ABSTRACT: With the development of the petroleum industry, oil pollution has become widespread. It is harmful to the digestive, immune, reproductive, and nervous systems of fishes, wild animals, and humans, causing severe threats to ecological safety and human health. Gordonia has increasingly attracted attention in the treatment of alkane pollution for its outstanding performance against hydrophobic refractory substances. However, the lack of knowledge about alkane uptake and degradation restricts the application of gordonia. In this paper, we studied the strain lys1-3 of Gordonia sihwaniensis isolated from coal chemical wastewater, which showed good alkane degradation performance by lys1-3. It is found that stimulated by an alkane, lys1-3 secreted biosurfactants, which emulsified large alkane particles to smaller particles. By active transport, unmodified alkane was transferred into cells and produced a large amount of acid, which was secreted out of the cells.

INTRODUCTION

The rapid growth of industrial, agricultural, and municipal activities in recent years relies on the expanding scale of crude oil exploitation and manufacturing. With the development of the petroleum industry, oil pollution has become an extremely serious issue. During the production, transportation, usage, and disposal of crude oil and its products, increasing numbers of oil spills occur and cause approximately 2 million tons of crude oil being poured into seawater each year. Since large oil spill events, small- and micro-level oil spills also raise concern, and 73–88% of them have a volume less than one cubic meter. Half of crude oil is released from natural seeps, the other half is caused by human activities, which include spills from pipelines, tankers, and coastal facilities. Crude oil consists of complex components, mainly petroleum hydrocarbons. They are harmful to the digestive, immune, reproductive, and nervous systems of fishes, wild animals, and humans, causing severe threats to ecological safety and human health. Particularly, polycyclic aromatic hydrocarbons (PAHs) are typical environmental persistent organic pollutants with carcinogenicity, teratogenicity, and mutagenicity. Therefore, oil pollution remediation is imminent.

In the last few years, many new strains have been reported to be used in the remediation of oil-polluted environment and some of the results have been patented and widely used. For example, Pseudomonas and Bacillus should be producing natural biosurfactants, or Acinetobacter, Flavimonas, and Alcanivorax may have the chemotactic capability and should be able to attach to and emulsify oil droplets. Recently, one of the most popular strains is gordonia, a Gram-positive aerobic organism, many kinds of which are isolated from environments such as contaminated soil, wastewater, estuary sand, mangrove rhizosphere, oil-producing wells, biofilters, and activated sludge, as well as from clinical samples. Many of them are excellent in degrading environmental pollutants, alkylpyridines, phthalates, xenobiotic compounds (e.g., 1,3,5-triazines), or slowly biodegradable natural polymers (e.g., rubber), as well as in transforming or synthesizing organic compounds (e.g., SACs), microbial surface active compounds, and carotenoids. Because of the ability to degrade refractory pollutants and synthesize organic compounds, these bacteria are potentially useful for environmental and industrial application in the last few years.

The poor solubility of petroleum hydrocarbons restricts the direct contact between cells and petroleum hydrocarbon molecules, reducing the possibility of its degradation. Figuring out solutions to either enhance the solubility of petroleum hydrocarbons or improve their accessibility by cells is of great concern. The hydrophobic substrates entering into cells is a prerequisite for degradation, so the uptake process of alkane is very important. However, most reports mainly focus on the isolation and identification of strains, their ability to deal with environmental pollutants or their metabolic pathways. Few
studies pay attention to the process of alkane uptake. On the other hand, due to the degradation mechanism of alkane by Gordonia still being unclear, the industrialized application of Gordonia to oil pollution is limited. In the previous experiments, eight strains for degrading long-chain alkanes were obtained through enrichment, separation, and purification of coal chemical wastewater. The degradation rate of long-chain alkanes by bacteria lys1-3 was the highest to 72.5%, which was identified as Gordonia sihwanienisis. Therefore, in response to the abovementioned questions, we studied the process of alkane uptake and degradation by lys1-3 for its further application.

### RESULTS

**Biosurfactant Produced by Lys1-3.** The emulsification of lys1-3 on n-hexadecane degradation was obvious and reached 100% after 4 days of culture. The emulsification effect is closely related to the production of the biosurfactant. The biosurfactant could reduce the surface tension of liquid usually. The higher the yield of the surfactant, the better the emulsification effect.

The surface tension of the fermentation broth decreased greatly from the initial 56.32 mN/m to 35.12 mN after 10 days of culture, as shown in Figure 1A. From the oil exhaust ring experiment, a maximum oil discharge ring of 7 cm was observed. Generally speaking, an oil discharge ring can be considered to be larger than 3 cm in surface-active components of the fermentation broth. It was found that the cell...
hydrophobicity of lys1-3 was only about 11% at the beginning of the reaction (Figure 1B). As the reaction progressed, the cell hydrophobicity showed a rising trend, reaching a maximum of 44.7% on the 5th day and then decreased slightly. Based on the analysis, it was speculated that surface-active substances were produced during the degradation of alkanes by lys1-3.

Therefore, as shown in Figure 2, the result of infrared spectra analysis (FT-IR) shows that 3311.0 cm\(^{-1}\) represents the NH stretching vibration caused by the intermolecular hydrogen bond, 3070.83 cm\(^{-1}\) represents the NH stretching band caused by the intramolecular hydrogen bond of N–H group, and 1652.18 and 1538.98 cm\(^{-1}\) are the amide bands. These absorption characteristics indicate that the hydrophilic part of this surfactant molecule is peptides. The absorption at 2960–2860 and 1470–1380 cm\(^{-1}\) on the spectrum represents the C–H stretching vibration of a fatty acid group, and 1738.17 cm\(^{-1}\) represents the hydroxyl absorption of lactone, indicating that the hydrophobic part of the surfactant molecule is an aliphatic group. Therefore, the surfactant is an ester peptide substance.

Way of n-Hexadecane Uptake by Lys1-3. Cells can transport substances to ensure the need of energy metabolism for their own growth and reproduction. Alkane-degrading bacteria usually need specific, induced transport systems to absorb alkanes.\(^3\) From Figure 3A, the concentrations of hydrocarbon on the cell and in the cell within 30 min were compared. It was found that the concentration of n-hexadecane absorbed on the cell surface was lower than that in the cell, which indicates that n-hexadecane entered the cell in the reversed direction of the concentration gradient. Thus, n-hexadecane should be transported by active transport.

Generally, no energy is consumed in passive transport, but in active transport or vesicular transport. The inverse concentration gradient consumes the metabolic energy to transport across the membrane, which comes from ATP (adenosine triphosphate) and requires the assistance of a specific carrier on the membrane.\(^3\) Therefore, we then investigated whether the process of transportation consumed energy.

The effect of NaN\(_3\) is to prevent oxidative phosphorylation of cells, so as to prevent the production of ATP. Thus, the
reduction in the concentration of n-hexadecane enriched by lys1-3 after adding NaN₃ indicates that n-hexadecane uptake is by active transport. Otherwise, it is by passive transport. We added different concentrations of NaN₃ to measure the change in alkane concentration. As shown in Figure 3B, NaN₃ led to a sharp drop in intracellular concentration of n-hexadecane. Therefore, lys1-3 transported n-hexadecane by active transport consuming ATP.

**Existence Form of Alkane in Strain Lys1-3.** Through the abovementioned experiment, a large number of alkanes were found to be accumulated in the cell. A further analysis was necessary for the form of alkane absorbed in the cell of lys1-3 by transmission electron microscopy (TEM). As shown in Figure 4, lys1-3 was cultivated with n-hexadecane (Figure 4b,d–f) or glucose (Figure 4a,c) as the carbon source. When alkane was used as the carbon source, small and similar transparent inclusions appeared in the bacteria (Figure 4b,d). However, no inclusion was found in the bacteria cultured with glucose. The ultrastructure showed a large number of viscous substances around the membrane when cultured with n-hexadecane, which were interspersed around spherical transparency (Figure 4b,c). We believe that viscous substances are surfactants secreted by lys1-3, and the small spherical transparent substances should be small oil droplets dispersed by surfactants, which are called compatibilized hydrocarbons. In the process of bacterial growth and metabolism, cells continued to secrete surfactants to emulsify alkanes. The small spherical compatibilized hydrocarbons easily accepted by microorganisms helped them bind to the membrane (Figure 4f). More and more surfactants were secreted extracellularly at the same time (Figure 4e).

**Localization of Hydrocarbon-Degrading Enzymes.** Many scholars are committed to the study of the degradation mechanism and conditions to improve the efficiency of microbial degradation of organic compounds. Kennedy and Finnerty thought that enzymes in the membrane can oxidize alkanes. Gopinath et al. showed that extracellular enzymes produced by saprophytic fungi can degrade pollutants. Visibly, an endoenzyme, a membranous enzyme, and an extracellular enzyme may be related to the degradation of pollutants.

In this paper, the location of alkane-degrading enzyme was studied, with n-hexadecane or glucose as the carbon source separately. As shown in Figure 5, the intracellular, extracellular, and membranous enzymes were extracted to observe the degradation of n-hexadecane in 72 h. Enzymes with n-hexadecane as the carbon source extracted from different parts exhibited different degradation abilities. The degradation rate of extracellular and membranous enzymes was less than 10%, whereas that of intracellular enzymes was 100%. Therefore, we can infer that hydrocarbon-degrading enzyme of lys1-3 is mainly intracellular.

When compounds in the environment enter the cells, the bacteria are induced to produce enzymes that degrade the chemicals, which is also a defense mechanism in biology. The production and activity of enzymes are affected by the kind and quantity of available carbon sources in the external environment. In comparison, when glucose was used as the sole carbon source, the degradation rate of n-hexadecane by intracellular, extracellular, and pericellular enzymes was all very low. This suggests that although lys1-3 can grow rapidly with easily degradable carbon sources such as glucose, it is difficult to produce hydrocarbon-degrading enzymes without alkane induction. In other words, with long-chain alkane as the only carbon source, hydrocarbon-degrading enzymes can be induced and produced in large quantities. When there are toxic and harmful substances in the environment, microorganisms can adjust their functions and secrete targeted degrading enzymes to consume harmful substances. This can provide a theoretical basis for the screening of alkane-degrading bacteria and the treatment of hydrocarbon pollution. Thus, the degradation behavior of lys1-3 mainly occurs in the cell.

**Degradation of Alkane by the Strain Lys1-3.** The pH changed significantly when lys1-3 was fermented with n-hexadecane as the sole carbon source. After 7 days, the pH decreased from 7.0 to 4.2. The results showed that a large number of acidic substances were produced in the process of degradation (Figure 6).

![Figure 6. pH variety of hexadecane medium by lys1-3.](https://doi.org/10.1021/acsomega.1c01708)

After pretreatment, the intracellular metabolites were analyzed by gas chromatography–mass spectroscopy (GC–MS). Metabolites of n-hexadecane are shown in Figure 7. The retention time and relative molecular weight of various metabolites are shown in Table 1. According to the comparison of mass spectrometry library, the peak at 18.66 min was n-hexadecane. The results showed and confirmed that lys1-3 could absorb and store unmodified n-hexadecane into the cell. In addition, there were hexadecene,
hexadecanol, and hexadecanoic acid in the cells. The presence of hexadecanoic acid indicates that the metabolism of \( n \)-hexadecane by lys1-3 should be by terminal oxidation. Alkanes were oxidized to alcohols first and then aldehydes and monobasic fatty acids. This is the most common metabolic pathway for linear alkanes.\(^{35,36} \) However, hexadecene in the metabolites also indicates that there may be other pathways to degrade hexadecane by lys1-3, which will be studied in the future.

According to the primers designed by Kloos, using the DNA of lys1-3 as a template, the target gene fragment with the size of 550bp was obtained by polymerase chain reaction (PCR) amplification. In order to determine the phylogenetic position

| no. | compound name       | \( t_R \) (min) | relative molecular weight |
|-----|---------------------|----------------|--------------------------|
| 1   | hexadecane          | 18.66          | 226                      |
| 2   | hexadecene          | 14.62          | 224                      |
| 3   | hexadecanol         | 21.40          | 242                      |
| 4   | hexadecanoic acid   | 22.32          | 256                      |

Figure 7. Chromatogram of GC–MS analysis of hexadecane degradation.

Figure 8. Phylogenetic analysis of the lys1-3 \( alkB \) fragment.
of alkane hydroxylase genes (alkB) isolated from lys1-3, the phylogenetic tree (Figure 8) was constructed by high homology genes selected from NCBI. The highest homology with gene S14-10 was up to 99%, and they could be clustered into the same cluster. According to the report, the gene is generally responsible for medium- and long-chain alkanes, in which different alkB genes may have different substrate ranges.37

### DISCUSSION

Alkanes are hydrophobic pollutants, and the first step for hydrocarbon remediation is to ensure that hydrocarbons can be absorbed and utilized. As gordonia can effectively degrade and repair contaminated sites, the uptake and degradation mechanism of alkanes by *G. sihwaniensis* is studied in this paper.

By analyzing the characteristics of the fermentation broth (Figure 1) and infrared spectrum analysis of extracellular substances (Figure 2), it was confirmed that ester peptide biosurfactants were produced during the degradation of alkanes by lys1-3. The biosurfactant could enhance the cell hydrophobicity and reduce the surface tension of the fermentation broth. Thus, the high hydrophobicity indicates that cells are more lipophilic and are likely to come in contact with the oil droplets or hydrocarbon particles.

By analyzing the composition of intracellular substances, we believe that lys1-3 can transfer the unmodified long-chain alkane to the oxidation site of alkane in a certain way, accumulate and store them in the form of inclusion bodies for oxidation and decomposition. It was observed by Ilori38 that there are similar inclusion bodies in *Pseudomonas aeruginosa* and *Micrococcus luteus*, which is the main component of the inclusion bodies *n*-hexadecane. According to Figure 4f, it is speculated that the cells could obtain alkane by the way of “phagocytosis”. Therefore, we proposed that the strain lys1-3 transported alkane through the endocytosis of vesicular transport in this paper. Similarly, Cameotra and Singh38 found the “internalization” of “biosurfactant layered hydrocarbon droplet”, suggesting a mechanism similar in appearance to active pinocytosis when they studied the mechanism of hexadecane uptake by pseudomonas species.40,42

Degrading hydrophobic substrates by gordonia mainly depends on self-induced biosurfactant to adjust the hydrophobicity and surface adhesion, which helps degrade all hydrophobic pollutants. Studies suggest that surfactants help accelerate the uptake of alkanes in the cell. Surfactants improve the bioavailability of hydrocarbons in two ways.32 On the one hand, hydrocarbons can be dispersed in the aqueous phase to increase the interface area with oil. On the other hand, the hydrocarbons adsorbed on the surface of solid particles can be eluted into the aqueous phase, making it easier to transport to cells.

Rhamnolipids and sophorolipids can be produced by *P. aeruginosa* and *Torulopsis bombicola*, respectively, to promote the metabolism of alkanes in this study. It is observed that the biosurfactant not only improved the bioavailability of the substrate through pseudo-compatibilization and emulsification, but also formed a surface-active layer to contact the surface of the cell membrane outside the hydrocarbon droplets. The bridge assists cells to absorb alkanes. This is similar to the conclusion presented by Cameotra and Singh in 2009.38

Three types of monoxygenase systems are found in microorganisms. Methane monoxygenase is present in methane-oxidizing bacteria. P450 cytochrome monoxygenase is mainly found in yeast, while alkane hydroxylase encoded by alkB gene is mainly present in bacteria.41 Combined with the results of GC (Figure 7 and Table 1), the degradation process of lys1-3 was performed by alkane hydroxylase. To maintain consistency with this conclusion, Kim29 verified the existence of this pathway on the basis of degradation products (hexanol, hexanal, and hexanoic acid with *n*-hexane and hexadecanol, hexadecanal, and hexadecenoic acid with *n*-hexadecane as carbon sources, respectively). *Gordonia SoCg* was thought to contain a single alkB gene that was induced in the presence of medium- and long-chain *n*-alkanes.
Thus, the whole degradation process of \( n \)-hexadecane by \( G. \) *sihwaniensis* was analyzed for the first time in this paper. In addition, we mapped the degradation mechanism pattern in detail (Figure 9).

Stimulated by alkane, cells secreted a large number of biosurfactants, which emulsified large alkane particles into smaller particles. Alkanes on the cell surface were transferred into the unmodified cell while consuming ATP by vesicular transport. Subsequently, \( n \)-hexadecane was converted into \( n \)-hexadecyl alcohol, and then \( n \)-hexadecyl aldehyde and \( n \)-hexadecanoic acid by the action of monooxygenase, and finally entered the tricarboxylic acid cycle, producing a large number of low-molecule organic acids. As we can see, degradation mainly occurs in cells. The results will contribute to efficient use of \( G. \) *sihwaniensis* in remediation of environmental pollution.

## CONCLUSIONS

The degradation process of petroleum hydrocarbons by \( G. \) *sihwaniensis* is described in this paper. First, \( n \)-hexadecane is adsorbed on the cell surface, which stimulates the cell to secrete surfactants. The large oil droplets should be emulsified into small oil droplets and transported into the cell by active transportation. Subsequently, \( n \)-hexadecane is converted into hexadecanol through a series of enzymatic actions, and it finally enters the tricarboxylic acid cycle, which is accompanied by the consumption of ATP. Alkane hydroxylase encoded by an \(alkB\) gene is a key enzyme in the terminal oxidation and degradation pathway of alkanes, which is located in cells.

## MATERIALS AND METHODS

The strain lys1-3 was used as the research object, which was cultured in LB medium to logarithmic growth period as seed solution. In the following experiment, 2% of seed solution was inoculated in inorganic salt medium containing different carbon sources and cultured in the medium at 35 °C and 180 rpm.

**Adsorption and Uptake of Alkane.** Lys1-3 was cultured with 100 mg/L \( n \)-hexadecane as the carbon source. The solution was centrifuged at different culture times. Cells were cleaned with inorganic salt medium once, ethanol/butyl alcohol/chloroform (volume ratio of 10:10:1) twice, and inorganic salt medium twice. First, the cleaned fluid was combined and measured by GC to determine the amount of alkane absorbed on the cell surface. Second, cells collected by removing the cleaned fluid were suspended in 10 mL of 10 mmol/L Tris–HCl (pH = 7.0), and walls were broken by ultrasound on the ice for 3 s each time at an interval of 7 s, with a total of 60 times. After 8000 rpm centrifugation for 10 min, the supernatant was extracted twice with equal volume of \( n \)-hexane and then the organic phase was collected and measured by GC and mixed with the internal standard of \( n \)-hexadecane. In addition, the enzyme with glucose as the carbon source was extracted and measured by the same method for the degradation of \( n \)-hexadecane.

**Quantitative Analysis of Extracellular Acid.** The fermentation broth was centrifuged at 12,000 rpm for 10 min to remove residual hydrocarbons, and then the bacteria were removed with 0.22 μm filter membrane. 25 mL of treatment solution was taken and two drops of phenolphthalein reagent were added. Then, acid–base titration was performed with 0.05 mol/L NaOH. The acid production was calculated by lys1-3 culture as the control.

**GC–MS Analysis of Intracellular Metabolites.** The bacteria were collected by centrifuging at 12,000 rpm for 10 min. The cells were washed once in the inorganic medium, washed twice in ethanol/butanol/chloroform (volume ratio of 10/10/1), and then washed twice in the sterilized inorganic medium to remove the alkane adsorbed on the cell surface. The cells were suspended in 10 mmol/L Tris–HCl (pH = 7.0) of 10 mL, and the walls were broken by ultrasound on the ice for 3 s each time at an interval of 7 s, with a total of 60 times, and finally centrifuged at 8000 rpm for 10 min. The supernatant was adjusted to pH < 2 and extracted three times with equal volume of MTBE (methyl tertiary butyl ether) and then the organic phase was combined. The organic phase was dehydrated and dried with anhydrous sodium sulfate, evaporated by rotation at 40 °C, and then measured by GC–MS.

Test conditions: GC conditions: the column was DB5; the column temperature program was 50 °C for 15 min, maintaining for 10 min from 3 °C/min to 260 °C; and the injection volume was 1 μL of He, 1 mL/min.

Mass spectrometry conditions: The MS energy was derived from a 70 eV electron ionization source of electron bombardment; ion source temperature was maintained at 140 °C; the emission current was 250 μA; the voltage of electron multiplier was 1000 V; mass scan range was set at 45–1000 m/z.

**Biosurfactant Analysis by Infrared Spectroscopy.** The bio-surfactant was extracted and purified from the fermentation broth of lys1-3, and the purified product was vacuum-dried and analyzed by infrared spectroscopy.

**Determination of Emulsifying Ability.** The fermentation liquid was centrifuged for 10,000 rpm at 4 °C for 10 min. 5 mL of the supernatant was added to a 20 mL test tube and 5 mL of \( n \)-hexadecane was added to the test tube. The volume of
the remaining n-hexadecane was determined after full oscillations for 2 min and standing for 24 h.

SECTION: AUTHOR INFORMATION

Corresponding Author
Xiaolin Wu — PetroChina Daqing Oilfield Co. Ltd., Institute of Exploration and Development, Daqing 163002, China; Email: wuxldq@petrochina.com.cn

Authors
Yinsong Liu — Laboratory of Enhanced Oil Recovery of Education Ministry, Northeast Petroleum University, Daqing 163318, China; orcid.org/0000-0002-0096-3662
Jingchun Wu — Laboratory of Enhanced Oil Recovery of Education Ministry, Northeast Petroleum University, Daqing 163318, China
Yikun Liu — Laboratory of Enhanced Oil Recovery of Education Ministry, Northeast Petroleum University, Daqing 163318, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c01708

SECTION: NOTES

The authors declare no competing financial interest.

SECTION: ACKNOWLEDGMENTS

I would like to express my gratitude to all those who helped me during the writing of this thesis. Specially, my deepest gratitude goes first and foremost to X.W. and Y.L., my supervisors, for their constant encouragement and guidance.

SECTION: REFERENCES

(1) Kennish, M. J. Pollution Impacts on Marine Biotic Communities; CRC Press: Florida, 1998.
(2) Li, S.; Grifoll, M.; Estrada, M.; Zheng, P.; Feng, H. Optimization on emergency materials dispatching considering the characteristics of integrated emergency response for large-scale marine oil spills. J. Mar. Sci. Eng. 2019, 7, 214.
(3) Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Van Mooy, B. A. S.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V. Tracking hydrocarbon plume transport and biodegradation at deep-water horizon. Science 2010, 330, 201–204.
(4) Urakawa, H.; Ratsch, J.; Sassen, S.; Feeney, M. E.; Sobeyck, P. A.; Mortazavi, B. Ecological response of nitratification to oil spills and its impact on the nitrogen cycle. Environ. Microbiol. 2019, 21, 18–33.
(5) Adlakha, J.; Singh, P.; Ram, S. K.; Kumar, M.; Singh, M. P.; Singh, D.; Sahai, V.; Srivastava, P. Optimization of conditions for deep desulfurization of heavy crude oil and hydride sulfited diesel by Gordonia sp. ITRI100. Fuel 2016, 184, 761–769.
(6) Liu, P.-W. G.; Yang, D.-S.; Tang, J.-Y.; Hsu, H.-W.; Chen, C.-H.; Lin, I.-K. Development of a cell immobilization technique with polyvinyl alcohol for diesel remediation in seawater. Int. Biodeterior. Biodegrad. 2016, 113, 397–407.
(7) Mohebali, G.; Ball, A. S. Bio desulfurization of diesel fuels—Past, present and future perspectives. Int. Biodeterior. Biodegrad. 2016, 110, 163–180.
(8) Wang, W.; Shao, Z. The long-chain alkane metabolism network of Alcanivorax dieselolei. Nat. Commun. 2014, 5, 5755.
(9) Chengalroyen, M. D.; Dabbs, E. R. The Biodegradation of Latex Rubber: A Minireview. J. Polym. Environ. 2013, 21, 874–880.
(10) Kotani, T.; Yurimoto, H.; Kato, N.; Sakai, Y. Novel Acetone Metabolism in a Propane-Utilizing Bacterium, Gordonia sp. Strain TY-5. J. Bacterial. 2007, 189, 886–893.
(11) Liu, J.; Zheng, Y.; Lin, H.; Wang, X.; Li, M.; Liu, Y.; Yu, M.; Zhao, M.; Pedentchouk, N.; Lea-Smith, D. J.; Todd, J. D.; Magill, C. R.; Zhang, W.-J.; Zhou, S.; Song, D.; Zhong, H.; Xin, Y.; Yu, M.; Tian, J.; Zhang, X.-H. Proliferation of hydrocarbon-degrading microbes at the bottom of the Mariana Trench. Microbiome 2019a, 7, 47.
(12) Nie, Y.; Chi, C.-Q.; Fang, H.; Liang, J.-L.; Lu, S.-I.; Lai, G.-L.; Tang, Y.-Q.; Wu, X.-L. Diverse alkane hydroxylase genes in microorganisms and environments. Sci. Rep. 2015, 4, 4968.
(13) Sowani, H.; Kulkarni, M.; Zinjarde, S. An insight into the ecology, diversity and adaptations of Gordonia Species. Crit. Rev. Microbiol. 2018, 44, 393–413.
(14) Aoyama, K.; Kang, Y.; Yazawa, K.; Gonoi, T.; Kamei, K.; Mikami, Y. Characterization of Clinical Isolates of Gordonia Species in Japanese Clinical Samples During 1998–2008. Micropathological 2009, 168, 175–183.
(15) Sowani, H.; Kulkarni, M.; Zinjarde, S.; Javdekar, V. Gordonia and Related Genera as Opportunistic Human Pathogens Causing Infections of Skin, Soft Tissues, and Bones. The Microbiology of Skin, Soft Tissue, Bone and Joint Infections; Elsevier, 2017; pp 105–121.
(16) Akhtar, N.; Akhtar, K.; Ghauri, M. A. Biodesulfurization of Thiophenic Compounds by a 2-Hydroxybiphenyl-Resistant Gordonia sp. HS126-4N Carrying dusABC Genes. Curr. Microbiol. 2018, 75, 597–603.
(17) Al-Mailém, D. M.; Kansour, M. K.; Radwan, S. S. Bioremediation of Hydrocarbons Contaminating Sewage Effluent Using Man-made Biofilms: Effects of Some Variables. Appl. Biochem. Biotechnol. 2014, 174, 1736–1751.
(18) Meena, S. S.; Sharma, R. S.; Gupta, P.; Karmakar, S.; Aggarwal, K. K. Isolation and identification of Bacillus megaterium YB3 from an effluent contaminated site efficiently degrades pyrene: Bacterial degradation of pyrene. Basic Microbiol. 2016, 56, 369–378.
(19) Silva, A. S.; Camargo, F. A. d. O.; Andreazza, R.; Jacques, R. J. S.; Baldoni, D. B.; Bento, F. M. Enzymatic activity of catechol 1,2-dioxygenase and catechol 2,3-dioxygenase produced by Gordonia polysoprenivorans. Quim. Nova 2012, 35, 1587–1592.
(20) Stobdan, T.; Sinha, A.; Singh, R. P.; Adhikari, D. K. Degradation of pyridine and 4-methylpyridine by Gordonia terrae IIIN1. Biodegradation 2008, 19, 481–497.
(21) Wu, X.; Li, M.; Bai, Q.; Lin, D.; Wang, Y.; Chao, W. Complete degradation of di-n-octyl phthalate by biochemical cooperation between Gordonia sp. strain JDC-2 and Arthrobacter sp. strain JDC-32 isolated from activated sludge. J. Hazard. Mater. 2010, 176, 262–268.
(22) Khan, M. I. Microbial Degradation and Toxicity of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine. J. Microbiol. Biotechnol. 2012, 22, 1311–1323.
(23) Andler, R.; Hiessl, S.; Yuel, O.; Tesch, M.; Steinbüchel, A. Cleavage of poly(ε-caprolactone) rubber as solid substrate by cultures of Gordonia polysoprenivorans. New Biotechnol. 2018, 44, 6–12.
(24) Hiessl, S.; Schultes, J.; Thürmer, A.; Halbsguth, T.; Bröker, D.; Angelov, A.; Liebl, W.; Daniel, R.; Steinbüchel, A. Involvement of Two Latex-Clearing Proteins during Rubber Degradation and Insights into the Subsequent Degradation Pathway Revealed by the Genome Sequence of Gordonia polysoprenivorans Strain VH2. Appl. Environ. Microbiol. 2012, 78, 2874–2887.
(25) Laurrattanarak, S.; Rongsayamanont, W.; Khondiee, N.; Paorach, N.; Soonglerdsongphra, S.; Sinyakong, O.; Luempromchai, E. Production and Application of Gordonia westfalica GY40 Bio surfactant for Remediation of Fuel Oil Spill. Water, Air, Soil Pollut. 2016, 227, 325.
(26) Pizzul, L.; Pilar Castillo, M. D.; Stenström, J. Characterization of selected actinomycetes degrading polyaromatic hydrocarbons in liquid culture and spiked soil. World J. Microbiol. Biotechnol. 2006, 22, 745–752.
(27) Saeki, H.; Sasaki, M.; Komatsu, K.; Miura, A.; Matsuda, H. Oil spill remediation by using the remediation agent JE1058BS that contains a biosurfactant produced by Gordonia sp. strain JE-1058. Bioresources. Technol. 2009, 100, 572–577.
(28) Sowani, H.; Mohite, P.; Damale, S.; Kulkarni, M.; Zinjarde, S. Carotenoid stabilized gold and silver nanoparticles derived from the
Actinomycete Gordonia amicalis HS-11 as effective free radical scavengers. *Enzyme Microb. Technol.* 2016a, 95, 164–173.

(29) Kim, J. H.; Kim, S. H.; Yoon, J. H.; Lee, P. C. Carotenoid production from n-alkanes with a broad range of chain lengths by the novel species Gordonia ajoucoccus A2T. *Appl. Microbiol. Biotechnol.* 2014, 98, 3759–3768.

(30) Silva, T. P.; Paixão, S. M.; Alves, L. Ability of Gordonia alkaniivorans strain 1B for high added value carotenoids production. *RSC Adv.* 2016, 6, 58055–58063.

(31) Hua, F.; Wang, H. Q.; Li, Y.; Zhao, Y. C. Trans-membrane transport of n-octadecane by Pseudomonas sp. DG17. *J. Microbiol.* 2013, 51, 791–799.

(32) Hua, F.; Wang, H. Q. Uptake and trans-membrane transport of petroleum hydrocarbons by microorganisms. *Biotechnol. Biotechnol. Equip.* 2014, 28, 165–175.

(33) Kennedy, R. S.; Finnerty, W. R.; Sudarsanan, K.; Young, R. A. Microbial assimilation of hydrocarbons. *Arch. Microbiol.* 1975, 102, 75.

(34) Gopinath, S. C. B.; Hilda, A.; Anbu, P. Extracellular enzymatic activity profiles in fungi isolated from oil-rich environments. *Mycoscience* 2005, 46, 119–126.

(35) Liu, Y.-F.; Galzerani, D. D.; Mbadinga, S. M.; Zaramela, L. S.; Gu, J.-D.; Mu, B.-Z.; Zengler, K. Metabolic capability and in situ activity of microorganisms in an oil reservoir. *Microbiome* 2018, 6, 5.

(36) Meng, L.; Li, H.; Bao, M.; Sun, P. Metabolic pathway for a new strain Pseudomonas synxantha LSH-7: from chemotaxis to uptake of n-hexadecane. *Sci. Rep.* 2017, 7, 39068.

(37) Ji, N.; Wang, X.; Yin, C.; Peng, W.; Liang, R. CrgA Protein Represses AlkB2 Monoxygenase and Regulates the Degradation of Medium-to-Long-Chain n-Alkanes in Pseudomonas aeruginosa SJTD-1. *Front. Microbiol.* 2019, 10, 400.

(38) Cameotra, S.; Singh, P. Synthesis of rhamnolipid biosurfactant and mode of hexadecane uptake by Pseudomonas species. *Microb. Cell Fact.* 2009, 8, 16.

(39) Ilori, M. O.; Amund, D.; Robinson, G. K. Ultrastructure of two oil-degrading bacteria isolated from the tropical soil environment. *Folia Microbiol.* 2000, 45, 259–262.

(40) Wang, W.; Shao, Z. Enzymes and genes involved in aerobic alkane degradation. *Front. Microbiol.* 2013, 4, 116.

(41) Park, S. J.; Lee, S. Y. Efficient recovery of secretory recombinant proteins from protease negative mutant Escherichia coli strains 4. *Biotechnol. Tech.* 1998, 12, 815.

(42) Cooper, D. G.; Macdonald, C. R.; Duff, S. J. B.; Kosaric, N. Enhanced production of surfactin from B. subtilis by continuous product removal and metal cation additions. *Appl. Environ. Microbiol.* 1981, 42, 408–412.