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Isolation and identification of novel geosmin-degrading bacteria

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1. ABSTRACT

Three novel geosmin-degrading bacteria were isolated from the sediments of Lake Kasumigaura, Japan. All strains were identified as *Acinetobacter* spp. by 16S rRNA gene sequence analysis and can biodegrade geosmin at an initial geosmin concentration of 2 mg/L after 2 days. Furthermore, at an initial geosmin concentration of 40 μg/L, geosmin removal was more than 68% by GSM-2 strain, and the degradation mechanism followed a pseudo-first-order mode. A rate constant of 0.026 reveals rapid geosmin degradation. This is the first report on geosmin degradation by *Acinetobacter* spp.

2. INTRODUCTION

Taste and odor problems in drinking water occur frequently worldwide (1-3). Geosmin (trans-1, 10-dimethyl-trans-9-decalol), a common compound that imparts a musty taste and odor to water, is a secondary metabolite produced by various cyanobacteria and actinomycetes in aquatic environments (4, 5). It may arouse psychosomatic effects, such as headaches, stress, or stomach upsets (6), resulting in consumer complaints and distrust regarding drinking water quality (3). Biological methods are demonstrated great promise due to simple technology and less possibility of contamination. Several

biological treatment studies for geosmin removal have recently been reported (3, 7-9). Though, microorganisms play important roles during biodegradation, studies identifying strains responsible for geosmin biodegradation are limited. One reason is that it is difficult to isolate bacteria by enrichment culture using geosmin as the sole carbon and energy source (10). Nonetheless, bacterial strains like *Bacillus cereus* (11), *B. subtilis* (12), *Arthrobacter atrocyaneus*, *A. globiformis*, *Chlorophenolicus* strain N-1053, *Rhodococcus maris* (13), a member of the *Alphaproteobacteria* (8), *Pseudomonas* sp. SBR3-tpnb, and *R. wratislaviensis* (10) are reported to be involved in geosmin degradation. However, a definitive biodegradation pathway remains unknown. A report on "likely" biodegradation of geosmin by a pathway similar to that of cyclohexanol (7, 14) and one detailing *Acinetobacter* strains capable of degrading cyclohexanol via monooxygenase enzymes (15) have been published. However, to the best of our knowledge till date, no reports suggest on the relationship between geosmin degradation and *Acinetobacter* spp. Here, for the first time, we report three isolates, identified as *Acinetobacter* spp., capable of degrading geosmin by enrichment culture using geosmin as the sole carbon and energy source. Some data on kinetic studies are also presented.

3. MATERIALS AND METHODS

3.1. Geosmin standard

Geosmin was purchased from WAKO pure Chemicals Ltd. Osaka. Japan. 20 mg of geosmin was dissolved in Milli-Q water (Resistivity 18.2MΩ.cm at 25°C) prepared with a water purification system (Purelite PRB-001A/002A) supplied by Organo, Japan. The stock solution was transferred into 500 mL brown air-tight glass bottle and stored in the dark at 4°C prior to use.

3.2. Enrichment and isolation

Sediment samples were collected from Lake Kasumigaura in Japan. Enrichment was performed in a mineral salts medium (MSM) (pH 7.6) containing (per liter of Milli-Q water) 210 mg $CaCl_2{\cdot}2H_20$, 130 mg $MgSO_4{\cdot}7H_20$, 50 mg $Na_2HPO_4{\cdot}12H_2O$, 10 mg K_2HPO_4, and 20 mg $(NH_4)_2SO_4$. Sediment sample (2 g) was added to a 300 ml conical flask filled with 100 ml sterile MSM. Geosmin was spiked as the sole carbon and energy source at a starting concentration of 40 μg l^{-1}. Enrichment was performed in a bio-shaker (25 ± 0.5°C) at 100 rpm, and acclimation was continued for 10 days. To isolate geosmin degraders, MSM was supplemented with 1.5% agar and autoclaved. After cooling, geosmin was added as the sole carbon and energy source at 400 μg l^{-1} and the medium was poured onto petri dishes. These dishes were inoculated with serially diluted inoculum containing the acclimated microorganisms and subsequently incubated in the dark at 25°C. After cultivation, all visible colonies were isolated and inoculated again on petri dishes. The process was repeated until isolated colonies were purified. To eliminate agar interference, single colonies were selected and transferred into liquid MSM with geosmin as the sole carbon and energy source. The isolates were then checked for survival according to the color of the MSM culture (The original MSM culture is transparent/clear and if the isolates grow using geosmin as sole carbon and energy source, the culture becomes turbid).

3.3. Degradation potential check

Three bacterial strains were isolated and subsequently named GSM-1, GSM-2, and GSM-3. The isolates were selected and transferred to MSM with geosmin and incubated for 2 days. Growing cultures were then washed twice by centrifugation at 3000 × *g* for 10 min with resuspension of bacterial pellets in sterile MSM. The cultures were incubated at 25°C for 24 h to deplete residual carbon. Then 1 ml of inoculum was added to a 50 ml conical flask filled with 30 ml sterile MSM containing geosmin (2 mg l^{-1}) to test the degradation capability of each isolated strain. Flasks were sealed using a silicon stopper to avoid volatilization and incubated at 25°C with constant shaking. To estimate geosmin losses due to factors other than biological degradation, an extra flask with inactivated bacterium (An equivalent amount to the active cultures) was included as control (121°C and 20 min autoclaving).

3.4. Kinetic study

1 ml of bacterial culture (washed and carbon-depleted as described above) was added to a 300 ml flask filled with 100 ml sterile MSM containing 40 μg l^{-1} geosmin and incubated in the dark at 25°C (for each sample in triplicate). Samples were taken from the flask at regular intervals (12 h) for geosmin analysis over 48 h. Relevant controls were maintained for comparison.

3.5. Geosmin concentration estimation by GC/MS

Geosmin concentration in the samples was quantitatively analyzed by gas chromatography–mass spectrometry system (GC/MS system) equipped with a pure and trap apparatus (P&G: O.I. Analytical 4660, Auto Sampler: O.I. Analytical 4551A, GC: Agilent Technologies 6890N, MS: Agilent Technologies 5973 inert).

3.6. Phylogenetic analysis

To identify selected isolates, a DNA isolation kit (ISOIL for Beads Beating Japan) was used for total DNA extraction. PCR amplification of partial 16S rDNA fragments was performed using primers 27F and 1492R (8). DNA sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3130 DNA Sequencer (Applied Biosystems). The sequence determined was subjected to BLAST in the NCBI nucleotide sequence database (http://www.ncbi.nlm.nih.gov). For more information on taxonomy, a phylogenetic tree was constructed using DNA sequences of all isolates along with related bacteria and some previously known geosmin-degrading bacteria and the MEGA4 software (16).

4. RESULTS AND DISCUSSION

4.1. Test for degradation potential

All strains could biodegrade geosmin at the initial geosmin concentration of 2 mg L^{-1} after 2 days (Table 1), possibly because of some geosmin-degrading enzymes in

Table 1. Determination of geosmin removal

Name	Removal(%)[1,2]	Standard Deviation (%)
GSM-1	45.02	±0.1
GSM-2	45.31	±0.2
GSM-3	36.27	±0.5

[1]The experiment was performed in triplicate, [2]The initial geosmin concentration was 2 mg L^{-1}

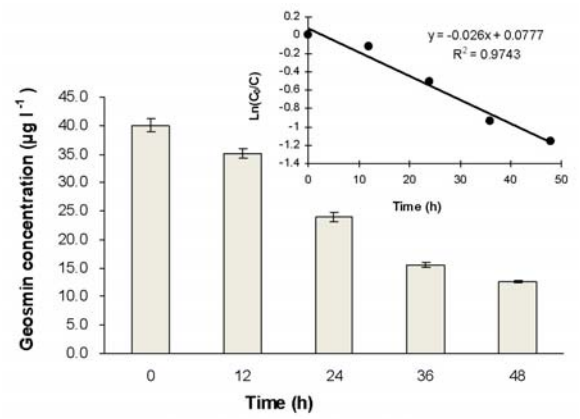

Figure 1. Kinetic study data for geosmin biodegradation. Error bars represent standard deviations of triplicate analyses. Ratio of inoculation, 1.0%; initial geosmin concentration, 40 µg l^{-1}; incubation time, 48 h; and temperature, 25°C. Inset: pseudo-first-order kinetic plot using data from the removal curve.

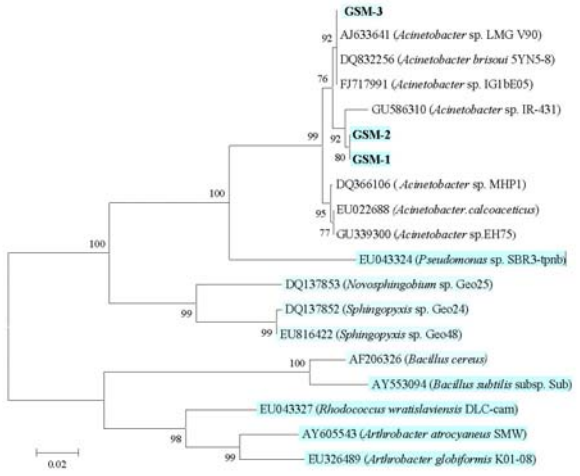

Figure 2. Neighbor-joining phylogenetic tree showing the position of isolates GSM-1, GSM-2, and GSM-3 in relation to other closely related organisms, including isolates previously implicated in geosmin degradation. Bacteria with blue background are geosmin-degrading strains. Numerical tree values represent bootstrap support. The scale bar at the bottom shows the number of nucleotide substitutions per site.

the culture. The geosmin removal capability of GSM-1 and GSM-2 showed no obvious difference, indicating that they have almost similar geosmin degrading abilities. The geosmin removal caused by these two strains was higher than GSM-3. This suggests that some geosmin-degrading enzymes in GSM-1 and GSM-2 are more active than those present in GSM-3.

4.2. Kinetic analyses

GSM-2 was chosen for kinetic studies (chosen arbitrarily). It was observed that in presence of GSM-2, geosmin decreased significantly with increased incubation time. It is interesting to observed that geosmin concentration was not significantly reduced for the first 12 h, but reduced from 40 to 12.6 µg l^{-1} (by >68%) after 48 h of incubation. This suggests that, GSM-2 possibly requires an adaptation period, and/or some enzymes involved in geosmin degradation were not completely activated initially. Experimental data were analyzed using a pseudo-first-order Lagergren equation. A straight line of Ln(C_0/C) versus reaction time suggests the applicability of this kinetic model (Figure 1) (8). The linearity of the relationship (R^2 = 0.9743) indicates a first-order nature of geosmin degradation. A rate constant of 0.026 reveals rapid geosmin degradation. Hoefel *et al.* (8) previously reported similar rate constants for geosmin degradation by isolated pure strains, but initial concentrations were expressed as ng l^{-1}. It must be mentioned that geosmin loss caused by volatilization and other factors was extremely less (<1%) during biodegradation in the kinetic study (data not shown).

4.3. Phylogenetic analysis

Phylogenetic analysis reveals that isolates GSM-1, GSM-2, and GSM-3 belong to *Acinetobacter* spp. (Figure 2). GSM-1 and GSM-2 are identical according to the analysis of 16S rRNA genes. The two isolates were 100% same over a 529 bp fragment of their 16S rRNA genes. They fall on a separate branch in the tree when compared with other already known geosmin degrading bacteria. It must also be mentioned that they cluster well with *Acinetobacter* spp. The DNA sequences of 16S rRNA gene fragments were deposited in GenBank (accession numbers HM209469–HM209471). Some *Acinetobacter* spp. have been reported to degrade pollutants, such as benzene, toluene, phenol, benzoate, crude oil, biphenyls, and swainsonine (17). Also, several strains degrading cyclohexanol using monooxygenase enzymes in reactions similar to the biological Baeyer–Villiger reaction have been earlier reported (15). In future, genes encoding monooxygenase enzyme in cyclohexanol degradation could possibly be targeted for elucidating the relationship between geosmin and cyclohexanol degradations.

5. CONCLUSION

This is the first report on the isolation and characterization of Acinetobacter bacteria capable of degrading geosmin individually. All the strains were isolated from the sediments of Lake Kasumigaura, Japan. GSM-2 is demonstrated to biodegrade geosmin via a pseudo-first-order mechanism. Further studies to identify genes involved in geosmin degradation and explore the degradation pathway are underway.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. R. W. Eaton and P. Sandusky: Biotransformations of 2-methylisoborneol by camphor-degrading bacteria, *Appl Environ Microbiol* 75, 583-588 (2009)

2. F. Juttner and S. B. Watson: Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters, *Appl Environ Microbiol* 73, 4395-4406 (2007)

3. B. McDowall, D. Hoefel, G. Newcombe, C. P. Saint and L. Ho: Enhancing the biofiltration of geosmin by seeding sand filter columns with a consortium of geosmin-degrading bacteria, *Water Res* 43, 433-440 (2009)

4. N. N. Gerber, Volatile substances from actinomycetes: their role in the odor pollution of water, *CRC Crit Rev Microbiol* 7, 191-214 (1979)

5. N. Sugiura, N. Iwami, Y. Inamori, O. Nishimura and R. Sudo: Significance of attached cyanobacteria relevant to the occurrence of musty odor in Lake Kasumigaura, *Water Res* 32, 3549-3554 (1998)

6. W. F. Young, H. Horth, R. Crane, T. Ogden and M. Arnott: Taste and odour threshold concentrations of potential potable water contaminants, *Water Res* 30, 331-340 (1996)

7. L. Ho, D. Hoefel, F. Bock, C. P. Saint and G. Newcombe: Biodegradation rates of 2-methylisoborneol (MIB) and geosmin through sand filters and in bioreactors, *Chemosphere* 66, 2210-2218 (2007)

8. D. Hoefel, L. Ho, P. T. Monis, G. Newcombe and C. P. Saint: Biodegradation of geosmin by a novel Gram-negative bacterium; isolation, phylogenetic characterisation and degradation rate determination, *Water Res* 43, 2927-2935 (2009)

9. N. Sugiura, H. Isoda and M. Takaaki, Degradation potential of musty odour in a drinking water source by a biofilm method, *Journal of Water Supply: Research and Technology – AQUA* 52, 181-187 (2003)

10. R. W. Eaton and P. Sandusky: Biotransformations of (+/-)-geosmin by terpene-degrading bacteria, *Biodegradation* 21, 71-79 (2010)

11. J. K. G. Silvey, A. W. Henley, W. J. Nunez, and R. C. Cohen. Biological Control: Control of Naturally Occurring Taste and Odors by Microorganisms. Proceedings of the National. Biological Congress, Detroit, USA (1970)

12. L.V. Narayan and W.J. Nunez: Biological control: isolation and bacterial oxidation of the taste and odor compound geosmin, *J Am Water Works Assoc* 66, 532-536 (1974)

13. I. Saadoun and F. el-Migdadi: Degradation of geosmin-like compounds by selected species of gram-positive bacteria, *Lett Appl Microbiol* 26, 98-100 (1998)

14. Q. Cheng, S. M. Thomas, K. Kostichka, J. R. Valentine and V. Nagarajan: Genetic analysis of a gene cluster for cyclohexanol oxidation in *Acinetobacter* sp. Strain SE19 by *in vitro* transposition, *J Bacteriol* 182, 4744-4751 (2000)

15. P. W. Trudgill. Microbial degradation of the alicyclic ring: structural relationships and metabolic pathways, p. 131–180. In D. T. Gibson, (ed.), Microbial Degradation of Organic Compounds. Marcel Dekker Inc., New York. (1984)

16. S. Kumar, M. Nei, J. Dudley and K. Tamura: MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences, *Brief Bioinform* 9, 299-306 (2008)

17. D. Abd-El-Haleem: *Acinetobacter*: environmental and biotechnological applications, *African Journal of Biotechnology* 2, 71-74 (2003)

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