



## Protatranes, effective growth biostimulants of hydrocarbon-oxidizing bacteria from Lake Baikal, Russia

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### ABSTRACT

Under natural conditions, biodegradation processes proceed slowly, especially in regions with low temperature. To activate vital processes in hydrocarbon-oxidizing microorganisms at low temperatures, biologically active compounds can be employed as growth stimulants. A low-temperature (10 °C) study has shown that tris-(2 hydroxyethyl) ammonium arylchalcogenylacetates, "protatranes" exert an effect on the growth of hydrocarbon-oxidizing strains of *Rhodococcus erythropolis* and *Pseudomonas fluorescens*, isolated from natural oil seepage on Lake Baikal. It has been found that "protatranes", at microconcentrations, increase the growth rate of *R. erythropolis* bacteria by 2–16 times. It has been established that compounds slightly effect the growth of *P. fluorescens*. The positive effect of "protatranes" compounds on the growth rate of hydrocarbon-oxidizing microorganisms at low positive temperatures can be used for the development of environmentally benign methods for the restoration of natural objects after their contamination with oil.

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### 1. Introduction

Among the numerous substances of anthropogenic origin, that affect the environment, oil and its products are the most widespread. Almost all elements of economic infrastructure, i.e. industry, transport, and defence enterprises, face a problem of environmental pollution with oil products during production and in the case of emergencies [1]. Estimations have shown that up to 50% of crude oil gets to marine ecosystems in a natural fashion, whereas the second half is due to oil spills during production, transportation, and storage [2,3].

There are a large number of methods to remediate environmental pollution with oil and oil products [4,5]. Biological methods are considered the most promising. They are mainly based on the application of biological products containing hydrocarbon-oxidizing microorganisms as well as the activation of the microbial community of the polluted object [1,6–11].

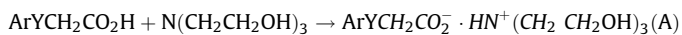
Apart from biological components, the above products usually contain adsorbents, conservation agents, stabilizers (sucrose, boric acid, benzoic acid, malachite green, and polyethylene glycol), growth stimulants (yeast extract and biotrine), cryoprotectants (sucrose, gelatin, and glycerol) and nitrogen-phosphorus-potassium compounds, which are necessary for development of microorganisms [12–15]. The bioavailability of poorly water soluble and adsorbed organic pollutants can be increased with the introduction of surfactants, which are either added to the pollution sites or are produced by microorganisms *in situ* [16]. At the same time, currently developed and patented compositions intended for remediation do not contain synthetic chemical compounds possessing physiological activity that affects the growth rate of hydrocarbon-oxidizing microorganisms.

Under natural conditions, biodegradation processes proceeds slowly, especially in regions with low temperature. To activate vital processes in hydrocarbon-oxidizing microorganisms at low temperatures, biologically active compounds can be employed as growth stimulants. In light of this, the chemical compounds synthesized at the Irkutsk institute of chemistry SB RAS can be considered as potential such materials. In particular, numerous tris-(2 hydroxyethyl) ammonium arylchalcogenylacetates, so-called "protatranes", attract special interest [17–20].

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Biogenic ethanolamines (in particular, triethanolamine) and biologically active arylchalcogenylacetic acids have been employed as the starting materials for the synthesis of protatranes. Ethanolamines participate in the processes of intracellular metabolism. Their structural motif is seen in phospholipids, choline, and acetylcholine, etc. [21]. Arylchalcogenylacetic acids possess a plethora of biological activity and have found applications in medicine and agriculture. For instance, aryloxyacetic acids (Y = O) are used as regulators of plants growth [22]. Sulfur-containing acids (Y = S, SO, SO<sub>2</sub>) also show various high pharmacological activities [23].

Protatranes (**A**) have been synthesized by the reaction of arylchalcogenylacetic acids with triethanolamine (Scheme):



where Ar=het(aryl); Y=O, S, SO, SO<sub>2</sub>, Se

**A** Compounds have an "atrane" structure (Fig. 1), usually found as colourless low-melting powders or viscous protonic alkanolammonium ionic liquids (PAIL) [24–29]. They are stable upon storage and highly soluble in water, alcohols, and other organic solvents [17–20,30].

Some **A** protatranes are non-toxic compounds (LD<sub>50</sub> = 1300–6000 mg/kg), which can be used in agriculture, medicine, clinical microbiology, and biotechnology due to their antioxidant, immunotropic, anti-allergenic, antitumor, anti-metastatic, protective, growth-, and enzyme-stimulating properties. Effective growth biostimulants of microorganisms in genera such as *Staphylococcus*, *Streptococcus*, *Neisseria meningitidis*, *Salmonella typhi spermophil*, and *Escherichia coli* have been synthesised from protatranes and their analogs, which allows for the diagnosis time of dangerous diseases to be shortened [17,18,31].

It has been suggested that synthesised biostimulants can influence the growth rate of hydrocarbon-oxidizing microorganisms and their activity, not only at room temperature, but also at low temperatures, characteristic of soils of the northern regions and waters of the Arctic seas and Lake Baikal.

Among the most promising places for the search of psychrophilic hydrocarbon-oxidizing microorganisms are areas of natural oil seepage on Lake Baikal, which have been known since the 18<sup>th</sup> century [32], where the temperature of the surface waters in open parts of the lake seldom exceeds 9 °C, even in the summer [33]. Owing to geological processes, oil, and products of its transformation, are constant components of the ecosystem of the eastern shore of the central hollow of the lake [34]. As shown earlier, in zones of oil seepage on Lake Baikal, there is a dynamic microbial community, which can effectively clean the water of pollution with oil, as evidenced by the stability of its area [35]. Aerobic oil destructors that show hydrocarbon-oxidizing activity at the lower temperatures (4 °C), and biological surfactants, have been isolated from samples of water and bottom sediments of these areas [36]. The ability to degrade hydrocarbons is inherent in genomes of the Baikal microorganisms, and this is dependent on the existence of *alkB* genes that are responsible for the synthesis of alkane-1-monooxygenases, which allows for the aerobic degradation of a wide range of normal alkanes [37,38].

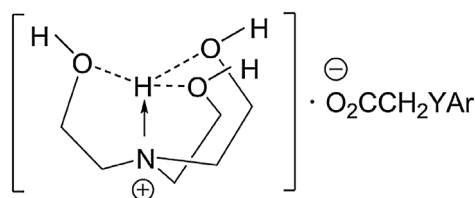
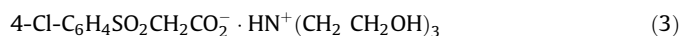
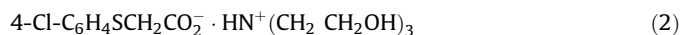
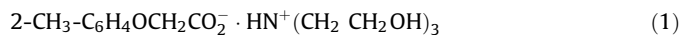


Fig. 1. The structure of protatranes **A**.

The presence of hydrocarbon-oxidizing activity genes in the genomes of microorganisms isolated from areas of natural oil seepage on Lake Baikal enables their use as test-objects for the development of new technologies for biodrugs design.

The present work is aimed at examining compounds from a number of **A** protatranes:



as growth biostimulants of hydrocarbon-oxidizing bacteria *Rhodococcus erythropolis* (No 4–08) and *Pseudomonas fluorescens* (No 5–05) isolated from Lake Baikal.

## 2. Materials and methods

### 2.1. General procedure for synthesis of compounds 1–3

A solution of tris-(2-hydroxyethyl)amine (trietanolamine) and the corresponding arylchalcogenylacetic acid (in 1:1 M ratio) in ethanol was heated at 65 °C for 15–30 min, and then allowed to stand at 20–22 °C for 1 h. The mixture was poured into diethyl ether (absolute), which was also allowed to stand for 12 h at 5–10 °C. The residue was filtered off, washed with ether, and dried in a vacuum to give colourless powders, which are highly soluble in water and alcohol [17–19].

### 2.2. Objects of investigation

The microorganisms *Rhodococcus erythropolis* (No 4–08) and *Pseudomonas fluorescens* (No 5–05) were isolated as pure cultures from the area of natural oil seepage near Cape Gorevoy Utes (Central Baikal).

The strain No 4–08 (*R. erythropolis*) was isolated from bitumen structures, which are formed during oil seepage at the bottom / water boundary [34]. The strain is capable of growing at wide range of temperatures (+4 – +37 °C) and various levels of salinity (3–5%). It possesses hydrocarbon-oxidizing activity: it oxidises *n*-alkanes C<sub>12</sub>–C<sub>29</sub> by 60–100% over 92 h at 22 °C, while C<sub>23</sub>–C<sub>29</sub> hydrocarbons are utilised completely under the action of this strain. The consumption of hydrophobic hydrocarbon substrates by *R. erythropolis* is accompanied by the synthesis of surface-active glycolipid compounds. It has been found that its genome contains *alk*-genes (alkanehydroxylases) responsible for the degradation of numerous *n*-alkanes [39].

The strain No 5–05 (*Pseudomonas fluorescens*) was isolated from waters near the oil seepage. The experiments have shown that the strain *P. fluorescens* (No. 5–05) oxidises up to 35% of *n*-alkanes C<sub>10</sub>–C<sub>24</sub> over 36 days at 4 °C. At the same time, short-chain alkanes are not accumulated in the medium, which indicates that this strain oxidizes these alkanes to simpler compounds [35].

### 2.3. Experimental growth conditions for the hydrocarbon-oxidizing strains of *R. erythropolis* and *P. fluorescens*

To evaluate the effect of compounds **1–3** on the growth of the microorganisms, the cultivation was carried out in mineral medium of the following composition (g/L): KH<sub>2</sub>PO<sub>4</sub>; 1.0, K<sub>2</sub>HPO<sub>4</sub>; 1.0; CaCl<sub>2</sub>·2H<sub>2</sub>O; 0.02, MgSO<sub>4</sub>·7H<sub>2</sub>O; 0.2, FeCl<sub>3</sub>; 0.05, NH<sub>4</sub>NO<sub>3</sub>; 1, pH 7.2. The mineral medium (100 ml) was added to experimental flasks (250 ml), along with oil (50 μL), a suspension of a strain (1 mL),

which was preliminarily prepared according to the opacity standard (up to  $10^4$  cells/mL), and compounds 1–3 in concentrations from 0.0001% ( $10^{-4}$  wt. %) to 0.00000001% ( $10^{-8}$  wt. %).

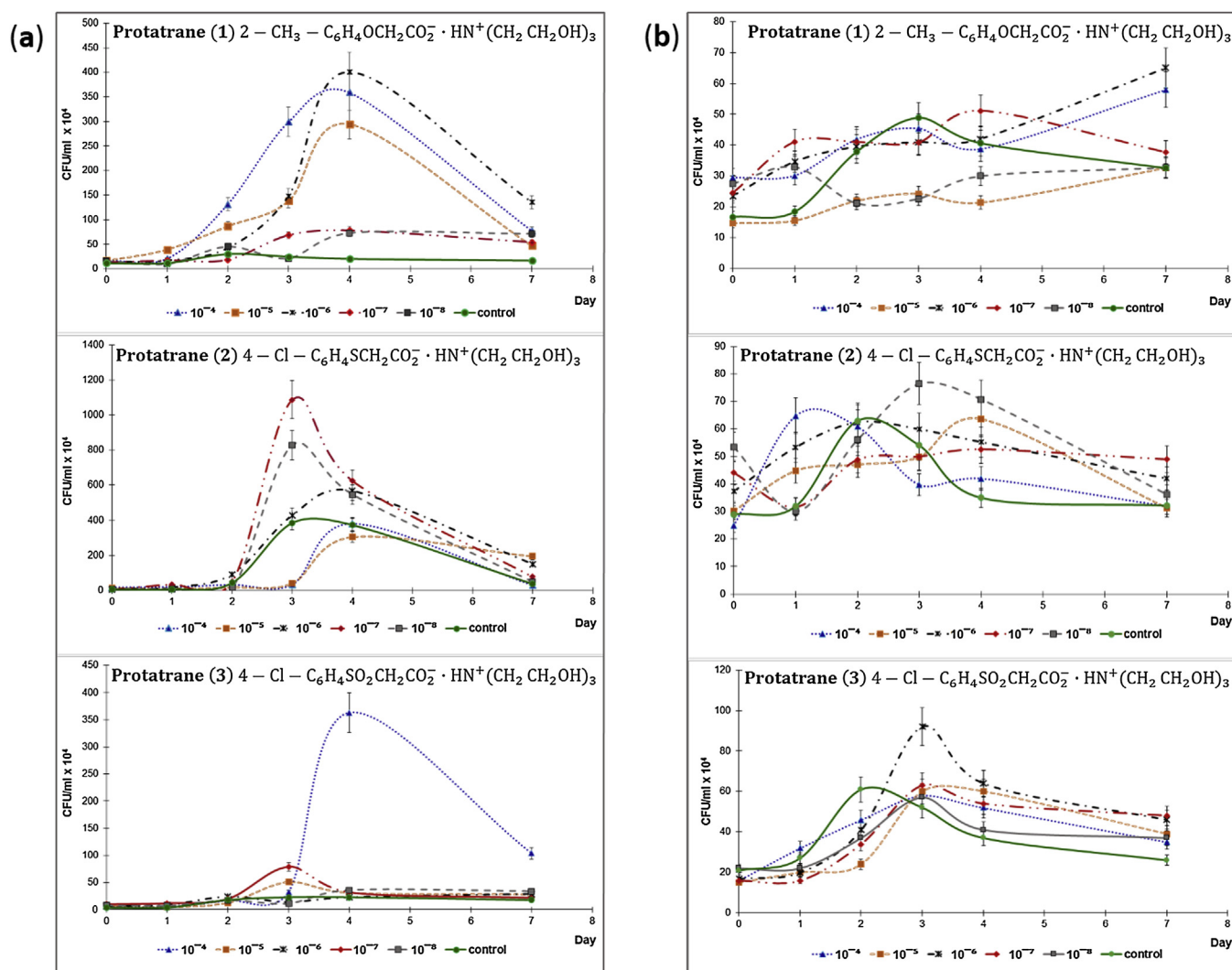
The cultivation was performed for 7 days at  $10^\circ\text{C}$  and with continuous aeration using an orbital shaker (BioSan OS-20, Latvia). The experiment was repeated three times for each compound, each concentration, and for the two strains of the microorganisms. For control purposes, the microorganisms were seeded on mineral medium with oil but without the introduction of compounds 1–3.

The number of the microorganisms was determined by cell counting of a fixed bacterial smear painted by fluorochrome dye (4,6-diamino-2-phenylindole, DAPI) using an epifluorescent microscope (AxioImager M1, Zeiss, Germany). To a sample, which had been dehydrated in a series of ethanol solutions, was added a working solution of the dye up to a final concentration of  $0.5\ \mu\text{g/mL}$ , which was allowed to stand for 3 min. The cells of microorganisms were directly counted at the start of the experiment (initial amount of the bacteria), and then at 1, 2, 3, 4, and 7 days.

#### 2.4. Evaluation of hydrocarbon-oxidizing activity of *R. erythropolis*

The hydrocarbon-oxidizing activity of *R. erythropolis* was determined after implementation of the main cultivation experiment in

the presence of compound 2 for the seventh day. The degree of oil utilization under the action of the microorganisms was evaluated by the decrease of *n*-alkane  $\text{C}_8\text{--C}_{33}$  homologs using a chromatographic method on the 7th day of the experiment. To isolate the *n*-alkane fraction, chloroform (4.5 mL) and squalane solution (0.5 mL) in chloroform ( $0.3\ \text{mg/mL}^{-1}$ ), used as the internal standard, were added to an experimental mixture. The mixture was vigorously shaken and allowed to stand until complete phase disengagement. Then an aliquot was collected from the lower phase (chloroform) and transferred to an autosampler flask of the chromatomass-spectrometer (Agilent, GC 6890, MSD 5973 Network). To determine the concentration of *n*-alkanes, the extract ( $2\ \mu\text{L}$ ) was introduced into a column of the chromatograph with a division of flow into the injector at a ratio of 1:10. The analysis was carried out in the following conditions: capillary column OPTIMA-17 ms, 30 m, internal diameter of 0.32 mm, the temperature of the injector was  $280^\circ\text{C}$ ; temperature of the column was  $50^\circ\text{C}$ , 5 min, then a gradient of temperature of  $15^\circ\text{C/min}$ , from 50 to  $310^\circ\text{C}$  and left in isothermal mode at  $310^\circ\text{C}$  for 15 min; a flow of helium  $1\ \text{mL/min}$ . Chromatograms were registered in the mode of selective ionic detection with  $m/z$ : 57 and 71. Concentration of *n*-alkanes was calculated as the average value for three parallel experiments. The determination error did not exceed 15%.



**Fig. 2.** Changes in the number of hydrocarbon-oxidizing bacteria of the *R. erythropolis* (A) and *P. fluorescens* (B) during oil destruction in the presence of different concentrations of protatranes 1–3.

### 3. Results

The investigations have shown that protatranes **1–3** exert a different effect on growth of two strains of the microorganisms (Fig. 2), which considerably differ in their physiological and biochemical properties from each other [35,38]. In addition, microorganisms of the genus *Rhodococcus* have previously shown higher growth rates and generation times than those of the genus *Pseudomonas* while growing on hydrocarbon substrates [40].

All three tested compounds influenced the growth rate of *R. erythropolis* strain (No. 4–08). Under conditions of periodic cultivation in mineral medium with oil but without addition of compounds **1–3** (control), a longer (up to 24 h) growth lag-phase was observed. Exponential growth of *R. erythropolis* began after 24 h, with its specific growth rate being increased by an order (from  $0.002 \text{ h}^{-1}$  to  $0.02 \text{ h}^{-1}$ ). After introduction of compound **1** in concentrations of  $10^{-4}$ – $10^{-6}$  wt. %, by the 1st day of cultivation, the lag-phase decreased and growth rate ranged from  $0.007$ – $0.03 \text{ h}^{-1}$ . Further, by the 4th day of cultivation, in the presence of compound **1** (concentration  $10^{-4}$ – $10^{-6}$  wt. %, growth rate  $0.03 \text{ h}^{-1}$ , generation time – 20.6 h), the number of the microorganisms increases by 7–9 times (Fig. 2a). At the same time, in the control sample on the 4th day the growth rate is  $0.005 \text{ h}^{-1}$  and generation time is 138 h. The introduction of compound **1** at a lower concentration ( $10^{-7}$ ,  $10^{-8}$  wt. %) did not exert a significant impact on growth of the microorganism.

The effect of compound **2** on the growth rate of *R. erythropolis* was observed by the third day of the experiment (Fig. 2a). In this case, concentrations of  $10^{-6}$ – $10^{-8}$  wt. % were the most effective, with the number of microorganisms increasing by 2–2.8 times. Notably, the growth rate is  $0.06$ – $0.07 \text{ h}^{-1}$ , which corresponds to a generation time of 10 h, while for the control  $\mu = 0.02 \text{ h}^{-1}$  and the generation time is 35 h. At higher concentrations ( $10^{-4}$ – $10^{-5}$  wt. %), growth of the microorganism is inhibited in comparison with the control.

When compound **2** is used at concentrations of  $10^{-4}$ – $10^{-5}$  wt. %, growth inhibition and low hydrocarbon-oxidizing activity is detected for *R. erythropolis*. If the bacteria are cultivated in the experimental medium containing  $10^{-4}$ – $10^{-6}$  wt. % of compound **2**, the number of normal alkanes decreases by 38–50% during the 7 days of the experiment. Meanwhile, the application of compound **2** in a concentration of  $10^{-7}$  wt. % increases the number of bacteria by 3 times and significantly decreases the number of *n*-alkanes (up to 74%). It should be noted that in the control samples

containing nutrient medium, oil, and microorganisms without compound **2**, the number of normal alkanes is reduced by 70%. Thus, most likely, the tested compound stimulates a growth of the microorganisms, but does not significantly affect their hydrocarbon-oxidizing activity (Fig. 3).

Compound **3** at a concentration of  $10^{-4}$  wt. % appears to be the most effective biostimulant. The addition of this compound significantly affects the growth of microorganisms (Fig. 2a). For instance, in the presence of compound **3**, the number of bacteria increases by 16 times on the 4th day of experiment. The growth rate is  $0.04 \text{ h}^{-1}$  and the generation time is 16 h, while for the control samples these parameters are  $0.01 \text{ h}^{-1}$  and 38 h, respectively. Lower or higher concentrations of this substance did not significantly influence the growth of the microorganisms.

The introduction of compounds **1–3** to the culture medium has a low effect on the growth of *P. fluorescens* strain (No. 5–05). In the presence of compound **1** at a concentration of  $10^{-6}$ – $10^{-7}$  wt. %, the growth rate (1st day of cultivation) increases to  $0.02 \text{ h}^{-1}$  in comparison with the control ( $0.004 \text{ h}^{-1}$ ). The increase in the number of microorganisms is observed only at concentrations of  $10^{-4}$  and  $10^{-6}$  wt. % and only by the seventh day of cultivation (Fig. 2b). At the same time, despite the increase in the number of the bacteria after 168 h of cultivation (concentration of compound **1** =  $10^{-4}$  and  $10^{-6}$  wt. %), the growth rate of the bacteria is low, being between  $0.004$  –  $0.006 \text{ h}^{-1}$  (generation time = 114–171 h) and comparable with that of the control sample ( $\mu = 0.004 \text{ h}^{-1}$ , generation time – 175 h).

Upon addition of compound **2** at concentrations of  $10^{-4}$  and  $10^{-6}$  wt. %, the number of microorganisms increases only in the first 24 h of the experiment (Fig. 2b). When compound **2** is introduced at a concentration of  $10^{-4}$  wt. %, the growth rate increases by an order ( $0.04 \text{ h}^{-1}$ ) as compared to the control ( $0.004 \text{ h}^{-1}$ ). After seven days of the experiment, the growth rate in the presence of compound **2** at various concentrations was comparable to that of the control.

The effect of compound **3** on characteristics of *P. fluorescens* strain (No. 5–05) is observed only at a concentration of  $10^{-6}$  wt. %. In the control samples, which contain none of the tested compound, growth acceleration was found by the 1st day of the experiment ( $\mu = 0.01 \text{ h}^{-1}$ ). In 48 h, the specific growth rate increases twice and reaches  $0.02 \text{ h}^{-1}$  (generation time = 31 h). In 72 h, the growth rate slows down, indicating that it has reached stationary phase. After the addition of compound **3** at a concentration of  $10^{-6}$  wt. %, the number of the bacteria increases

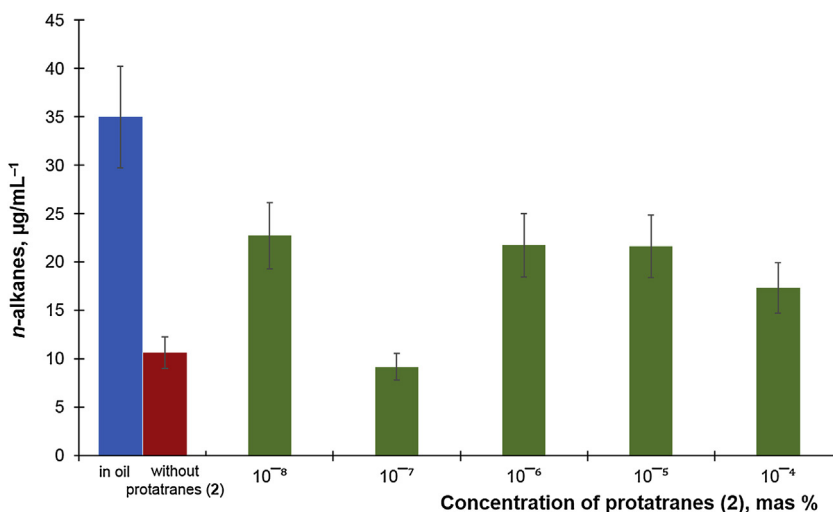


Fig. 3. Content of *n*-alkanes in oil before the experiment ( $\mu\text{g/mL}^{-1}$ ) [blue]; after seven days of cultivation of *R. erythropolis* without the addition protatranes **2** [red]; after seven days of cultivation of *R. erythropolis* in the presence of protatranes **2** in different concentrations [green].



by the 3rd day of the experiment (Fig. 2b). Introduction of compound **3** to the medium inhibits the growth phase for this strain by one day.

#### 4. Discussion

*Pseudomonas* spp. and *Rhodococcus* spp. are often isolated from hydrocarbon-contaminated sites and hydrocarbon-degrading cultures [41]. These two genera have a broad affinity for hydrocarbons and can degrade selected alkanes, alicyclics, thiophenes, and aromatics. Typically, *Rhodococcus* spp. are more hydrophobic and have a higher affinity for hydrocarbon-water interfaces than *Pseudomonas* spp., suggesting that these strains utilize different modes of hydrocarbon access [41,42]. In spite of the fact that *Rhodococcus* and *Pseudomonas* species are the most frequent participants in the processes surrounding hydrocarbon biodegradation, in marine ecosystems the obligate marine group of hydrocarbonoclastic bacteria (OMHCB) has been found. *Rhodococcus* sp. and *Pseudomonas* sp. cannot be referred to this group, since they belong to the heterotrophic community and are present both in contaminated and uncontaminated ecosystems. Hydrocarbons are biodegraded by *Rhodococcus* and *Pseudomonas* bacteria via syntrophic metabolism [43,44]. Unlike other heterotrophic microorganisms, *Pseudomonas* spp. and *Rhodococcus* spp. contain a number of enzymes capable of oxidizing hydrocarbons (alkanehydroxylases), and are able to absorb a hydrophobic substrate. The latter ability is closely connected to the structural peculiarities and metabolic organization of the hydrocarbon-oxidizing bacteria. Direct contact of cells with a substrate plays a crucial role in the processes of hydrocarbon oxidation. Therefore, the ability or inability of a microorganism to absorb hydrocarbons depends on the structure and composition of the cell wall and, first of all, is defined by the presence of a hydrophobic cellular surface [45,46]. The ability of *Rhodococcus* sp. to emulsify and degrade various hydrocarbons is mainly due to structural features of their cellular shell. The cell wall of these bacteria is highly lipophilic and has a high affinity for hydrophobic substrates, which is absent in other bacteria [47]. Apart from lipids, the cell wall of *Rhodococcus* sp. includes mycolic acids [48], which provide advantages to hydrocarbon-oxidizing bacteria in the course of substrate absorption. The formation of bioemulsifiers is also characteristic of *Pseudomonas* bacteria [11]. By emitting bioemulsifiers to the external environment, the bacteria reduce the hydrophobic properties of hydrocarbons and promote their solubilisation. However, a more complex cell wall structure, and the absence of teichoic and mycolic acids, hinders the intake of some substances into the cell. It is probable that the effect of the synthesized protatranes **1–3** on growth rate is defined by the structural peculiarities of the cell wall of the two different microorganisms.

As mentioned above, protatranes **1–3** can be referred to as PAIL [24–29]. Previously, it has been shown that some PAIL, having unusual physical and chemical properties and consisting of biologically active ammonium cations and proton acid anions, can penetrate faster through cellular membranes in comparison with other compounds [28]. It is assumed that successful transport of PAIL is due to the fact that they penetrate through a membrane in the form of hydrogen-bonded complexes. This renders ionic pairs and their aggregates “more neutral” in character, thus facilitating penetration through a model membrane. Possibly, the transport of biostimulators **1–3** inside *Rhodococcus* sp. bacteria occurs similarly.

A high specific biological activity of protatranes **A**, in particular **1–3**, is explained by a favourable combination of several effects in their molecules. First, ethanolamines and arylchalcogenylacetic acids exert a so-called double action [49]. Second, protatranes have

an unusual tricyclic structure containing the intramolecular bond N→H. Such a structure leads to a high dipole moment of the molecule and increased electronegativity of the equatorial oxygen atoms of the protatrane skeleton. The positive end of the dipole is a nitrogen atom. Evidently, hemisorbption of the protatranes on the surfaces of biological membranes, and their further penetration into a living cell, occurs due to the hydrogen bonds and dipole-dipole interaction with polar groups of proteins and lipids. An in-depth study of the mechanism of the physiological action of “atranes”, in particular “protatranes” **A**, on microorganisms, plants, fish and animals is the subject of our further research [50].

The results obtained show that protatranes **A** are promising biostimulators of the growth, development, and activities of oil destructive bacteria. Among the advantages of synthetic biostimulators **1–3** are their low cost, high solubility in water, stability upon storage, non-toxicity, and efficiency at low ( $1 \cdot 10^{-4}$ – $1 \cdot 10^{-8}$  wt. %) concentrations. The found positive effect of “protatranes” compounds on the growth rate of hydrocarbon-oxidizing microorganisms at low positive temperatures can be used for the development of environmentally benign methods for restoration of natural objects after their contamination with oil.

#### 5. Conclusion

In conclusion, we have demonstrated that synthesised protatranes **A** compounds **1–3** are biostimulators of the growth of hydrocarbon-oxidizing *R. erythropolis* bacteria isolated from the area of oil seepage on Lake Baikal. The addition of compounds **1–3** to cultural medium at a low temperature (10 °C) in microconcentrations ( $10^{-4}$ – $10^{-8}$  wt. %) can significantly (by 2–16 times) increase the rate of *R. erythropolis* generation time, depending on the cultivation duration, concentration, and type of anion. The effect of compounds **1–3** on growth of *P. fluorescens* is less pronounced. Probably, the positive influence of biologically active protatranes **1–3** on *R. erythropolis* is due to structural peculiarities of the cell wall of these bacteria. The original composition and unique structure of compounds **1–3** promotes their penetration into the cell of oil destructive bacteria.

#### Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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#### References

- [1] C. Vogt, H.H. Richnow, Bioremediation via *in situ* microbial degradation of organic pollutants, Adv. Biochem. Eng. Biotechnol. 142 (2014) 123–146, doi: [http://dx.doi.org/10.1007/10\\_2013\\_266](http://dx.doi.org/10.1007/10_2013_266).
- [2] K.A. Kvenvolden, C.K. Cooper, Natural seepage of crude oil into the marine environment, Geo-Mar. Lett. 23 (2003) 140–146, doi: <http://dx.doi.org/10.1007/s00367-003-0135-0>.
- [3] I.A. Nemirovskaya, Hydrocarbons in the Ocean, Scientific World, Moscow, 2004 328 p.
- [4] A. Rosa, J.A. Triguís, Bioremediation process on Brazil Shoreline, Environ. Sci. Pollut. Res. 14 (2007) 470–476, doi: <http://dx.doi.org/10.1065/espr2007.02.377>.

- [5] N. Couto, J. Fritt-Rasmussen, P.E. Jensen, M. Højrup, A.P. Rodrigo, A.B. Ribeiro, Suitability of oil bioremediation in an Arctic soil using surplus heating from an incineration facility, *Environ. Sci. Pollut. Res.* 21 (2014) 6221–6227, doi:http://dx.doi.org/10.1007/s11356-013-2466-3.
- [6] A.M. Boronin, A.E. Filonov, I.A. Kosheleva, A.N. Shkidchenko, A.B. Gafarov, S.L. Sokolov, I.F. Puntus, V.G. Grishchenko, V.V. Dmitriev, M.U. Arinbasarov, in: C. A. Brebbia (Ed.), *Bioremediation of Land Oil Spills: Diversity of Microorganisms Degrading Oil Hydrocarbons*, WIT Press, Boston, 2002, pp. 169–177.
- [7] S.S. Belyaev, I.A. Borzenkov, T.N. Nazina, E.P. Rozanova, R.R. Ibatullin, M.V. Ivanov, Use of microorganisms in the biotechnology for the enhancement of oil recovery, *Microbiology* 73 (2004) 590–598, doi:http://dx.doi.org/10.1016/b978-0-12-386545-8.00020-8.
- [8] A. Scoma, M.M. Yakimov, N. Boon, Challenging oil bioremediation at deep-sea hydrostatic pressure, *Front. Microbiol.* 7 (2016) 1203, doi:http://dx.doi.org/10.3389/fmicb.2016.01203.
- [9] D.A. Filatov, E.B. Krivtsov, N.N. Sviridenko, A.K. Golovko, L.K. Altunina, Biogenic oxidation of the high-viscosity oil of the Ashal'chinskoe field and its hetero compounds, *Petrol. Chem.* 57 (2017) 649–656, doi:http://dx.doi.org/10.1134/s0965544117080059.
- [10] T.N. Nazina, D.Sh. Sokolova, T.L. Babich, E.M. Semenova, A.P. Ershov, S.Kh. Bidzhieva, I.A. Borzenkov, A.B. Poltarau, M.R. Khisametdinov, T.P. Tourova, Microorganisms of low-temperature heavy oil reservoirs (Russia) and their possible application for enhanced oil recovery, *Microbiology* 86 (2017) 748–761, doi:http://dx.doi.org/10.1134/S0026261717060121.
- [11] S.J. Varjani, V.N. Upasani, Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant, *Biores. Technol.* 232 (2017) 389–397, doi:http://dx.doi.org/10.1016/j.biortech.2017.02.047.
- [12] J.K. Kim, C.H. Cui, Q. Liu, M.H. Yoon, S.C. Kim, W.T. Im, Mass production of the ginsenoside Rg3 (S) through the combinative use of two glycoside hydrolases, *Food Chem.* 141 (2013) 1369–1377, doi:http://dx.doi.org/10.1016/j.foodchem.2013.04.012.
- [13] C. Liao, X. Liu, Prevent the degradation of algicidal ability in *Scenedesmus*-lysing bacteria using optimized cryopreservation, *Environ. Sci. Pollut. Res.* 23 (2016) 5925–5930, doi:http://dx.doi.org/10.1007/s11356-015-5822-7.
- [14] C. Liao, X. Liu, L. Shan, Optimization of liquid media and biosafety assessment for algae-lysing bacterium NP23, *Can. J. Microbiol.* 60 (2014) 593–597, doi:http://dx.doi.org/10.1139/cjm-2014-0322.
- [15] X. Liu, J. He, C. Liao, Optimization of cryoprotectants to harvest high active cells of a *Scenedesmus*-lysing *Enterobacter* sp. During long-term preservation, *Algal Res.* 13 (2016) 298–302, doi:http://dx.doi.org/10.1016/j.algal.2015.12.014.
- [16] A. Decesaro, T.S. Machado, A.C. Cappellaro, C.O. Reinehr, A. Thomé, L.M. Colla, Biosurfactants during in situ bioremediation: factors that influence the production and challenges in evaluation, *Environ. Sci. Pollut. Res.* 24 (2017) 20831–20843, doi:http://dx.doi.org/10.1007/s11356-017-9778-7.
- [17] A.N. Mirskova, S.N. Adamovich, R.G. Mirskov, M.G. Voronkov, Pharmacologically active salts and ionic liquids based on 2-hydroxyethylamines, arylchalcogenylacetic acids, and essential metals, *Russ. Chem. Bull.* 63 (2014) 1869–1883, doi:http://dx.doi.org/10.1007/s11172-014-0679-3.
- [18] A.N. Mirskova, S.N. Adamovich, R.G. Mirskov, O.P. Kolesnikova, U. Schilde, Immunoactive ionic liquids based on 2-hydroxyethylamines and 1-R-indol-3-ylsulfanylacetic acids. Crystal and molecular structure of immunodepressant tris-(2-hydroxyethyl)ammonium indol-3-yl-sulfanylacetate, *Open Chem.* 13 (2015) 149–155, doi:http://dx.doi.org/10.1515/chem-2015-0018.
- [19] S.N. Adamovich, I.A. Ushakov, A.V. Vashchenko, Novel guanidinium salts of biologically active (het)arylchalcogenylacetic acids, *Mendeleev. Comm.* 27 (2017) 88–89, doi:http://dx.doi.org/10.1016/j.mencom.2017.01.028.
- [20] S.N. Adamovich, A.N. Mirskova, R.G. Mirskov, Method for obtaining of 1-R-indole-3-yl-sulfanylacetates of (2-hydroxyethyl)ammonium. Patent RU 2642778, 2018.
- [21] M. Kunaszewska, Complexogenic properties of ethanolamines, *Sci. Bull. Ldz Techn. Univ.* 31 (1976) 1–65, doi:http://dx.doi.org/10.1002/chin.197602279.
- [22] N.N. Melnikov, *Pesticides: Chemistry, technology, and applications*, Chemistry, Moscow, (1987), pp. 712.
- [23] F.A. Khaliullin, E.K. Alekhin, E.É. Klen, L.A. Ryabchinskaya, V.A. Kataev, A.S. Bogdanova, Synthesis and immunotropic activity of (benzimidazolyl-2-thio)acetic acid derivatives containing thietane cycles, *Pharm. Chem.* 35 (2001) 11–14, doi:http://dx.doi.org/10.1002/chin.200137131.
- [24] N. Bicak, A new ionic liquid: 2-hydroxyethylammonium formate, *J. Mol. Liquids* 116 (2005) 15–18, doi:http://dx.doi.org/10.1016/j.molliq.2004.03.006.
- [25] M. Petkovic, K.R. Seddon, L.P.N. Rebelo, C.S. Pereira, Ionic liquids: a pathway to environmental acceptability, *Chem. Soc. Rev.* 40 (2011) 1383–1403, doi:http://dx.doi.org/10.1002/chin.201127270.
- [26] A. Pinkert, K.L. Ang, K.N. Marsh, S. Pang, Density, viscosity and electrical conductivity of protic alkanolammonium ionic liquids, *Phys. Chem. Chem. Phys.* 13 (2011) 5136–5143, doi:http://dx.doi.org/10.1039/c0cp02222e.
- [27] J. Stoimenovski, D.R. MacFarlane, Enhanced membrane transport of pharmaceutically active protic ionic liquids, *Chem. Commun.* 47 (2011) 11429–11431, doi:http://dx.doi.org/10.1039/c1cc14314j.
- [28] J. Stoimenovski, P.M. Dean, E.I. Izgorodina, D.R. MacFarlane, Protic pharmaceutical ionic liquids and solids: aspects of protonics, *Faraday Discuss.* 154 (2012) 335–352, doi:http://dx.doi.org/10.1039/c1fd00071c.
- [29] H. Niedermeyer, C. Ashworth, A. Brandt, T. Welton, P.A. Hunt, A step towards the a priori design of ionic liquids, *Phys. Chem. Chem. Phys.* 15 (2013) 11566–11578, doi:http://dx.doi.org/10.1039/c3cp50521a.
- [30] I.A. Ushakov, V.K. Voronov, S.N. Adamovich, R.G. Mirskov, A.N. Mirskova, The NMR study of biologically active metallated alkanol ammonium ionic liquids, *J. Mol. Struct.* 1103 (2016) 125–131, doi:http://dx.doi.org/10.1016/j.molstruc.2015.08.074.
- [31] S.N. Adamovich, A.N. Mirskova, O.P. Kolesnikova, *Antitumor agent*. Patent RU 2623034, 2017.
- [32] V.D. Ryazanov, *Ozokerite and Oil Deposits in Cisbaikalia* University Press, Russia, (1928) 116 p.
- [33] M. Kozhov, *Geography and hydrology of baikal, Lake Baikal and Its Life*. Monographiae Biologicae, Springer, Dordrecht, 1963, pp. 5–55, doi:http://dx.doi.org/10.1007/978-94-015-7388-7\_2.
- [34] O.M. Klystov, A.G. Gorshkov, A.V. Egorov, T.I. Zemskaya, N.G. Granin, G.V. Kalmychkov, S.S. Vorob'eva, O.N. Pavlova, M.A. Yakup, M.M. Makarov, V.I. Moskvich, M.A. Grachev, Oil in the Lake of world heritage, *Dokl. Earth Sci.* 415 (2007) 682–685, doi:http://dx.doi.org/10.1134/S1028334X07050042.
- [35] O.N. Pavlova, A.V. Lomakina, A.G. Gorshkov, M.Yu. Suslova, A.V. Likhoshvai, T.I. Zemskaya, Microbial communities and their ability to oxidize n-alkanes in the area of release of gas- and oil-containing fluids in Mid-Baikal (Cape Gorevoi Utes), *Biol. Bull.* 39 (2012) 458–463, doi:http://dx.doi.org/10.1134/s1062359012050123.
- [36] O.N. Pavlova, T.I. Zemskaya, A.G. Gorshkov, T.Ya. Kostornova, O.M. Khlystov, V. V. Parfenova, Comparative characterization of microbial communities in two regions of natural oil seepage in Lake Baikal, *Biol. Bull.* 35 (2008) 287–293, doi:http://dx.doi.org/10.1134/s1062359008030096.
- [37] A.V. Lomakina, T.V. Pogodaeva, I.V. Morozov, T.I. Zemskaya, Microbial communities of the discharge zone of oil and gas-bearing fluids in low-mineral Lake Baikal, *Microbiology* 83 (2014) 278–287, doi:http://dx.doi.org/10.1134/s0026261714030126.
- [38] A. Likhoshvai, T. Khanaeva, A. Gorshkov, T. Zemskaya, M. Grachev, Do oil-degrading *Rhodococcus* contribute to the genesis of deep water bitumen mounds in Lake Baikal? *Geomicrobiol. J.* 30 (2013) 209–213, doi:http://dx.doi.org/10.1080/01490451.2012.665149.
- [39] A. Likhoshvai, A. Lomakina, M. Grachev, The complete alk sequences of *Rhodococcus erythropolis* from Lake Baikal, *Springer Open J.* 621 (2014) 1–5, doi:http://dx.doi.org/10.1186/2193-1801-3-621.
- [40] A.P. Astashkina, A.A. Bakibayev, E.V. Plotnikov, Yu.V. Kolbysheva, A.B. Mukashev, Study of the hydrocarbon-oxidizing activity of bacteria of the genera *Pseudomonas* and *Rhodococcus*, *Procedia Chem.* 15 (2015) 90–96, doi:http://dx.doi.org/10.1016/j.proche.2015.10.014.
- [41] J.D. Hamme, O.P. Ward, Physical and metabolic interactions of *Pseudomonas* sp. Strain JA5-B45 and *Rhodococcus* sp. Strain F9-D79 during growth on crude oil and effect of a chemical surfactant on them, *Appl. Environ. Microbiol.* 67 (2001) 4874–4879, doi:http://dx.doi.org/10.1128/aem.67.10.4874-4879.2001.
- [42] A. Acosta-González, S.-M. Martirani-von Abercron, R. Rosselló-Móra, R.-M. Wittich, S. Marqués, The effect of oil spills on the bacterial diversity and catabolic function in coastal sediments: a case study on the Prestige oil spill, *Environ. Sci. Pollut. Res.* 22 (2015) 15200–15214, doi:http://dx.doi.org/10.1007/s11356-015-4458-y.
- [43] M.M. Yakimov, K.N. Timmis, P.N. Golyshin, Obligate oil degrading marine bacteria, *Curr. Opin. Biotechnol.* 18 (2007) 257–266, doi:http://dx.doi.org/10.1016/j.copbio.2007.04.006.
- [44] S. Santisi, S. Cappello, M. Catalfamo, G. Mancini, M. Hassanshahian, L. Genovese, L. Giuliano, M.M. Yakimov, Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium, *Braz. J. Microbiol.* 46 (2015) 377–387, doi:http://dx.doi.org/10.1002/cfen.200700042.
- [45] F.J. Aranda, J.A. Teruel, M.J. Espuny, A. Marqués, Á. Manresa, E. Palacios-Lidon, A. Ortiz, Domain formation by a *Rhodococcus* sp. Biosurfactant trehalose lipid incorporated into phosphatidylcholine membranes, *Chin. J. Biochem. Biophys.* 1768 (2007) 2596–2604, doi:http://dx.doi.org/10.1016/j.bbame.2007.06.016.
- [46] M.S. Kuyukina, I.B. Ivshina, *Rhodococcus* biosurfactants: biosynthesis, properties, and potential applications, in: H. Alvarez (Ed.), *Biology of Rhodococcus*, Springer, Berlin, Heidelberg, 2010, pp. 291–313, doi:http://dx.doi.org/10.1007/978-3-642-12937-7\_11.
- [47] E.V. Serebryakova, I.V. Darmov, N.P. Medvedev, S.M. Alekseev, S.I. Rybak, Evaluation of the hydrophobicity of bacterial cells by measuring their adherence to chloroform drops, *Microbiology* 71 (2002) 202–204, doi:http://dx.doi.org/10.1023/A:1015154406214.
- [48] W. Whitman, M. Goodfellow, P. Kämpfer, H.-J. Busse, M. Trujillo, W. Ludwig, K. Suzuki, A. Parte (Eds.), *Bergey's Manual of Systematic Bacteriology, The Actinobacteria*, Springer-Verlag, New York, 2012, pp. 2083, doi:http://dx.doi.org/10.1007/978-0-387-68233-4\_3.
- [49] M. Seter, M.J. Thomson, J. Stoimenovski, D.R. MacFarlane, M. Forsyth, Dual active ionic liquids and organic salts for inhibition of microbially influenced corrosion, *Chem. Commun.* 48 (2012) 5983–5985, doi:http://dx.doi.org/10.1039/c2cc32375c.
- [50] S.N. Adamovich, New atranes and similar ionic complexes. Synthesis, structure, properties, *Appl. Organomet. Chem.* 33 (2019) e4940, doi:http://dx.doi.org/10.1002/aoc.4940.