



Colonial–morphological characteristics of dissociatives of the *Rhodococcus ruber* - 8/4/1 strain

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ABSTRACT. This article provides a study of the molecular, physiological, biochemical and morphological features of R-, S- and M-dissociants of *Rhodococcus ruber* - 8/4/1. The sequence similarity of the genome fragments of R- and S-dissociant forms encoded by 16S rRNA was demonstrated. MALDI spectra were obtained for the *R. ruber*-8/4/1 strain and its dissociants. Under standard analysis conditions, the MALDI profiles of R-, S- and M- forms dissociants of the *Rhodococcus ruber* - 8/4/1 were similar. The determination of the resistance of the strain dissociants to external factors showed that R-forms were more stable under UV irradiation and increased temperature. The S-forms showed stability upon the conversion of acrylic acid nitrile into acrylamide (AA), whereas the M-forms were stable under a decrease in the cultivation temperature, and an increase in the concentration of NaCl in the medium. The S-forms proved to be the most active in the transformation of acrylonitrile into AA.

Keywords: dissociation; forms; strain; *Rhodococcus ruber* - 8/4/1; biotransformation; nitrile hydratase.

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Introduction

Bacteria of the genus *Rhodococcus* have a wide range of metabolic capabilities. They synthesize enzymes (dehydrogenases, oxygenases, nitrile hydratase) with broad substrate specificity and resistance to toxic compounds. They are also active biodegradants of hydrocarbons and their derivatives, which are hard to reach and toxic to other microorganisms (Kim et al., 2018; Cappelletti et al., 2020). Adaptation of bacteria of the genus *Rhodococcus* to changing environmental conditions and to toxic compounds can occur due to changes the process of dissociation, that is, the splitting of a homogeneous population into other forms. Depending on the morphology of the colonies, the following main dissociants of *Rhodococcus* are distinguished: R - form (rough) has a rough type of colonies, S form (smooth) - smooth, M form (mucoid) - mucous. The splitting of bacteria of the genus *Rhodococcus* into R, S and M forms of dissociants, which are based on significant differences in the size and structure of cell membranes, is also accompanied by a change in their physiological and biochemical characteristics: the rate of release of substances from the cell; cell resistance to external influences; growth rate; activities of a number of membrane-dependent enzymes (Kolomytseva et al., 2005; Ivshina et al., 2021). According to the results of studies conducted (Iwabuchi et al., 2002), it was shown that exopolysaccharides and mycolic acids can significantly affect the hydrophobic properties of *Rhodococcus* cells and help in the transformation of cell morphotypes, due to changes in the habitat of strains and adaptive interaction between cells and substrates.

There has been an increase of interest in this particular bacterial characteristic, in connection with its practical application. For instance, the activities of the R- and S-forms of the *Rhodococcus sp.* USPTU 21 cultures are different. When (S)-1-heptenyl-3-acetate is obtained, the enzymatic reaction proceeds faster with a high yield of (S)-1-heptenyl- 3-acetate in the presence of the dissociant S – forms, and then the dissociant R- forms (Sharaeva et al., 2012). In contrast, the dissociant M - forms is the most productive for a *Bacillus mesentericus* strain synthesising a proteolytic enzyme complex (Milko et al., 2007), and the dissociant S - form of *Rhodococcus sp.* shows activity in the transformation of steroids (Voishvillo et al., 1993). The dissociant R - forms, with maximum cell wall thickness, dominates in bacterial populations that destroy toxic substances of pyridine by a *Rhodococcus aquosus* strain (Sharaeva et al., 2012). According to the results of

studies conducted during the biodegradation of diclofenac by the *Rhodococcus ruber* IEGM 346 strain, it was shown that the effect of diclofenac on *Rhodococcus* leads to morphological changes in cells. In the presence of diclofenac, significant cell aggregation occurred, as well as a change in the ζ -potential, an increase in hydrophobicity and total lipid content in cells (Ivshina, Tyumina, Kuzmina, & Vikhareva, 2019). The present work studied the phenotypic heterogeneity of *Rhodococcus rhodochrous* CNMN-Ac-05 in the presence of C60 fullerene. It was found that with an increase in the concentration of C60 fullerene to 100, the appearance of R-forms increased, indicating the strain's stress state. In these variants of the experiment, a decrease in the total number of CFU was observed (Postolachi, Rastimesina, & Josan, 2021). The dissociation process can lead to a decrease in the efficiency of microbiological production due to a change in the composition of the population and a high survival rate of the less active dissociator. During the cultivation of the *Rhodococcus ruber* TH3 strain in the fermenter, it was found that undesirable cell flocculation is associated with the process of colony dissociation, which occurred only in rough type cells (R-TH3), and not in smooth type cells. (S-TH3). It has been established that the hydrophilicity of S-TH3 cells is mainly due to the presence of EPS in the outermost layer of cells (Jiao, Chen, Yu, & Shen, 2017). Thus, knowledge of the biological characteristics of microorganisms associated with cell dissociation makes it possible to regulate the processes of maintaining the stability of useful properties, as well as to intensify various biotechnological processes.

The strain *Rhodococcus ruber* - 8/4/1, a highly active producer of nitrile hydratase (230 IU mg⁻¹ mL⁻¹), was isolated into a pure culture from soil samples of Navoiyazot JSC. The biomass of the strain effectively converts acrylonitrile to acrylamide (AA). A patent for the strain was obtained (Makhsumkhanov, Alimova, Pulatova, Kambaralieva, & Tashbaev, 2018). In the process of working with the culture, it was found that the strain has the ability to dissociate.

The purpose of this study is to assess the variability of the colonial and morphological characteristics of the dissociants of the strain *R. ruber* - 8/4/1 under the influence of various environmental conditions, and their ability to biosynthesis nitrile hydratase

Material and methods

The object of research was the bacterial strain *R. ruber* - 8/4/1, deposited under No. SKB-318 in the Collection of Microorganisms of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan, and its R-, S-, and M-forms. The pure bacterial cultures were maintained on nutrient agar medium (HiMedia, India) and Munz agar medium with 2% n-hexadecane from the volume of the nutrient medium.

To identify the variability in the colonial-morphological characteristics of dissociatives of *R. ruber* - 8/4/1 strain and to study their biological properties, we used the method of multiple subcultures. The morphology of cells and the developmental cycle of dissociants were investigated on live specimens of 24-, 48-, 72- and 96-hour cultures by light microscopy. The frequency of the occurrence of dissociants was studied on a solid nutrient medium (meat-peptone broth (MPB) with the addition of wort in a 1:1 ratio). The composition of the population was determined by plating bacteria on a solid nutrient medium, which made it possible to establish the percentage of dissociatives in the *R. ruber* - 8/4/1 culture. For each variant of the experiment, 10-12 plates were used, each of which was added with 1.0 mL of a cell suspension diluted in physiological solution to 10⁷ cells mL⁻¹. The plates were stored in a thermostat for 6-8 days at 28°C.

To investigate the growth, development, and nitrile hydratase biosynthesis of dissociants, we grew R-, S- and M- forms of *R. ruber* - 8/4/1 cultures in 50 mL of nutrient medium with the following composition (g L⁻¹): glucose, 10.0; urea, 6.0; peptone, 2.0; K₂HPO₄, 0.5; KH₂PO₄, 0.5; MgSO₄·7H₂O, 1.0; and CoCl₂·6H₂O, 0.03 at a pH of 7.0-7.5 and a temperature of 28°C on shaker incubator (150 rpm) for 72-96 hours. Cells of dissociant cultures grown on agar medium (MPB with the addition of wort in a 1:1 ratio) were used as inoculum. The inoculum was added at a concentration of 3%.

The growth ability of dissociants in the presence of various concentrations of NaCl was studied in tryptone soy broth diluted with distilled water (HiMedia, India) in a 1:1 ratio. The dissociatives were cultured in tubes containing 2, 4, 6, 8 and 10% NaCl in 10 mL of nutrient medium.

To study the effect of UV radiation on the dissociation process, we used dissociants grown in a nutrient medium for 48 hours. A cell suspension in a vegetative form at a concentration of 10⁷ cells mL⁻¹ at a wavelength of $\lambda = 254$ nm (2 x 15 W) was exposed to UV irradiation in different time modes using UVC-30 lamps. Irradiation was carried out in exposure modes of 3-, 10- and 20-second exposure. Irradiated and control cultures were plated on agar medium (MPB with the addition of wort in a ratio of 1:1) and cultured at 28°C for 6-8 days.

The optical density (OD) of the cell suspension was determined on a UV-5100 spectrophotometer (Metash, China) using a 1.0 cm cuvette. Bacterial growth was assessed based on the OD of the cell suspension at $\lambda = 540$ nm, considering the dilution. The absolutely dry biomass was determined by weighing on an analytical balance. The dry biomass was obtained by centrifuging living cells for 10 min. at 12,000 rpm which content was then subjected to drying until reaching constant cell mass.

Genetic analysis using polymerase chain reaction was carried out at the Centre for Advanced Technologies (Uzbekistan, Tashkent). The chromosomal DNA of the bacterium was prepared via the phenol–chloroform method (Kayumov & Gimadutdinov, 2016). To amplify the 16S rRNA gene region by PCR, we used the following universal primer pairs: 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-GACGGGCGGTGTGTRCA-3'. For PCR analysis, we prepared a Mastermix solution containing (per sample): water, 7.32 μ L; 10x PCR buffer, 1 μ L; BSA, 0.2 μ L, dNTPs, 0.08 μ L; primers, 0.2 μ L; and TaqPol, 0.2 μ L. The electrophoretic separation of PCR products was carried out in 1.5% agarose gel in Tris borate buffer at an electric field strength of 6 V cm^{-1} . PCR products were extracted from the gel using a gel extraction kit (Thermo Fischer, USA); the resulting PCR products were sequenced using a SeqStudio Genetic Analysr and a BigDye Terminator v. 3.1 Cycle Sequencing Kit (Thermo Fischer, USA).

To confirm that the isolated dissociants of the *R. ruber* - 8/4/1 strain belong to the same species, a proteomic analysis was performed using MALDI-TOF MS (EXPS2600 ZYBIO) based on the technology of time-of-flight mass spectrometry with laser desorption/ionization with the use of a matrix.

Nitrile hydratase activity was determined using the following procedure: 1 mL of the bacterial culture broth was centrifuged at 12,000 rpm for 10 min. The cells were then washed with 0.01 M phosphate buffer (pH 7.5) and resuspended in the same buffer. Acrylonitrile (AN) (10–15 μ L) was added to 1 ml of the cell suspension. The reaction was carried out at 20–25°C for 10 min. and then stopped by adding 0.1 mL of 1 N HCl. The unit of specific nitrile hydratase activity was the amount of nitrile hydratase contained in 1 mg of dried cells that catalyzed the formation of 1 μ mol acrylamide (AA) per minute ($\mu\text{M}^{\text{amide}} \text{mg}^{-1} \text{dry weight} \cdot \text{min}^{-1}$).

The AA concentration in the supernatant was analyzed by high-performance liquid chromatography (HPLC) on an LKB HPLC system using a Silosorb SPHC₁₈ column (150x4 mm, 8 μ m) or DISCOVERY-C₁₈ (75x4 mm, 5 μ m) pressure 40–60 bar, a UV detector with a 206 nm filter, and a mobile phase flow of 1.0 mL min^{-1} of the following composition: 0.01 M phosphate buffer or ddH₂O, 97%; CH₃CN, 3%; pH, 2.5 (adjusted with phosphoric acid). Serial dilutions of pure preparations of acrylonitrile and AA were used as controls. The retention time was 2.0 min. for AA and 3.0 min. for acrylonitrile. The HPLC calibration curve was used to quantify the AA in the sample.

All experiments were carried out three times unless mentioned in the text. Data were analyzed using GraphPad Prism by one-way analysis of variance (ANOVA) and Tukey to determine statistical significance. A p-value of < 0.05 was considered to be statistically significant. The correlation index was calculated using the Pearson coefficient (ρ).

Results and discussion

The predominant growth of one or another dissociant in a developing culture is determined by two main factors: its nutritional requirements and its resistance to changes in the physical and chemical factors of the environment (Milko & Milko, 2014). Thus, we studied the morphological and cultural properties of dissociants R-, S-, and M forms of the *R. ruber* - 8/4/1 strain. The effect of pH, UV, and various concentrations of NaCl on the dissociative variability of the strain was studied.

Morphological–cultural and physiological–biochemical properties of R-, S- and M - forms dissociants of the *Rhodococcus ruber* - 8/4/1 strain

The ability of the strain to form dissociants was observed during the storage of culture cells in 10% glycerol at -18°C, and when studying the effect of different concentrations of AA on the resistance of the strain's cells. The intensity of the process of strain cleavage during its long-term storage in 10% glycerine showed that the strain had three forms. The R-form colonies are rough, with a folded matte surface and uneven edges. In a liquid nutrient medium, they form sediments, sometimes films, while the medium remains transparent. S-form colonies are smooth, with even and sharply defined edges, convex, and shiny. Homogeneous growth was observed in liquid nutrient media. M-forms have colonies that are larger and slimy, spreading, shiny, lighter

than S-forms, and show homogeneous growth on liquid nutrient media (Figure 1). The colour of the colonies ranged from dark pink to orange-red. Cells obtained from colonies of the same type were subcultured on a solid medium. A clone that retains colonial morphological characteristics for three to four passages was considered a dissociant.

The frequency of the occurrence of S-, R- and M-forms of the *R. ruber* - 8/4/1 culture was studied. The initial S-forms dissociate into R- and M-forms with a constantly high frequency, and the process of dissociation towards the formation of R-forms was more intense than towards the formation of M-forms. R- and M-forms are reversed into S-forms, and the frequency of this process varies. The S-forms dissociated into R- and M-forms, with frequencies of occurrence of 2 and 0.5%, respectively. The R-forms transformed into the S-forms, with a dissociation frequency of no more than 0.2%.



Figure 1. Morphology of colonies of dissociants of the *Rhodococcus ruber* - 8/4/1 strain. *Rhodococcus ruber* - 8/4/1 strain was cultivated on the solid medium containing nutrient agar at 28 °C for 8-10 days in the growing chamber. Colonies are distinguished by their morphology forms: A - S form, colonies are smooth, with even edges, convex, shiny, with sharply defined edges, homogeneous; B - R form, colonies are rough, with a folded matte surface, with uneven edges; C - M form, colonies form larger and slimy, spreading, shiny.

When studying the growth and development of dissociatives in a liquid nutrient medium, we found that, with an initial number of cells of 10^4 cells mL^{-1} , the S-form had a higher growth rate compared with R-. Thus, after 24 hours of culture, the cell titre was 1.4×10^6 cells mL^{-1} . After 72 hours of cultivation, the cell titre increased to 2.1×10^8 cells mL^{-1} . After 72 hours of cultivation, the cell titre of the R-forms of the dissociant was no more than 1.4×10^5 cells mL^{-1} . The M-forms of the dissociant were less stable, with a cell titre of no more than 10^3 cells mL^{-1} ; when the cells were precipitated during centrifugation, no sedimentation occurred.

The investigation into cell morphology and development cycle of the studied forms during cultivation in a liquid nutrient medium showed no significant difference in the morphology of the R- and S-forms. In the initial stage of growth, the germination and branching of the original coccoid and short rod-shaped cells began with the formation of one to three growth tubes. In 24-48-hour cultures, branching cells and a small number of shorter fragments were present (Figures 2 and 3).

The disintegration of mycelium cells was observed after 72-96 hours of cultivation, with the formation of coccoid-shaped cells in the medium. During the cultivation of the S-form dissociant, the number of coccoid-shaped cells was significantly greater than during the cultivation of the R-form dissociant.

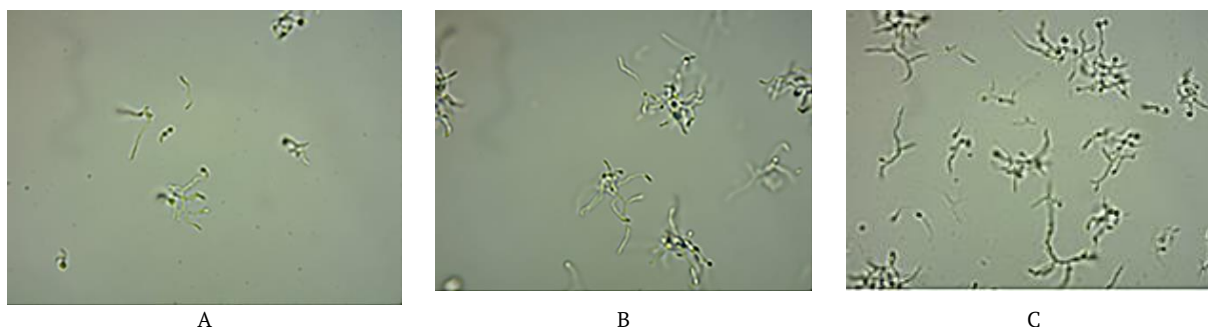


Figure. 2. Given pictures shows the analyses of cell morphology of the R-form of *Rhodococcus ruber* - 8/4/1 strain in the dynamics of growth and development. The R-form of the culture of *Rhodococcus ruber* - 8/4/1 were grown in 50 ml of nutrient liquid medium of the following composition (g L^{-1}): glucose - 10.0, urea - 6.0, peptone - 2.0, K_2HPO_4 - 0.5, KH_2PO_4 - 0.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 1.0, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ - 0.03, (pH=7.0-7.5) at a temperature of 28°C, on shaker incubator (150 rpm) for 72- 96 hours. A - 24 hours, B - 48 hours, C - 72 hours.

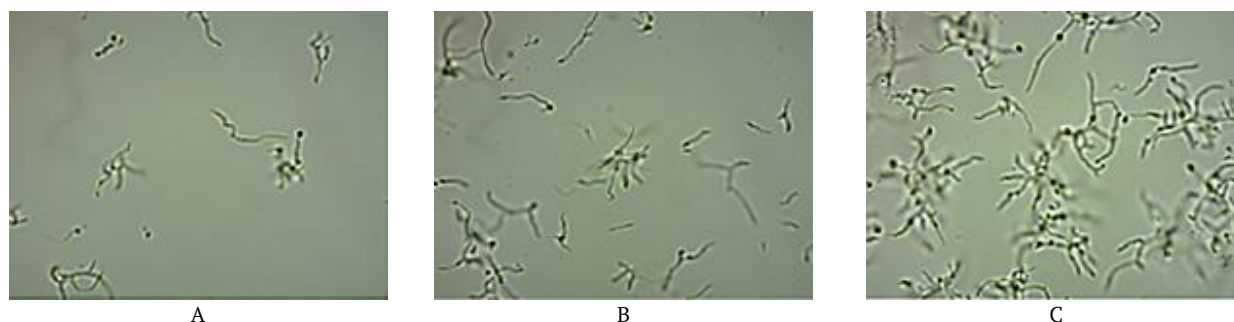


Figure 3. Given pictures shows the analyses of cell morphology of the S-form of *Rhodococcus ruber* - 8/4/1 strain in the dynamics of growth and development. The S-form of the culture of *Rhodococcus ruber* - 8/4/1 were grown in 50 mL of nutrient liquid medium of the following composition (g L⁻¹): glucose - 10.0, urea - 6.0, peptone - 2.0, K₂HPO₄ - 0.5, KH₂PO₄ - 0.5, MgSO₄*7H₂O - 1.0, CoCl₂*6H₂O - 0.03, (pH=7.0-7.5) at a temperature of 28°C, on shaker incubator (150 rpm) for 72- 96 hours. A - 24 hours, B - 48 hours, C - 72 hours.

Molecular properties of R-, S- and M- forms dissociants of *Rhodococcus ruber* - 8/4/1

To confirm that the three isolated dissociants belonged to the same species, we carried out a polymerase chain reaction of fragments of their genes encoding 16S rRNA, using a system of universal primers; the type strain *R. ruber* - 8/4/1 served as a control. The results showed that the sequence of 16S rRNA gene fragments of the R- and S- forms dissociants of the bacterium was identical to the sequence of the type strain.

Proteomic analysis of cells of bacterial R-, S- and M- forms dissociants of *Rhodococcus ruber* - 8/4/1

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) can analyze the protein composition of a bacterial cell and has emerged as a new technology for species identification. By measuring the exact sizes of peptides and small proteins, which are assumed to be characteristic for each bacterial species, it allows determination to the species level within a few minutes when the analysis is performed on whole cells, cell lysates, or crude bacterial extracts (Mellmann et al., 2008).

The phenotypic variability of bacteria causes variations in the composition of the proteins they synthesize. Therefore, the analysis of the protein spectra of dissociants can be used for their identification. Some bacterial strains having the same colony morphology can differ in peptide profile and substrate specificity when cultivated on different nutrient media (Böhme et al., 2013; Kryazhevskikh et al., 2015). Screening of the *Rhodococcus opacus* strain for dissociants showed that, within the same colony morphology, the strains had different substrate specificities in chlorophenol decomposition. For such dissociants, the MALDI test was the only effective detection method (Kryazhevskikh et al., 2015).

In the present work, MALDI spectra were obtained for *R. ruber* - 8/4/1 and its dissociants. The analysis of the MALDI spectra of the vegetative cells of the dissociatives of the studied strain included the determination of the similarity of the protein spectra. Under standard conditions of analysis in the genus–species–strain–dissociant series, the MALDI profiles in dissociants of the *R. ruber* - 8/4/1 were similar. Notably, no specific universal criteria for all microorganisms have been established for determining the species based on the analysis of mass spectra.

Influence of external environmental factors on the growth of R-, S- and M- forms dissociants of *Rhodococcus ruber* - 8/4/1

Dissociation involves two processes: the emergence of dissociatives as a result of rearrangements in the cell genome and selection, and their emergence under the influence of physicochemical factors and environment composition.

Bacteria of the genus *Rhodococcus* are viable in nature in a wide range of pH values, from 4 to 12 (Khudokormov, Karaseva, Samkov, Volchenko, & Kozitsyn, 2013; Ivshina et al., 2019). The influence of the medium's pH on the growth and variability of the R-, S- and M- form dissociants of the *R. ruber* - 8/4/1 strain was studied. The three dissociants differed significantly in their sensitivity to the pH value of the nutrient medium. In the R-form, the zone of optimal pH value was 7.5-9.0. In the S-forms, the optimal pH value shifted towards slightly acidic (6.0-7.5). At an alkaline pH of 9.0, their growth was almost completely suppressed. The broadest zone of optimum pH was observed in the M-forms, which varied within the pH range of 5.5-8.0. At weakly acidic pH values in the medium, the number of cells of this form was significantly reduced, and at a pH below 5.0, their growth was completely absent (Figure 4).

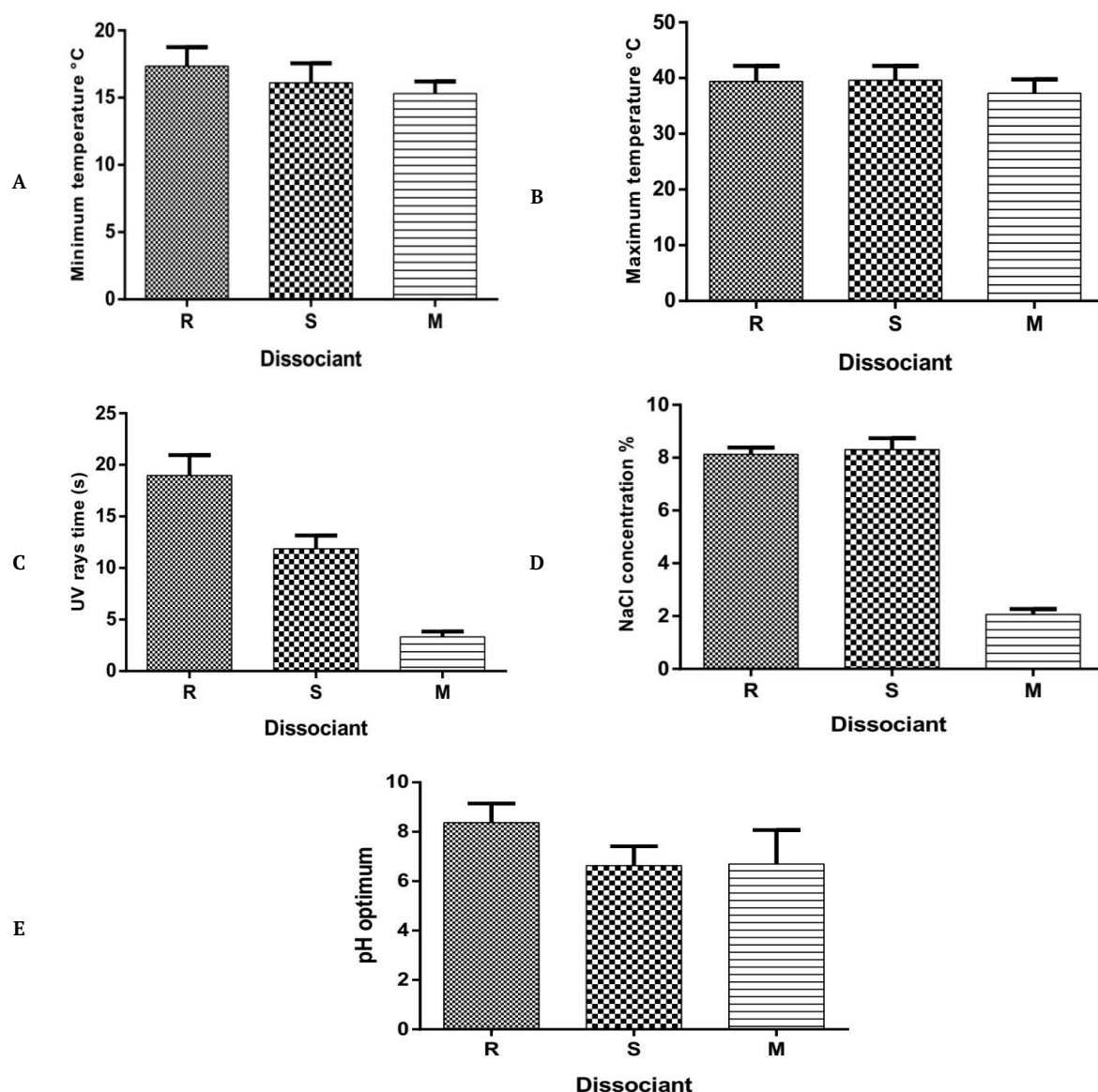


Figure 4. Influence of external environmental factors on the growth of R-, S- and M- forms dissociants of the *Rhodococcus ruber* - 8/4/1 strain. A. Temperature, °C Minimum. B Temperature, °C Maximum. C. UV rays (intensity at which half of the cells survive), exposure time (s). D. Maximum concentration of NaCl, %. E. pH medium.

The study of the influence of the cultivation temperature on the growth of dissociants showed that the optimum temperature for their growth and development was 28-30°C. However, the dissociants of the R- and S-forms were able to grow at 42-40°C, respectively, whereas the growth of the M-forms was not observed at a cultivation temperature above 37°C. Notably, when the dissociants were grown at high temperatures, the ability of the cells to transform AN into AA decreased.

The effect of different UV doses on the dissociation and survival of the *R. ruber* - 8/4/1 strain at different time intervals was studied. Under the influence of UV, the original S-form of the strain dissociated with the formation of R-forms, and the longer the exposure time to UV rays, the greater the transition of the S- to the R-form. When exposed to UV rays for 20s, the survival rate of R-form cells was no more than 50%, whereas complete cell death was observed in the M-form cells. The transition of the R-forms to S- and M-forms was not observed. The maximum exposure time of cell survival was no more than 3s for the M-forms and no more than 10s for the S-forms. The different sensitivities to UV rays were probably related to the structure of the cell wall of the dissociatives, given that the R-forms were thicker than the S-, and M-forms. Thus, the results showed that the *R. ruber* - 8/4/1 strain, when stored on a glycerol-containing medium, as well as under the influence of UV irradiation, dissociates into the R-, S- and M-forms. The sensitivity of the R-, S- and M-forms of dissociatives to UV irradiation has been shown.

The ability of the dissociants of the *R. ruber* - 8/4/1 strain to grow in the presence of various NaCl concentrations showed that the R- and S-forms were able to grow in a NaCl concentration of up to 8%, whereas the growth of strains slows down at 9%-10% NaCl. The M-form of the strain showed weak growth at 3% NaCl.

Biotransformation of acrylonitrile into acrylamide of R-, S- and M- forms dissociants of the *Rhodococcus ruber* - 8/4/1 strain

A characteristic feature of bacteria of the genus *Rhodococcus* and other microorganisms to dissociation may be the reason for the decrease in the activity of cultures. In this regard, the knowledge of the biological characteristics of dissociantes will optimize the processes of obtaining biotransformation of various chemical compounds (Milko, Maksimovich, Lopatina, & Porodenko, 2010, Milko & Milko, 2017).

The ability of the R-, S- and M- forms dissociants of the *R. ruber* - 8/4/1 strain to transform AN into AA was studied, and the resistance of the forms to high concentrations of AA that accumulated in the reaction medium during biotransformation of acrylonitrile was investigated (Figure 5).

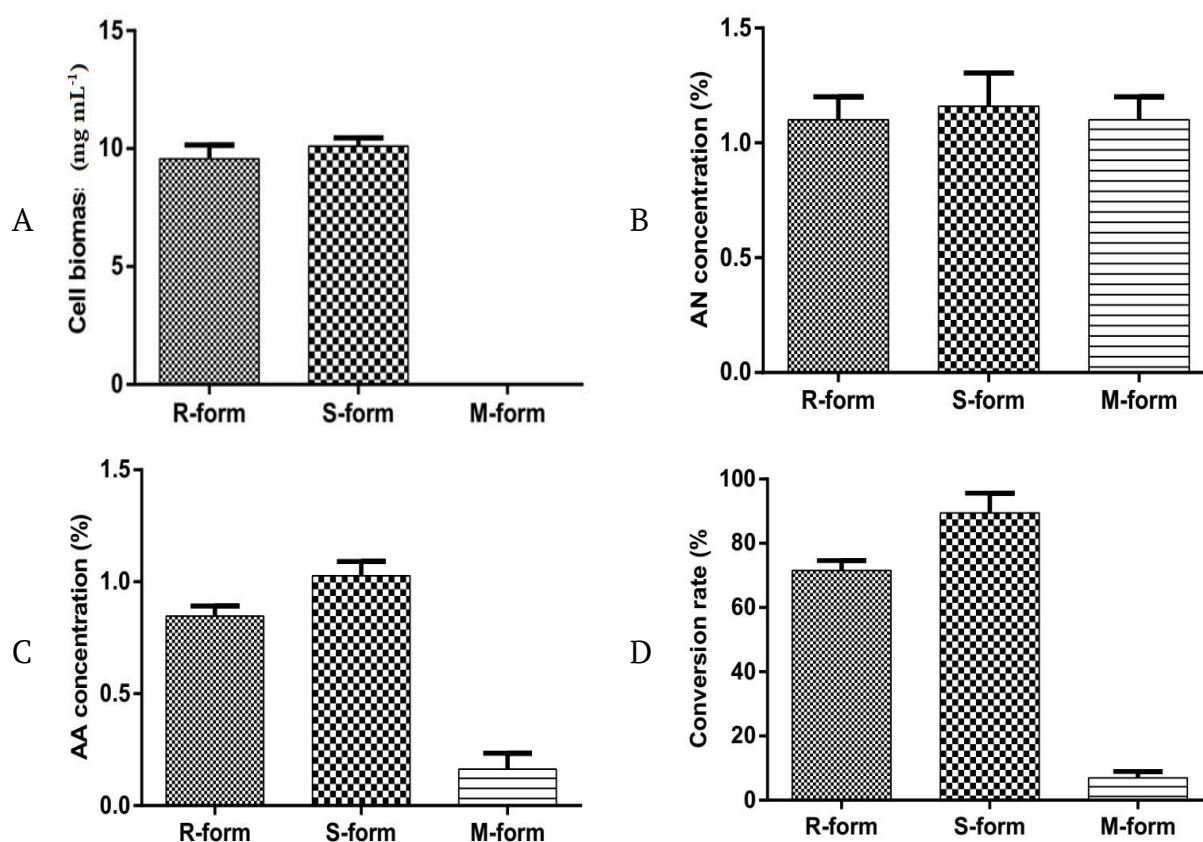


Figure 5. The ability of R-, S- and M- forms dissociants of *R. ruber*-8/4/1 to transform AN into AA. A. Cell biomass (mg mL⁻¹). B. AN concentration (%). C. AA concentration in the medium (%). D. Conversion rate (%).

The ability of the R- and S-forms of dissociants to transform AN into AA was studied. At a cell concentration of 10 mg mL⁻¹, the degree of conversion of AN to AA was 71% in the colonies of R-form, and 92% of the S-form. Given that the M-form of the dissociant forms mucous colonies during centrifugation, the process of transforming AN into AA does not cause precipitation.

The process of transforming AN into AA was studied in 1 ml of the culture liquid of the dissociant corresponding in optical density (OD) with R- and S-forms at a concentration of 10 mg mL⁻¹. Upon transformation of AN into AA, the M-form of the dissociant formed no more than 8% AA. During the transformation of AN into AA, AA accumulated in the medium, which had a suppressive effect on cells. This is due to the resistance of the S-, R-, and M-forms to the concentration of AA in the medium up to 15, 10-12%, and no more than 3%, respectively, which has been experimentally established (Figure 5).

The R-form had selective advantages with UV irradiation and an increase in the growth temperature, the S-form was superior in the conversion of AN to AA, and the M-form was superior under a decrease in the cultivation temperature and an increase in the concentration of NaCl in the environment. The results showed

that the S-forms of the *R. ruber* - 8/4/1 strain had the greatest activity in the transformation of AN into AA. Currently, the knowledge about the mechanism of resistance of the S-form to AA is scarce.

The *Rhodococcus* bacterium cell wall, being an intermediary in the interaction of the cell with the substrate, protects the cell from harmful environmental factors and is a complex structure. The cell wall contains glycolipids, polysaccharides, and fatty acids, which improve the hydrophilicity of the cell surface, thereby ensuring the diffusion of the substrate through the cell wall.

The R-form of *Rhodococcus* bacterium has a hydrophobic surface, whereas the surface of the S-form is more hydrophilic. Sunairi et al. (1997) showed that glycolipids and polysaccharides contained in the cell wall of S-forms of bacteria reduce the hydrophobicity of the bacterial cell surface and serve as a protective mechanism of the cell in relation to complex organic substances.

Conclusion

Thus, the *R. ruber* - 8/4/1 strain, when stored in 10% glycerol and under the influence of UV irradiation, dissociates with the formation of the R-, S-, and M- forms. The sensitivity of the R-, S-, and M-forms of dissociatives to UV irradiation was determined. We found that the initial S-form dissociates into R- and M-forms with a constant and high frequency, and the process of dissociation towards the formation of R-forms is more intense than towards the formation of M-forms. We assumed that, under natural conditions, the dissociative transitions exhibited by microorganisms enhance the survival capabilities of a certain type of microorganism. This is attributed to the adaptative response of cells at the population level to dynamic environmental conditions.

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