



Characterization Of A Bacterium – Producer Of Amidase

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ABSTRACT

We analyzed 11 samples of wastewater, sludge and soil contaminated with acrylic acid nitrile, acrylamide and acrylic acid from the territory of Navoiyazot JSC (Navoi, Uzbekistan). As a result, 19 strains belonging to the bacteria of the genus *Rhodococcus* were isolated into a pure culture. In the process of selection, 2 strains of bacteria were selected that exhibit the highest amidase activity for acrylic acid (0.712 U / mg and 0.868 U / mg). Strains of *Rhodococcus* sp. - 8/4/1 and *Rhodococcus* sp. - 3/4/3 are potential amidase producers.

KEYWORDS

Strain, Acrylic Acid, Producer, Amidase, Enzyme Activity.

INTRODUCTION

Rhodococci possess a high level of adaptation to extreme conditions of existence [1,2], are characterized by such unique biological properties as pleomorphism, the ability to coaggregate, have a complex morphogenetic

development cycle, and these characteristics determine the presence of various methods of cell cooperation [3]. Cooperation promotes the establishment of cell contact with each other, their retention in colonies, adsorption

on the surface of drops of hydrophobic substrates and soil particles, as well as the formation of biofilms on the surface of carriers used in biotechnological processes.

Rhodococci have been isolated from various sources, such as soil, groundwater, marine sediments, internal organs of insects, sick and healthy animals or plants [1, 2, 5]. They are widely used in the bioconversion of various organic substrates [6]. The multi-ton synthesis of acrylamide (AA) over many years, launched at several production sites around the world, is regarded as the most outstanding example of the cellular process for this group of bacteria, as well as the possibility of being used as biocatalysts for the production of other useful chemicals, in particular, acrylic acid. Acrylic acid is a raw material for the production of polymers and copolymers for various purposes, widely used in all spheres of the national economy [7-10].

In this regard, the aim of this work is to isolate local strains of bacteria - producers of nitrile transforming enzymes, in particular amidases, and to assess the possibility of their use for obtaining acrylic acid.

MATERIALS AND RESEARCH METHODS

To isolate microorganisms with amidase activity, wastewater, sludge and soil contaminated with nitrile acrylic acid, acrylamide and acrylic acid JSC "Navoiyazot" (Navoi, Uzbekistan) were used.

The strains were isolated by the method of enrichment culture and direct seeding. In the case of direct seeding, a soil sample of 10 g was suspended in 100 ml of sterile phosphate buffer, defended at a temperature of 25-27 °C

for 1 hour, the supernatant liquid in an amount of 100 µl was applied to the surface of the agar medium Nutrient agar (Himedia, India) containing acetonitrile at a concentration of 2-5%, acrylonitrile at a concentration of 0.1-1% and rubbed with a spatula. In the case of using sludge or effluents as samples, the samples were not diluted in phosphate buffer, inoculation was made directly into nutrient agar.

To grow enrichment cultures and isolate nitrile-converting strains of microorganisms, 10 g or 10 ml of the sample was added to nutrient media: Tryptone-soy broth (Himedia, India) or peptone-containing (PS) medium (g / L): peptone - 5.0, KH₂PO₄ - 0.5, MgSO₄ * 7H₂O - 0.5, NaCl - 0.5, CoCl₂ * 6H₂O - 0.01 and glucose - 10.0 with the addition of acetonitrile and acrylonitrile, the pH of the medium is 7.0-7.3.

Identification of strains was carried out on the basis of cultural, morphological and biochemical characteristics according to Bergey's guide, guidelines of I.B. Ivshina and O.A. Nesterenko [1, 2, 11].

The morphology and life cycle of the strains were studied during 6, 12, 18, 48, 72 hours of cultivation on nutrient agar (HIMEDIA) and liquid nutrient medium PS using light microscopy.

The transformation of NAA into AA and Acrylic acid was carried out using the biomass of the studied strains, preliminarily grown in a liquid nutrient medium PS, in 10 mM potassium phosphate buffer, pH 7.5, with a concentration of NAA of 1-2%. The reaction was carried out at a temperature of 20-25 °C for 10 and 30 min and was stopped by the addition of 0.1 ml of 1 N

hydrochloric acid. HCl. The samples were centrifuged at 12000 rpm for 7 min. The concentration of AA and Acrylic acid was determined in the supernatant using high performance liquid chromatography (HPLC). Serial dilutions of pure preparations of NAC, AA, and Acrylic acid were used as a control.

RESULTS AND DISCUSSION

To isolate active strains of microorganisms capable of transforming nitriles, samples of wastewater, silt and soil contaminated with NAC, AA and Acrylic acid were analyzed. Sampling was carried out from the territory of production of JSC "Navoiyazot" in the areas where the production facilities for the production of NAC and Acrylic acid are located. In order to isolate active strains capable of transforming NAA into AA and Acrylic acid, 11 samples were analyzed. Individual colonies of microorganisms were isolated by the method of enrichment culture and direct seeding. As a result, 19 strains were isolated from wastewater, silt and soil samples into a pure culture.

All investigated strains are gram-positive aerobes, immobile, non-acid-resistant, non-spore-forming. Cells are rod-shaped, weakly branching. V-shaped arrangement of cells is characteristic. A distinctive feature of the studied strains is a three-stage morphogenetic development cycle (cocci - rod-shaped, filamentous or branching cells - cocci). On nutrient agar at a temperature of 28 °C, pH = 7.0, colonies of a soft consistency without aerial mycelium with pink, dark pink, orange-

red non-defending pigments are formed. On the basis of morphological and cultural properties, the isolated strains were assigned to the genus *Rhodococcus*.

Isolated in pure culture strains of bacteria belonging to the genus *Rhodococcus* were studied for their ability to synthesize amidase. The ability of isolated strains of bacteria of the genus *Rhodococcus* to transform NAA into AA and Acrylic acid was studied using cell biomass. The cultures were grown in a liquid nutrient medium PS for 72 - 96 hours, then the cells were precipitated by centrifugation, the sediment was used for the ability of cultures to transform NAC into AA and Acrylic acid.

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 activity 0.712 U / mg and 0.868 U / mg, respectively. It is known that some strains of rhodococci, such as *R. rhodochrous* M-33 and *R. ruber* GT1, have high nitrile hydrolysis activity, which determined their use in the industrial synthesis of acrylamide [13, 14].

During the biotransformation of NAC, acrylamide and acrylic acid were detected in the medium. This made it possible to establish the presence of a nitrile hydratase-amidase metabolic pathway in the strain, i.e. enzyme system nitrile hydratase / amidase of the *Rhodococcus* sp. - 8/4/1 converts NAC to AA in two stages: nitrile hydratase converts NAC to AA, which is then converted to Acrylic acid by the action of amidase.

Screening of isolated strains of bacteria of the genus *Rhodococcus* with amidase activity

Nº	Bacterial culture strains	Amidase activity (U / mg)
1	<i>Rhodococcus</i> sp. - 8/4/1	0,712
2	<i>Rhodococcus</i> sp. - 8/2/1	-
3	<i>Rhodococcus</i> sp. - 8/2/2	-
4	<i>Rhodococcus</i> sp. - 1/1	0,050
5	<i>Rhodococcus</i> sp.- п 1/5/1	0,037
6	<i>Rhodococcus</i> sp.- 3/5/1	-
7	<i>Rhodococcus</i> sp.- 3/1 CAAK	0,324
8	<i>Rhodococcus</i> sp.- 3/4/3	0,868
9	<i>Rhodococcus</i> sp - 2/5	0,460
10	<i>Rhodococcus</i> sp.- 2/3/6	0,226
11	<i>Rhodococcus</i> sp. - 5/3	-
12	<i>Rhodococcus</i> sp.- 52	-
13	<i>Rhodococcus</i> sp. - 3/5/2	-
14	<i>Rhodococcus</i> sp.3/2/2	0,115
15	<i>Rhodococcus</i> sp. 2/3/6 cp	0,114
16	<i>Rhodococcus</i> sp. 3/2/1Sf	0,115
17	<i>Rhodococcus</i> sp. 2/4/2	0,112
18	<i>Rhodococcus</i> sp. п 2/7/2	0,230
19	<i>Rhodococcus</i> sp. 3/7 (32)	-
(-) undefined		

CONCLUSION

The strains of *Rhodococcus* sp. - 8/4/1 and *Rhodococcus* sp. - 3/4/3 are potential amidase producers. Further improvement of this strain will serve to obtain a potential biocatalyst for the biocatalytic synthesis of acrylic acid.

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