide and by amidase to acid and ammonium.

The study of amidases and their producers requires genetic analysis and regulation of a diverse structure, properties, enzyme activity, and integration with other metabolic processes. Bacteria with high amidase activity play an important role in the biocatalytic extraction of various carboxylic acids.

In order to isolate active strains of microorganisms capable of transforming nitriles, 11 samples from wastewater, silt and soils contaminated with acrylonitrile, acrylamide and acrylic acid were taken from territory of «Navoiyazot» JSC (Navoiy, Uzbekistan) and analyzed. The strains were isolated by enrichment culture and direct seeding.

Acrylamide (acrylic amide), acrylonitrile and acetonitrile were used to select bacterial strains producing amidases on a solid nutrient medium.

To obtain a pure culture, morphologically different colonies were selected and their homogeneity was checked by double subculture on nutrient agar. The purity of the cultures was controlled by seeding nutrient agar on agar medium.As a result, 19 strains belonging to the bacteria of the genus Rhodococcus have been isolated into a pure culture.

The identification of strains was carried out on the basis of cultural, morphological and biochemical characteristics according to the Bergey determinant, the guidelines of I.B. Ivshina and O.A. Nesterenko.

The concentration of acrylic acid was determined in the supernatant using high performance liquid chromatography (HPLC). Serial dilutions of pure acrylamide and acrylic acid preparations were used as controls. As a result of the screening, 2 strains with amidase activity for acrylic acid were selected. It was found that strains of Rhodococcus sp. – 8/4/1 and Rhodococcus sp. – 3/4/3 synthesize amidase with an activity of 0.712 U/mg and 0.868 U/mg, respectively.

During the biotransformation of NAA, acrylamide and acrylic acid were found in the medium. This made it possible to establish the presence of the nitrile hydratase-amidase metabolic pathway in the strain; nitrile hydratase/amidase enzyme system of Rhodococcus sp. – 8/4/1 nitrile hydratase converts NAA into AA, which is further converted into AA by the action of amidase.

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