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Effects of Aromatic Compounds Degradation on Bacterial Cell Morphology

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Abstract: The aim of the present study was to evaluate in parallel the capacity of three bacterial strains originating from oil-polluted soils to degrade monoaromatic compounds and the alterations in the bacterial cell morphology as a result of the biodegradation. The strain *Gordonia* sp. 12/5 can grow well in media containing catechol, *o*-, *m*-, and *p*-cresol without significant morphological changes in the cells, as shown by scanning electron microscopy. This implies good adaptation of the strain for growth in hydrocarbon-containing media and indicates it is a proper candidate strain for further development of purification methodologies applicable to ecosystems contaminated with such compounds. The growth of the two *Rhodococcus* strains in the presence of the above carbon sources is accompanied by changes in cell size characteristic of stress conditions. Nevertheless, their hydrocarbon-degrading capacity should not be neglected for future applications. In summary, the established ability to degrade monoaromatic compounds, in parallel with the morphological changes of the bacterial cells, can be used as a valuable indicator of the strain's vitality in the presence of tested aromatic compounds and, accordingly, of its applicability for bioremediation purposes.

Keywords: *Gordonia*; *Rhodococcus*; catechol; cresols; SEM; biodegradation

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1. Introduction

Our lives and wellness depend on a healthy and clean environment. Because of this, our society has seen a notable rise in ecological consciousness in recent decades. Environmentally friendly lifestyles and protection have become top priorities. Our community today places a high priority on environmental conservation, with its primary tenets being sustainable development and lowering the risk to human health.

Many scientists from all over the world involved in microbiology are concerned about restoring the purity of industrially polluted soils. For many years, there have been reports of intense work in this direction, and so far, there is abundant literature information on different types of microorganisms capable of degrading multiple chemical substances in different habitat conditions. Over the years, pollution and ways to achieve its removal have increased and diversified in parallel. Particular attention is paid to oil pollution, especially in countries with large oil reserves and, therefore, large oil production [1–3]. Microorganisms belonging to all taxonomic domains have been isolated and identified as xenobiotic destroyers with different abilities.

It has been said that hydrocarbons occupy the dominant share of oil's chemical composition, and aromatics (all hydrocarbons containing one or more rings of the benzenoid structure) constitute 3–30% of them [4]. The negative impact of the presence of aromatic and polyaromatic compounds in our surrounding environment has long been described

in detail [5,6]. They constitute a substantive part of oil and petroleum products, which surround us and affect our quality of life.

Cresols, and especially *p*-cresol, are among the most frequently found in significant quantities, not only in petroleum products and waste from their processing. They are also a major component in obtaining gas from coal, in the production of explosives, pesticides, and a number of other widely carried out industrial activities.

One of the most common phenol derivatives is catechol. It is used independently and is also included as an intermediate in many chemical reactions. It is actively involved both in the processing and in the synthesis of a number of products of the chemical industry, pharmaceuticals, the production of plant protection preparations, etc. This contributes to its significant presence in various components of the environment.

The impact of these phenolic compounds on plant and animal life, on food chains, and thus on humans has been determined to be very harmful. This is a strong motivation to search for new scientific approaches and technologies for their removal from contaminated soils and waters. Commonly used physical and chemical cleaning methods may not achieve such complete degradation and cause the appearance of other toxic compounds. For this reason, biodegradation and, accordingly, bioremediation occupy a large part of the modern trends for cleaning the environment. Microorganisms occupy the most significant place in these processes, as they possess a huge range of enzymes, and thanks to this, some of them manage to digest even highly toxic pollutants, such as various phenolic derivatives. The goal is to convert such compounds into environmentally normal compounds, such as carbon dioxide, acetate, succinate, and water, which are assimilated in normal microbial metabolism.

That is why microorganisms are essential for the degradation of environmental chemical pollutants. This capability of microorganisms can be efficiently used for soil and water bioremediation, which lowers or prevents environmental contamination and is closely related to the circular economy concepts for restoring ecosystem health. The isolation of various microorganisms from different climatic zones and the discovery of their new abilities to efficiently and economically remove toxic chemical products help enrich our knowledge in this scientific field and address such global problems.

Gram-negative bacteria, *Pseudomonas*, are able to degrade numerous compounds of various compositions and structures [7]. The diversity of genera and species of bacteria with relevant potential for the destruction of hazardous chemical wastes is increased by the search for microbial strains with various physiological and metabolic traits. The positive nocardioform actinomycetes species of the genus *Rhodococcus* are among the most promising of these. Many other ecosystems have been found to have the *Rhodococcus* bacterium. This genus's strains have the capacity to biotransform, use, and eliminate a wide range of substances from the environment. Studies on its mechanisms of tolerance to aromatic compounds show its significant potential to degrade phenol and phenolic derivatives [8–13]. Other actinobacteria associated with the decomposition of harmful aromatic compounds are representatives of the genus *Gordonia*, which have also been described to a lesser extent. *Gordonia* strains capable of degrading or transforming various n-alkanes and polyaromatics have been investigated [14–16]. Different substrate specificities have been observed for both *Rhodococcus* and *Gordonia* stars [17–20]. Although the problem of bacterial biodegradation of petroleum products and their main constituents, such as aromatic compounds, has been the subject of numerous studies, the concomitant changes in the structure of bacterial cells as a result of their assimilation have only been considered in very rare cases. On the other hand, previous studies revealed that bacterial cells often react to unfavorable conditions by changing their morphology: starvation, oxidative stress, and antibiotic treatment are among the signals that can induce variations in bacterial cell structure [21–23]. On the level of a bacterial population, such morphological plasticity has been considered a strategy of the bacterial strain to adapt to the requirements of the changed environment [23].

Earlier, the strains of *Rhodococcus* and *Gordonia* investigated in this work were described as capable of utilizing oil and petroleum products to varying degrees. Some of these

microbial cultures have shown the ability to degrade 60–80% of oil and diesel and 46% of engine oil [24]. The strains *Rhodococcus erythropolis* 14/1 and *Gordonia* sp. 12/5 have shown the capacity to degrade phenol as well [25]. The current study aims to reveal the potential of the tested strains to degrade some of the most toxic phenol derivatives, such as cresols and catechol, and the alterations in bacterial cell structure that occur during degradation.

2. Materials and Methods

The three bacterial strains used in the investigation were isolated from oil-contaminated soils from the Kumkol field, Kyzylorda region, Kazakhstan. The two of the investigated strains are registered in the Gene Bank database of NCBI under the numbers as follows: *Rhodococcus erythropolis* 14/1 (MF 188993.1), *Gordonia* sp. 12/5 (MF 188989.1). The taxonomic affiliation of the strain *Rhodococcus* sp. 1G/1 is determined in this study.

2.1. Chemicals

Merck–Schuchardt, Hohenbrunn, Germany, supplied the monoaromatic hydrocarbons used in the experiments. Catechol, *o*-cresol, and *m*-cresol have an analytical standard for synthesis with purity above 99%. The purity of *p*-cresol is equal to or above 98%. The chemical compounds with analytical grade purity used as components of nutrient medium VD were purchased from Merck Bulgaria EAD.

The used agar–agar has been produced by Carl Roth GmbH, Karlsruhe, Germany. From Sigma-Aldrich (St. Louis, MO, USA) were purchased 2N Folin-Ciocalteu reagent (quality level MQ200), 0.1 M Na cacodylate buffer, 25% glutaraldehyde, and OsO4 (99%).

2.2. Media and Culture Conditions

The studied strains were cultivated in a liquid mineral nutrient medium (VD): 1 g/L NH_4NO_3, 1 g/L K_2HPO_4, 1 g/L KH_2PO_4, 0.2 g/L $MgSO_4$, 0.02 g/L $CaCl_2 \times 6H_2O$, 10 g/L NaCl, 0.01 g/L $FeCl_3$, pH = 7.0–7.2 [24].

For inoculum preparation, the microbial strains were grown to the mid-log growth phase in a liquid meat peptone broth (MB) containing 1% glucose. The bacterial cells were centrifuged and washed twice (at 8000 rpm) with fresh saline (0.9% NaCl) to eliminate all residual nutritional media components. Then, the bacteria were diluted aseptically with 10 mL of a liquid mineral nutrient medium (VD). Bacterial cultures were maintained on a solid culture medium of the same composition. Also, 1.5% agar–agar was added for medium solidification. The medium was supplemented with 0.3 g/L of either catechol, *o*-cresol, *m*-cresol, or *p*-cresol, each separately, as the sole carbon source.

To ensure equal amounts of the initial biomass, the initial optical density of the cultures was calibrated to an OD_{540} of 0.235–0.31, determined with a Jenway 6306 spectrophotometer (Cole-Parmer Instrument Co. Europe, St. Neots, UK). The inoculum size is equal to 2–2.5 $\times$ 10^7 CFU/mL. Control samples contained 0.3 g/L glucose. Cultivation was done in Erlenmeyer flasks (100 mL) using a rotary shaker (180 rpm) at 28 °C.

The qualitative data from growth and degradation experiments were collected within three independent experiments. They were processed on MS Excel and are represented as the mean $\pm$ the standard deviation.

2.3. DNA Isolation, PCR, and Sequence Analyses

The bacterial cells of strain *Rhodococcus* sp. 1G/1 were grown in medium MB (Sigma-Aldrich, St. Louis, MO, USA) for 24 h on a rotary shaker. After centrifugation at 8000 rpm for 10 min, the precipitate was washed with a 0.9% sodium chloride solution. The Illustra™ bacteria genomicPrep Mini Spin Kit (GE Healthcare, Chicago, IL, USA) was used to isolate genomic DNA.

The species affiliation of the examined bacterial strains was determined by using primers for PCR amplification of conserved regions within 16S rRNA genes. The amplification was carried out on an "Eppendorf" Mastercycler Personal (Eppendorf AG, Hamburg,

Germany). PuReTaq TM Ready-To-Go™ PCR Beads (GE Healthcare, Chicago, IL, USA) were used for the experiment.

With a final volume of 25 µL, the reaction mixture comprises 50 ng of genomic DNA, 10 pmol of primers, and 1 bead from the Ready-To-Go Kit in 22 µL bi-distilled H_2O. The PCR conditions applied were as follows: initial step at 95 °C for 5 min; denaturation at 30 s, 95 °C; annealing at 30 s, 55 °C; extension at 2 min, 72 °C. After 35 iterations of the denaturation, annealing, and extension processes, the reaction was completed at 72 °C after 5 min.

The obtained PCR products were stored at 4 °C. The purification of the PCR products was performed using GFX™ PCR DNA and Gel Band Purification Kits (Amersham Biosciences, Amersham Biosciences, Amersham, UK). The sequencing of amplified DNA oligonucleotide fragments was performed by the Sanger dideoxy sequencing technology at Macrogen Inc., Amsterdam, The Netherlands. The row data were edited by the software program Bioedit 7.2. DNA sequences were processed by the BLAST analysis with the Gene Bank database at the National Center for Biotechnology Information (NCBI).

2.4. Determination of Phenols Concentration

The monophenolic compound quantity was defined by the reaction with a Folin-Ciocalteu reagent. The sample was diluted ten times with distilled water. One milliliter of Folin-Ciocalteu reagent was added to 10 mL of it. Two milliliters of 10% Na-carbonate were added to the diluted probe, and the reaction continued for 1 h in the dark. The absorption at λ = 750 nm was assessed spectrophotometrically [26].

2.5. Scanning Electron Microscopy (SEM)

The strains were cultivated in a mineral nutrient medium (VD) supplemented with 0.3 g/L of the aforementioned monoaromatic hydrocarbons as sole carbon sources for different time intervals to reach either the experimentally determined mid-log phase or the middle of the interval of the final utilization (Table 1).

Table 1. Time points of bacterial growth for SEM analysis.

Strains	Catechol	*o*-Cresol	*m*-Cresol	*p*-Cresol
Gordonia sp. 12/5	48 h	72 h	36 h	12 h
Rhodococcus sp. 1G/1	12 h	no degradation	no degradation	48 h
Rhodococcus erythropolis 14/1	24 h	72 h	no degradation	no degradation

Control samples were prepared identically, but the VD medium contained 0.3 g/L glucose. The bacteria were pelleted, washed in three changes of 0.1 M Na cacodylate buffer, pH 7.2 (CB), and fixed for 2 h in 4% glutaraldehyde in CB. After three rounds of CB washing, the samples were post-fixed for an hour in CB-buffered 1% OsO4 before being dehydrated in a graded ethanol series. The bacteria were then mounted on carbon foil stuck to metal holders and vacuum-coated with gold using Edwards's sputter coater. The observations were made on a Lyra/Tescan scanning electron microscope at an accelerating voltage of 20 kV [27]. For morphometrical analysis, the lengths of 50 bacterial cells per sample were measured.

The cell-size distribution plots in SEM analyses were prepared using OriginPro 2021 software. Each cell size distribution plot includes every one of the measured values of 50 cells and shows their distribution in individual cell populations.

3. Results

3.1. Growth and Degradation

In the current research, several monophenolic derivatives, including catechol, *o*-cresol, *m*-cresol, and *p*-cresol, have been found to be effective carbon sources for the growth of the strains.

In our previous studies, the two evaluated strains in this analysis were taxonomically classified. The ability of the identified strains to degrade and assimilate phenol, as the only carbon source included in the medium, was tested and established [25]. The identification of the strain *Rhodococcus* sp. 1G/1 was realized in this study. It is registered in the Gene Bank database of NCBI under Accession number OR 487093. Early research on the studied strains' growth in a mineral medium with 0.3 g/L glucose as a carbon source revealed very good growth. Within 48 h at λ = 540 nm, the determined spectrophotometric increase in cell density reached values of 1.2 to 1.5.

The growth curves of *Rhodococcus* sp. 1G/1, *Rhodococcus erythropolis* 14/1, and *Gordonia* sp. 12/5 strains with 0.3 g/L of catechol, *o*-cresol, *m*-cresol, or *p*-cresol added as carbon sources in a carbon-free culture medium are shown in Figures 1–3. The 100-h growth profiles showed that all three strains could grow well in the medium supplemented with catechol.

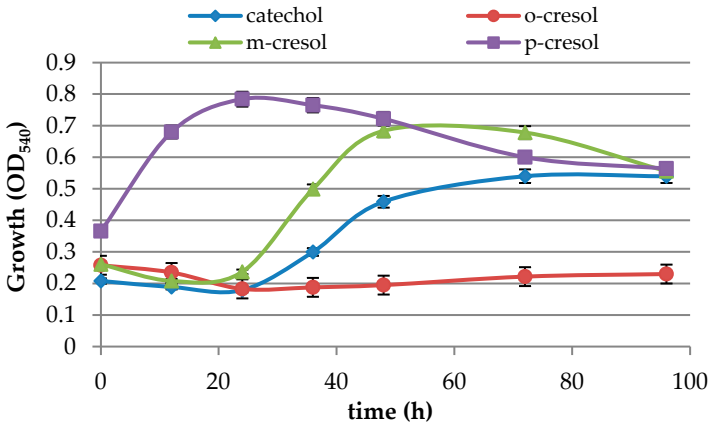

Figure 1. Growth curves of *Gordonia* sp. 12/5.

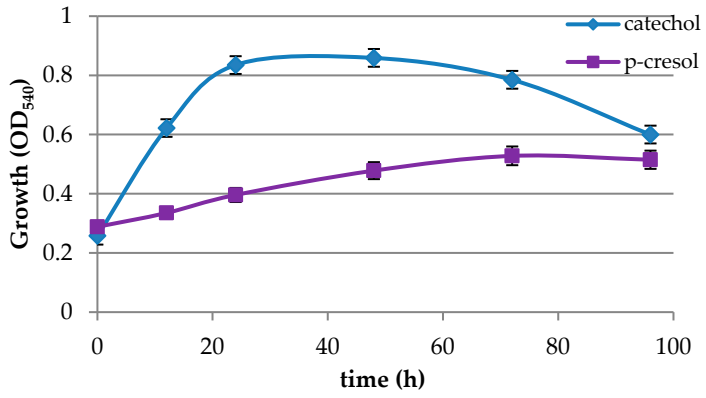

Figure 2. Growth curves of *Rhodococcus* sp. 1G/1.

It was observed that in the growth of the two strains of *Rhodococcus* in the presence of 0.3 g/L catechol as the only carbon source in the nutrient medium, there was no indication of a lag phase in the growth curve. The apparently easy uptake of catechol leads to very good growth of both strains, as evidenced by reaching an optical density of up to 0.9 at ƛ = 540 nm. Regardless of the different developmental dynamics of the representatives of *Rhodococcus*, studies show depletion of catechol at the same time (Table S1).

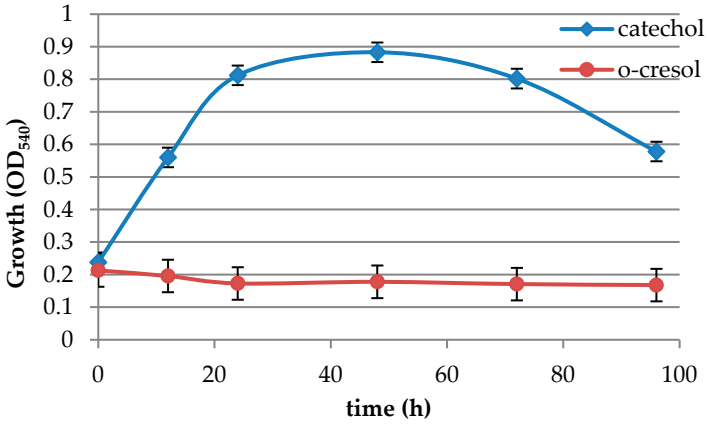

Figure 3. Growth curves of *Rhodococcus erythropolis* 14/1.

In contrast, a relatively long lag phase of growth was observed when the *Gordonia* strain was cultivated under these conditions. This affects the subsequent development of the culture and, accordingly, the accumulation of cell biomass. The achieved optical density is of the order of 0.56 at ƛ = 540 nm.

When methylated phenols were used as the only carbon source, their various dynamics were seen.

Examination of the investigated strain's degradation capacity clearly showed the differences in the utilization potential of catechol and methylated mono-phenols by the three strains. Figure 4 illustrates the broad substrate specificity exhibited by a strain of *Gordonia* sp. 12/5.

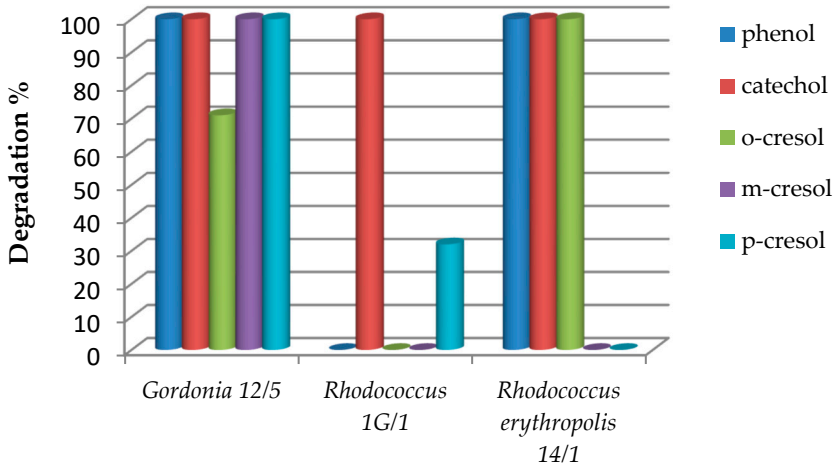

Figure 4. The strains' degradation ability related to hydroxylated and methylated phenols. The results shown are obtained within 96 h at an initial compound concentration of 0.3 g/L.

From Figure 1, showing the growth of *Gordonia* sp. 12/5 on the tested substrates, it is clearly seen that there is practically no adaptation phase (lag phase) when cultured in a medium with *p*-cresol. The development of the strain goes into a short phase of intense growth (log phase), which is also related to the rapid depletion of this substrate within 24 h. The growth of strain *Gordonia* sp. 12/5 in a medium, including *m*-cresol, begins after a 24-h adaptation phase, which affects the time required for complete depletion of the substrate. A very slow strain development was noticed when *Gordonia* sp. 12/5 was grown with another isomer of cresol (*o*-cresol), and the corresponding degradation was recorded after taking six times as long as *p*-cresol's elimination time (Figure 1, Table S1). From the experiments conducted with *Gordonia* sp. 12/5 cultured with *o*-, *m*-, and *p*-cresol, it is concluded that the length of the degradation time counted from the inoculation of the medium with the

bacterial culture until the time when the elimination of the carbon source from the medium is recorded is directly dependent on the duration of the lag phase.

The *Rhodococcus* sp. 1/G1 strain targets a more limited number of substrates, but its ability to deal with *p*-cresol, though partial, is noteworthy. In the strain, *Rhodococcus* sp. 1G/1, which degrades 30% of 0.3 g/L *p*-cresol, no direct correlation was observed between the reported degradation time and the length of the adaptation phase. The adaptation phase is comparable to that observed in the degradation of *m*-cresol by strain *Gordonia* sp. 12/5, but the growth phase proceeds more slowly and with a weaker accumulation of biomass, which also affects the reported significantly longer time for degradation (Figure 2, Table S1).

A clear-cut difference has been noticed in the rate of growth and degradation of *o*-cresol by the strain *Rhodococcus erythropolis* 14/1 (Figures 3 and 4). Although virtually no increase in biomass was observed, no *o*-cresol was identified in the medium within 144 h, indicating that its degradation had occurred. A similar discrepancy was observed in the growth and degradation curves, illustrating the uptake of *o*-cresol by strain *Gordonia* sp. 12/5. Nevertheless, at 144 h, the reduction of the carbon substrate in the growth medium was found to be 70%.

It is noteworthy that the degradation time of the monophenolic carbon compounds used differs significantly between the three strains studied, regardless of the same initial concentration of 0.3 g/L (Table S1).

3.2. Effects of the Monophenolic Substances on the Cells Morphology

The SEM observations illustrated different effects exerted by the monophenolic compounds on the microbial strains' cells. Morphological changes occurring in the middle of the logarithmic phase of growth were observed.

The cells of *Gordonia* sp. strain 12/5 were elongated, with a strap structure in the middle (Figures 5A and 6a). Their length was between 0.5 and 5 µm, with an average of 2.5 ± 0.8 nm. After cultivation to mid-log phases with catechol, *m*-cresol, or *p*-cresol as sole carbon sources, the bacteria did not apparently change very much (Figure 5B,D,E). However, the average cell size (1.8 ± 0.7) and the cell-size distribution patterns (Figure 6b–d) evidenced the occurrence of structural changes within the cell populations, characterized by both a reduction in the average cell lengths and more cell-to-cell variation in cell lengths.

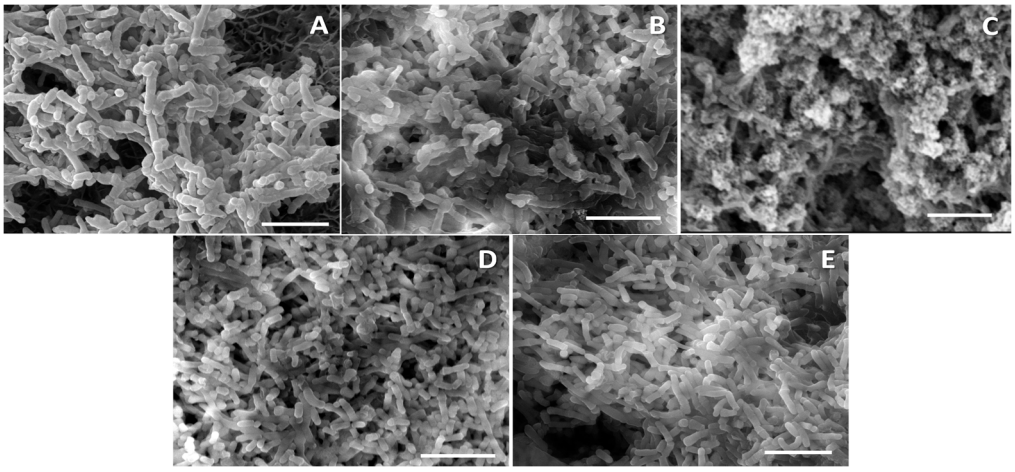

Figure 5. *Gordonia* sp. 12/5 cultivated in the presence of (**A**) glucose, (**B**) catechol, (**C**) *o*-cresol, (**D**) *m*-cresol, (**E**) *p*-cresol; scale bar = 5 µm.

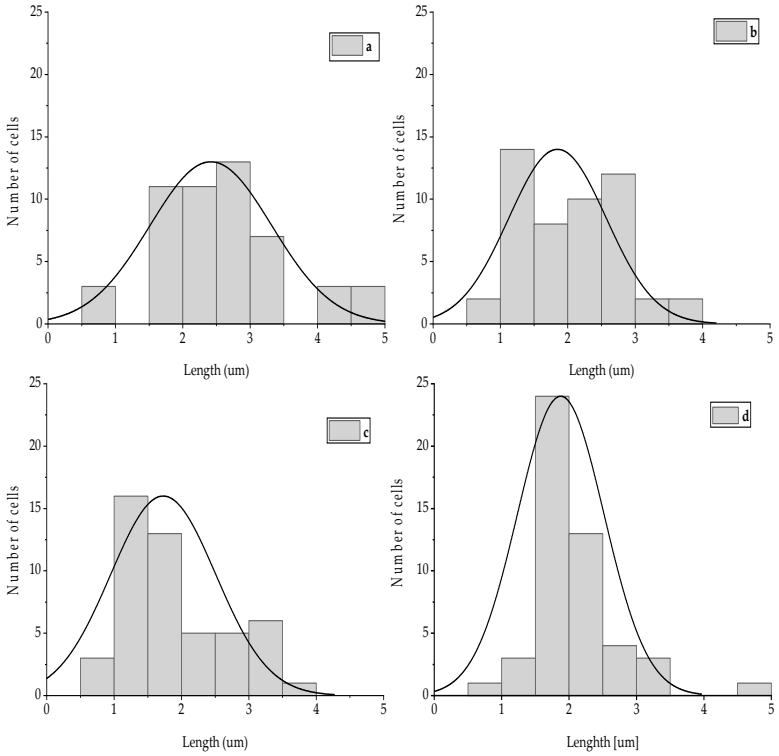

Figure 6. Distribution plot of bacterial cell lengths: *Gordonia* sp. 12/5 bacterial cells cultivated in (**a**) glucose, (**b**) catechol, (**c**) *m*-cresol, (**d**) *p*-cresol. Bars represent the number of cells within a given range of cell lengths, and the curve—the theoretical distribution of cell lengths within the entire cell population.

Nevertheless, the morphological evidence in accordance with the biodegradation data (Figure 4) confirmed the fitness of the strain for survival in the course of the biodegradation of the three hydrocarbons. Unlike this, when *o*-cresol was used as the sole carbon source, it caused severe alterations to the bacterial cell population, with a lot of cell debris in the sample visible at SEM (Figure 5C).

Both strains of *Rhodococcus* were represented by elongated rods after cultivation in VD The cells from strain 1G/1 ranged in length between 1 and 2.8 μm, averaged 2 ± 0.7 μm (Figures 7A and 8a), and were 0.6 ± 0.1 μm wide. Strain *Rh. erythropolis* 14/1 has larger cells. The length ranged between 1.5 and 6 μm, with single filamentous cells protruding up to 8.5 μm (Figures 9A and 10a), and the average width was 0.9 ± 0.2 μm.

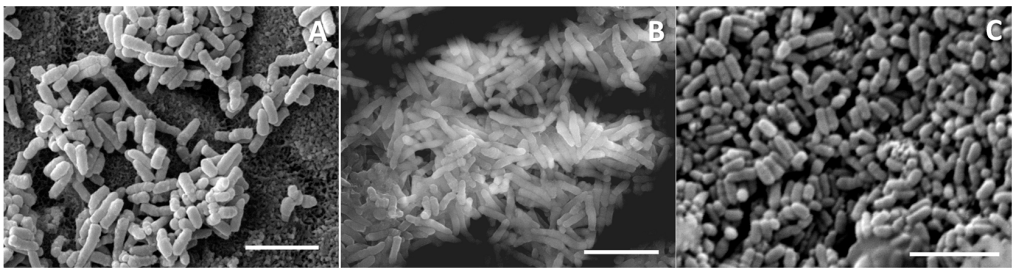

Figure 7. *Rhodococcus* sp. 1G/1 cultivated in the presence of (**A**) glucose, (**B**) catechol, (**C**) *p*-cresol; scale bar = 5 μm.

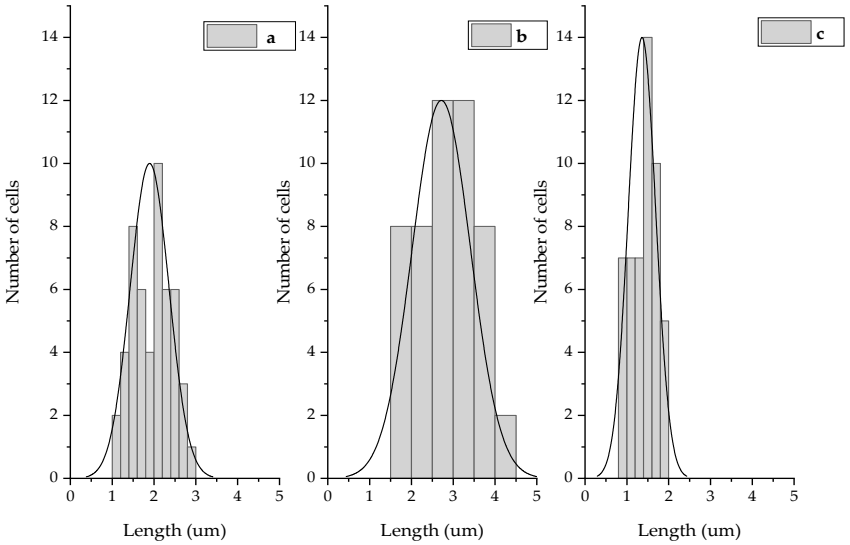

Figure 8. Distribution plot of bacterial cell lengths: *Rhodococcus* sp. 1G/1 bacterial cells cultivated in (**a**) glucose, (**b**) catechol, and (**c**) *p*-cresol. Bars represent the number of cells within a given range of cell lengths, and the curve—the theoretical distribution of cell lengths within the entire cell population.

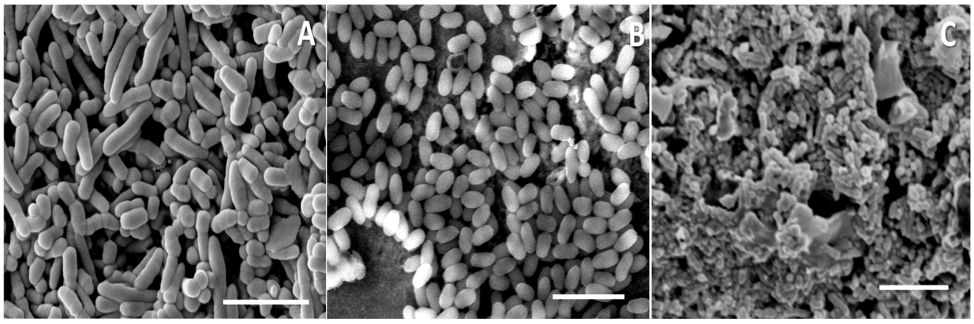

Figure 9. *Rhodococcus erythropolis* 14/1 cultivated in the presence of (**A**) glucose, (**B**) catechol, (**C**) *o*-cresol; scale bar = 5 μm.

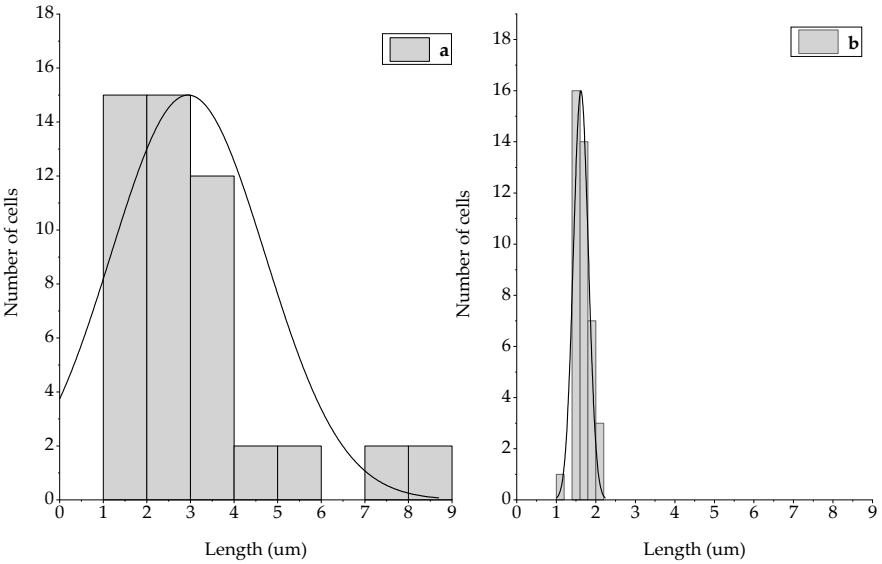

Figure 10. Distribution plot of bacterial cell lengths: *Rhodococcus erythropolis* 14/1 bacterial cells cultivated in (**a**) glucose, (**b**) catechol. Bars represent the number of cells within a given range of cell lengths, and the curve—the theoretical distribution of cell lengths within the entire cell population.

Biodegradation data (Figure 4) showed that strain 1G/1 could assimilate catechol and *p*-cresol. However, the structural alterations brought about by the use of the two hydrocarbons were very distinct. During catechol consumption (Figure 7B), the cells became elongated, within the range of 1.5 to 4.5 μm, with an average of 2.8 ± 0.8 μm, while in the presence of *p*-cresol (Figure 7C), short rods (average 1.3 ± 0.3 μm) were observed.

Strain of *Rhodococcus* sp. 1G/1 showed the narrowest substrate specificity, completely degrading catechol and partially *p*-cresol. It should be noted that in the uptake of these substrates, the cells retain their integrity in spite of the reduced cell size (Figure 7C).

The data in Figure 4 showed that strain *Rh. erythropolis* 14/1 degrades both catechol and *o*-cresol. During catechol consumption (Figures 9B and 10b), the cells changed morphologically to small ovoid shapes, measuring 1.6 ± 0.2 μm by 0.9 ± 0.1. Degradation of *o*-cresol during the tested interval (Figure 4) caused deleterious changes in the bacterial structure (Figure 9C). That coincided well with the lack of visible bacterial growth in the presence of this hydrocarbon (Figure 3). Strain *R. erythropolis* 14/1 is a slow catechol and *o*-cresol degrader. This process is accompanied by dramatic changes in cell morphology. Nevertheless, this strain should not be neglected due to its ability to completely degrade 0.3 g/L of *o*-cresol within the tested interval.

4. Discussion

The harmful effects on human and animal health of toxic phenol derivatives, especially methylated phenols such as *o*-, *m*-, and *p*-cresol, have been described. In turn, catechol is a compound that is a crucial step in the biodegradation pathways of many aromatic compounds. It is also a very poisonous substance [28,29]. All these compounds find a variety of industrial applications, which also leads to their widespread presence in wastewater. The ability to remove them from contaminated soils and waters before releasing them into nature or reusing them in industry is of great importance. This is illustrated by their inclusion in the lists of the Agency for Toxic Substances and Disease Registry (ATSDR), the Environmental Protection Agency (EPA), and the World Health Organization as highly toxic pollutants with proven or potential carcinogenic effects [30–33].

The effective bacterial destructors of cresols described in the last ten years are, e.g., *Serratia marcescens* ABHI001 [34], *Gliomastix indicus* MTCC 3869 [35], *Stenotrophomonas* sp. [36], *Pseudomonas monteilii* CR13 [37], and *Alcaligenes faecalis* [38], which are able to utilize cresols from 10–1500 mg/L in different experimental conditions and at different times. In all of them, the importance of the initial concentration of cresol for its successful and complete degradation was noted. For the most toxic of them, *p*-cresol, this concentration varies between 85 mg/L and 400 mg/L.

The representatives of the genus *Rodococcus* have been the focus of numerous research investigations and reviews related to the genus's capacity to catabolize a variety of difficult-to-degrade, environmentally hazardous compounds. These bacteria often find real use in biotechnological processes linked to the purification of industrially polluted waters and soils [8,11,39].

A *Rhodococcus erythropolis* M1 strain capable of degrading 0.1 g/L *p*-cresol within 20 h has been described [40]. The degradation of a mixture of *o*-, *m*-, and *p*-cresols by *Rhodococcus* strains has also been demonstrated [41]. An interesting study of the range of substrate specificity in a strain of *Rhodococcus erythropolis* shows that it has an enzyme system capable of degrading *p*-chlorophenol, *p*-nitrophenol, resorcinol, and *p*-cresol even though they are not substrates contributing to the growth of culture [42].

In spite of the many publications on the bacterial degradation of monophenolic compounds, there are very few articles that discuss the capacity of *Gordonia* bacteria to degrade substances like phenol, catechol, or other monophenolic compounds. According to the current scientific literature [43], the degradation of methylated phenols has also not been investigated.

The growth and biodegradation data, combined with the morphological data, confirm the suitability of strain *Gordonia* sp. 12/5 to grow well in media containing catechol

and *o*-, *m*-, and *p*-cresol. These features indicate it is a suitable candidate strain for further development of methodologies for the purification of ecosystems contaminated with these compounds.

The strains studied by us show a high capacity for the degradation of the studied spectrum of monophenolic compounds and are among the effective destructors of these widespread toxic environmental pollutants. There are only a few references that address the problem of the biodegradation of petroleum products in relation to the cellular structure of the biodegrading strains. During five-year monitoring of a bioremediation process, Chaerun et al. obtained SEM data showing no change in the morphology and size of the hydrocarbon-degrading bacterial consortium present in the contaminated site [44]. Cryogel-embedded *Rhodococcus wratislawiensis* BN38 preserved its shape and size after many cycles of use for phenol biodegradation [9]. These data suggested that the characteristics of cell morphology during cyclic assimilation of hydrocarbons can be considered one of the indicators of the adaptability of the bacterial strain as well as its applicability in bioremediation processes [45–47].

The variation in cell morphology within a bacterial population is another intriguing feature. This was typically occurring in our samples (Figure 6, Figure 8, and Figure 10). Similar variations have been observed in bacteria exposed to various environmental changes, and this phenomenon is known as "morphological plasticity" [23].

It has been suggested that different cell shapes may provide different advantages under the changed circumstances. For instance, the elongated bacterial form, with its increased surface area, may provide an adaptive advantage for the bacterial cell when changing the sources of nutrients [23,48]. The occurrence of morphological diversity, ranging from cocobacilli to filamentous forms, has earlier been reported for *Acinetobacter* sp. SA01 during phenol degradation [49]. An effect of phenol at a concentration of 300 mg/L was observed, leading to the dominance of filamentous bacteria in nitrifying granules, a decrease in the activity of nitrite-oxidizing bacteria in them, agglomeration, and loss of biomass [50]. The mycolic acid-rich cell wall and the high content of phospholipids in the membrane improve the adaptation of *Rhodococcus* bacteria in an unfavorable environment. In addition, they possess genetically determined transport systems called efflux pumps that help regulate the concentration of toxic compounds [45]. A new putative mechanism for the transport of phenol in *Rhodococcus opacus* strains has been described, which significantly increases the utilization of phenol [46,47]. *Gordonia* strains that produce free and capsular exo-polysaccharides have been described, which is an important advantage for their environmental and biotechnological applications as it creates natural protection under various environmental stresses, including the presence of toxic chemicals with phenolic structures [51,52].

In the present materials, cell size distribution variations were noted for *Gordonia* sp. 12/5 during the biodegradation of catechol, *m*-cresol, and *p*-cresol, with the occurrence of single filamentous forms (Figures 4 and 6). *Gordonia* sp. strain 12/5 cultured in VD completed with glucose (Figures 5A and 6a) had a cell morphology similar to that described in other electron microscopy studies [51,53,54].

Bacterial cell growth is under the control of molecular mechanisms known as the elongasome and divisome [23]. In them, peptidoglycan's function in controlling cell shape is crucial. The elongosome contains actin-homologous cytoskeletal components as well as the enzymes and regulatory systems necessary for peptidoglycan synthesis, whereas the divisome is connected to proteins that resemble tubulin-like proteins [48]. Regulation of processes in one direction or another may illustrate different strategies for bacterial survival. Thus, in the presence of antimicrobial agents or under oxidative stress, bacterial populations can respond with the appearance of filamentous forms together with other cell types [23,55].

One important signal for cell shape modulation appears to be the availability and nature of nutrients. Thus, starvation is generally considered to cause the occurrence of dwarf cells [22]. Some specific kinds of nutrition limitations, like nitrogen, phosphate, or

water limitations, can result in an inhibition of cytokinesis with the occurrence of giant filamentous cells [21,54]. During bacterial growth, the stationary phase is characterized by cellular changes in response to starvation. The transition to the stationary phase is accompanied by the accumulation of stress factors. Part of the adaptation of the bacteria in this situation is manifested in the formation of a denser cell envelope that can withstand the harsh environment [22,56]. The peptidoglycan becomes thicker, and the inner membrane loses fluidity and becomes rigid [22]. Under these circumstances, the most often encountered morphological change is that rod-shaped bacteria get shorter and change from bacillar to coccoidal form [57].

This has been observed in strains belonging to the two genera represented in the present study as well. Species of *Gordonia* and *Rhodococcus* gained reduced cell size during the stationary phase [51,54,58,59]. In the present study, rod shape shortening to coccoid forms was observed in *Rhodococcus* strains, 1G/1 cultured on *p*-cresol and 14/1 cultured on *o*-cresol (Figures 7C and 9C). Sampling was performed during the mid-exponential phase, but the morphological changes were similar to those typical of bacteria in the stationary phase. This evidence implies that for the strains examined here, feeding on these monocyclic hydrocarbons as a sole carbon source provides some stress-like signals similar to starvation. The peculiarities of the cell wall and membrane determine, to a great extent, the ability of microbes to withstand the effects of phenol and its derivatives. The toxic effects of aromatic and aliphatic hydrocarbons, phenols, and alcohols due to the interaction of these compounds with the membrane and membrane components have been studied for many years [60]. Thus, *Pseudomonas* and *Bacillus* cells grown in a phenolic mineral medium formed congregates, unlike those grown in a rich organic medium without phenol. The authors believe that this is due to the strain's efforts to prevent damage triggered by the high phenolic concentration and the corresponding intermediate metabolites [61]. In the present study, a similar effect was observed when the strains *Gordonia* sp. 12/5 and *Rhodococcus erythropolis* 14/1 were cultivated in a medium containing *o*-cresol as the sole carbon source. Other authors have described that at phenol concentrations higher than 750 ppm, biofilms have been formed, and phenotypic changes occur due to the emerging secretion of an extracellular substance containing proteins and polysaccharides [62].

5. Conclusions

The results of the degradation experiments performed prove that the studied strains have a significant degradation capacity with regard to toxic monoaromatic compounds. They are distinguished by their characteristics not only among actinobacteria but also among other known bacteria as destroyers of cresols. An important advantage of these strains is their ability to utilize catechol and cresols without the need for long adaptation and pre-incubation in a medium containing toxic carbon substrates. These characteristics make them appropriate for use in bioremediation processes.

It can be concluded that, along with the provided evidence for the biodegradation of hydroxylated and methylated monoaromatics, the morphological changes of bacterial cells subjected to their toxic effects can be used as a valuable indicator for the strain's adaptability to survive in the presence of such compounds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9110957/s1, Table S1: Time intervals for maximum achieved utilization of monophenolic substrates.

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