AEM
Journals.ASM.org

n-Alkane Chain Length Alters *Dietzia* sp. Strain DQ12-45-1b Biosurfactant Production and Cell Surface Activity

Xing-Biao Wang,[a,b,c] Yong Nie,[a] Yue-Qin Tang,[a] Gang Wu,[b] Xiao-Lei Wu[a]

Department of Energy and Resources Engineering, College of Engineering, Peking University, Beijing, People's Republic of China[a]; State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, People's Republic of China[b]; Graduate University of Chinese Academy of Sciences, Beijing, People's Republic of China[c]

Upon growth on *n*-hexadecane (C_{16}), *n*-tetracosane (C_{24}), and *n*-hexatriacontane (C_{36}), *Dietzia* sp. strain DQ12-45-1b could produce different glycolipids, phospholipids, and lipopeptides. Interestingly, cultivation with C_{36} increased cell surface hydrophobic activity, which attenuated the negative effect of the decline of the emulsification activity. These results suggest that the mechanisms of biosurfactant production and cell surface hydrophobicity are dependent upon the chain lengths of the *n*-alkanes used as carbon sources.

Recently, the biodegradation of crude oil constituents, such as alkanes, through bioremediation of oil-polluted environments (1–3) and microbial enhanced oil recovery (MEOR) technology (4) has received worldwide attention. To date, a number of microorganisms have been reported to degrade alkanes of different chain lengths (5). A critical step in the biodegradation process requires microorganisms to access hydrophobic alkanes by at least two possible mechanisms. First, microorganisms produce surface-active materials, including glycolipids, phospholipids, and lipopeptides, to emulsify alkanes and achieve surfactant-mediated access (6–12). Second, they increase the hydrophobic activity of the cell surface to directly interact with alkanes (5, 13).

Although extensive research has been conducted on the production of different biosurfactants and the cell surface hydrophobic activities, these studies were mainly restricted to alkanes with chain lengths shorter than 18 carbon atoms (C_{18}) (14–18). This raises the question of how bacteria, such as those belonging to the genus *Dietzia*, access hydrocarbons with chain lengths longer than C_{18}. It is unclear whether accessing longer alkanes requires the production of surface-active materials that are similar to those produced when shorter alkanes (i.e., $<C_{18}$) are degraded. In addition, whether cell surface hydrophobicity contributes to the accession of longer alkanes is unknown. The aim of this study was, therefore, to address these questions because degradation of alkanes longer than C_{18} is important for effective MEOR and bioremediation. We used a broad-spectrum alkane-degrading *Dietzia* sp. strain, DQ12-45-1b (19, 20), and the results of our study revealed that biosurfactant production and cell surface hydrophobic activity changed when different-chain-length *n*-alkanes were used as the sole carbon sources.

After *Dietzia* sp. strain DQ12-45-1b was incubated in mineral salt medium (MSM) (21) amended with 0.3% (vol/vol) *n*-hexadecane (C_{16}) and 0.05% (wt/vol) *n*-tetracosane (C_{24}) and *n*-hexatriacontane (C_{36}) as the sole carbon sources, respectively, the cultures were sampled at different time points and analyzed for bacterial growth, alkane degradation, cell surface hydrophobic activities, and emulsifying capacity of the culture broth. The biosurfactants were also extracted from the culture broth, and the moieties of the glycolipid-like biosurfactant were additionally analyzed. The transcripts of the glycolipid synthesis-related genes were also analyzed by real-time reverse transcription-PCR (RT-PCR). All experiments were performed in triplicate with various controls. Detailed experimental procedures and methods are described in the supplemental material.

Strain DQ12-45-1b could degrade C_{16}, C_{24}, and C_{36} *n*-alkanes for growth (see Table S1 in the supplemental material), as was previously reported (19, 20). Along with the growth and degradation of alkanes, the surface tension of the culture broth decreased from approximately 62 mN m^{-1} to 27.78 ± 1.97 and 45.97 ± 2.19 mN m^{-1} at day 30 for C_{16} and C_{24} cultures, respectively, and to 48.66 ± 0.51 mN m^{-1} at day 45 for the C_{36} culture (see Fig. S1 in the supplemental material); this finding suggested that shorter alkanes resulted in a higher emulsifying activity, which allowed cells to access alkanes more easily. The C_{16}, C_{24}, and C_{36} culture broths yielded 95.7, 25.4, and 15.8 mg $liter^{-1}$ of crude biosurfactant, respectively. Among them, 2 glycolipid compounds (16-A and 16-B) (Fig. 1A) were detected in the C_{16} culture, while levels of phospholipid and lipopeptide materials were negligible in the C_{16} culture (Fig. 1B and C). In the C_{24} culture, glycolipid (24-C) (Fig. 1A) and phospholipid (Fig. 1B) compounds were detected at relatively equal amounts, with no instance of lipopeptide being detected (Fig. 1C). One glycolipid (36-D) (Fig. 1A) and 2 lipopeptide (Fig. 1C) compounds were detected in the C_{36} culture; however, no phospholipids were detected (Fig. 1B). These results suggested that the production of biosurfactant by strain DQ12-45-1b was related to the length of the hydrocarbons. The biosurfactant activities of these materials were confirmed by oil displacement tests performed after they had been scraped out of the preparative thin-layer chromatography (TLC) plates (see the supplemental material).

Since glycolipid-like materials were detected in all 3 cultures, the structures of the 4 glycolipids (16-A, 16-B, 24-C, and 36-D)

Received 13 August 2012 Accepted 12 October 2012
Published ahead of print 26 October 2012
Address correspondence to Xiao-Lei Wu, xiaolei_wu@pku.edu.cn.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.02497-12.
Copyright © 2013, American Society for Microbiology. All Rights Reserved.
doi:10.1128/AEM.02497-12

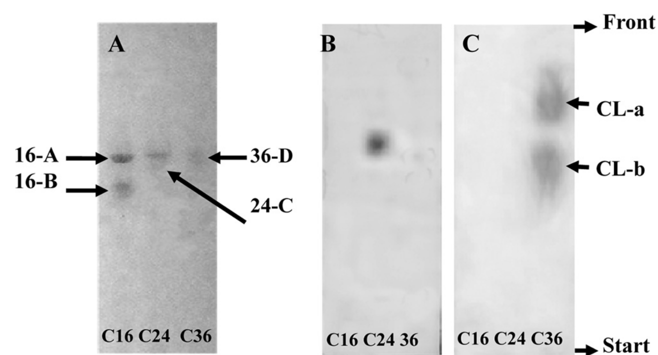

FIG 1 Thin-layer chromatography (TLC) analysis of crude biosurfactant extract from the cultures sampled at day 15. Panels A, B, and C show the presence and relative amounts of glycolipids, phospholipids, and lipopeptides from the *n*-hexadecane (C_{16}), *n*-tetracosane (C_{24}), and *n*-hexatriacontane (C_{36}) cultures, respectively. In panel A, 2 glycolipids (16-A, R_f, 0.59; 16-B, R_f, 0.51) were detected from the C_{16} culture, whereas glycolipids 24-C (R_f, 0.60) and 36-D (R_f, 0.59) were detected from the C_{24} and C_{36} cultures, respectively. In panel B, phospholipids (R_f, 0.62) were detected only in the C_{24} culture. In panel C, lipopeptides were detectable only in the C_{36} culture, with 2 compounds detected: CL-a (R_f, 0.79) and CL-b (R_f, 0.56).

were analyzed by gas chromatography-mass spectrometry (GC-MS). Three saccharide moieties were detected, including α-D-glucopyranoside-β-D-fructofuranose (disaccharide unit) for both 16-A and 16-B, 6-deoxy-mannose for 24-C, and 2-methoxime-gluconic acid for 36-D. The fatty acid moieties were also different among the glycolipids. In glycolipid 16-A, only hexadecanoic acid was detected, in contrast to the 5 acids detected in glycolipid 16-B, some of which had unsaturated bonds. Six fatty acids with chain lengths ranging from C_{12} to C_{19} and 11 fatty acids with chain lengths ranging from C_{12} to C_{24} were detected in 24-C and 36-D, respectively. These fatty acids also contained some unsaturated bonds (Table 1). Of note, the fatty acid moieties in 16-B contained fatty acids with chain lengths longer than C_{16}, indicating that they have originated from fatty acid synthesis processes.

The transcripts of phosphomannomutase (AlgC)-encoding gene homolog YMF1348 (accession no. JQ414011) and NADPH-dependent ketoacyl reductase (RhlG)-encoding gene homolog YMF0365 (accession no. JQ414010), which are key genes in glycolipid biosynthesis (see Table S2 in the supplemental material), were detected by real-time RT-PCR (see Fig. S2 in the supplemental material). Both YMF1348 and YMF0365 were significantly induced by *n*-alkanes compared to when cells were grown in a medium containing glucose. However, the 2 genes had different patterns of expression. In general, the transcriptional levels of both YMF1348 and YMF0365 were higher in cells grown on C_{16}, with the lowest values being obtained for cells grown on C_{36}, corresponding to the different amounts of glycolipids detected (see Fig. S2). The different transcription levels might be related to the different amounts of the precursors acetyl coenzyme A (acetyl-CoA) and malate in cells, which may suggest the different upregulation of genes in fatty acid biosynthesis, as detected in *Alcanivorax borkumensis* SK2 (22).

Although glycolipids, phospholipids, and lipopeptides have been reported as key microbial biosurfactants (6, 8, 10, 12), only 1 or 2 types (e.g., glycolipids, phospholipids, or lipopeptides) were simultaneously detected in the degradation of hydrocarbons with chain lengths of $<C_{18}$ (15, 16, 23). However, strain DQ12-45-1b could produce all the 3 types in various amounts when C_{16}, C_{24}, and C_{36} were used as the sole carbon sources. Moreover, the simultaneous detection of different glycolipids, both with different saccharide and acid moieties, in a *Dietzia* strain has not been reported before.

The hydrophobicity of the cell surface, which is measured as the surface hydrophobicity rate, plays an important role in the microbial attachment onto other hydrophobic surfaces, such as solid *n*-alkanes (24). In this study, the cell surface hydrophobicity rates slightly increased with the incubation time from approximately 0.5% to 8.5% ± 5.5% and 8.3% ± 4.6% for C_{16} and C_{24} cultures, respectively, compared to approximately 4% in the control without any hydrocarbon. In contrast, the hydrophobicity rate in C_{36} cultures could be as high as 23.9% ± 3.7% (Fig. 2), which caused more cells to attach to the solid surface of C_{36} than to C_{24} (see Fig. S3 in the supplemental material). These results could be attributed to the fact that glycolipids produced by C_{36} contain longer organic acid moieties, resulting in higher hydrophobic activities, or that lipopeptides (including the surfactin, iturin, and fengycin classes) changed the cell hydrophobicity and cell contact with hydrocarbons (6); therefore, when C_{36} was used as the sole carbon source, the decline in the emulsification activity (see Fig. S1 in the supplemental material) was compensated for by an increase in the cell surface hydrophobic activity (Fig. 2), which attenuated the negative impact of the long-chain length of C_{36} and maintained cell growth, as indicated in our previous studies (19, 20).

On the basis of these results, we hypothesized that bacteria capable of degrading hydrocarbons of various chain lengths have similar functions for producing different biosurfactants and changing cell surface hydrophobic activity. These functions could be attributed to the unique regulation of different genes and pathways. Similar results were reported when investigating the influence of other environmental factors on biosurfactant production (22, 25–28). However, further investigation is required to verify this hypothesis.

Nucleotide sequence accession numbers. The nucleotide se-

TABLE 1 Moieties of the glycolipids produced from C_{16}, C_{24}, and C_{36} by strain DQ12-45-1b

	Moiety of[a]:			
	n-C_{16}			
Fatty acid	16-A	16-B	*n*-C_{24} 24-C	*n*-C_{36} 36-D
$C_{12}H_{24}O_2$	ND	ND	+	+
$C_{14}H_{28}O_2$	ND	+	+	+
$C_{15}H_{30}O_2$	ND	ND	+	+
$C_{16}H_{30}O_2$	ND	ND	ND	+ (7:1)
$C_{16}H_{32}O_2$	+	+	+	+
$C_{17}H_{34}O_2$	ND	ND	ND	+
$C_{18}H_{32}O_2$	ND	+ (9:1, 12:1 and 9:1, 15:1)	ND	+ (9:1, 11:1)
$C_{18}H_{34}O_2$	ND	+ (9:1)	ND	+ (8:1)
$C_{18}H_{36}O_2$	ND	+	+	+
$C_{19}H_{38}O_2$	ND	ND	+	+ (10-methyl)
$C_{24}H_{48}O_2$	ND	ND	ND	+

[a] The saccharide moieties are as follows: *n*-C_{16} 16-A and 16-B, α-D-glucopyranoside-β-D-fructofuranose; *n*-C_{24} 24-C, 6-deoxymannose; and *n*-C_{36} 36-D, 2-methoxime-gluconic acid. ND, not detected. The format "n:1" represents an unsaturated bond at the "*n*th" carbon atom.

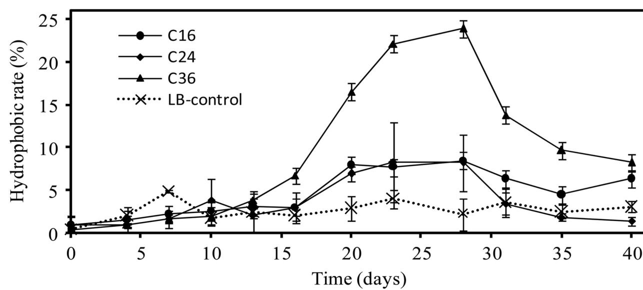

FIG 2 Change in cell surface hydrophobicity rates of *Dietzia* sp. strain DQ12-45-1b grown on *n*-hexadecane (C_{16}), *n*-tetracosane (C_{24}), and *n*-hexatriacontane (C_{36}), or grown on LB as the control.

quences of YMF0365 and YMF1348 have been deposited in the GenBank database under accession no. JQ414010 and JQ414011, respectively.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (31070107) and the National High Technology Research and Development Program (863 Program: 2012AA02A703).

REFERENCES

1. **Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N, Jansson JK, Probst A, Borglin SE, Fortney JL, Stringfellow WT, Bill M, Conrad ME, Tom LM, Chavarria KL, Alusi TR, Lamendella R, Joyner DC, Spier C, Baelum J, Auer M, Zemla ML, Chakraborty R, Sonnenthal EL, D'haeseleer P, Holman HY, Osman S, Lu Z, Van Nostrand JD, Deng Y, Zhou J, Mason OU.** 2010. Deep-sea oil plume enriched indigenous oil-degrading bacteria. Science **330**:204–208.
2. **Lu Z, Deng Y, Van Nostrand JD, He Z, Voordeckers J, Zhou A, Lee YJ, Mason OU, Dubinsky EA, Chavarria KL, Tom LM, Fortney JL, Lamendella R, Jansson JK, D'haeseleer P, Hazen TC, Zhou J.** 2012. Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume. ISME J. **6**:451–460.
3. **Rodríguez-Blanco A, Antoine V, Pelletier E, Delille D, Ghiglione JF.** 2010. Effect of temperature and fertilization on total vs. active bacterial communities exposed to crude and diesel oil pollution in NW Mediterranean Sea. Environ. Pollut. **158**:663–673.
4. **Lazar I, Petrisor IG, Yen TF.** 2007. Microbial enhanced oil recovery (MEOR). Petrol. Sci. Technol. **25**:1353–1366.
5. **Wentzel A, Ellingsen T, Kotlar H-K, Zotchev S, Throne-Holst M.** 2007. Bacterial metabolism of long-chain *n*-alkanes. Appl. Microbiol. Biotechnol. **76**:1209–1211.
6. **Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R.** 2010. Microbial biosurfactants production, applications and future potential. Appl. Microbiol. Biotechnol. **87**:427–444.
7. **Desai JD, Banat IM.** 1997. Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. Rev. **61**:47–64.
8. **Mukherjee S, Das P, Sen R.** 2006. Towards commercial production of microbial surfactants. Trends Biotechnol. **24**:509–519.
9. **Neu TR.** 1996. Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiol. Rev. **60**:151–166.
10. **Pacwa-Plociniczak M, Plaza GA, Piotrowska-Seget Z, Cameotra SS.** 2011. Environmental applications of biosurfactants: recent advances. Int. J. Mol. Sci. **12**:633–654.
11. **Rosenberg E, Ron EZ.** 1999. High- and low-molecular-mass microbial surfactants. Appl. Microbiol. Biotechnol. **52**:154–162.
12. **Singh A, Hamme JD, Ward OP.** 2007. Surfactants in microbiology and biotechnology. Part 2. Application aspects. Biotechnol. Adv. **25**:99–121.
13. **Rosenberg RO, Mikkilineni R, Berne BJ.** 1982. Hydrophobic effect on chain folding. The trans to gauche isomerization of n-butane in water. J. Am. Chem. Sci. **104**:7647–7649.
14. **Dastgheib SMM, Amoozegar MA, Khajeh K, Ventosa A.** 2011. A halotolerant *Alcanivorax* sp. strain with potential application in saline soil remediation. Appl. Microbiol. Biotechnol. **90**:305–312.
15. **Hua X, Wu Z, Zhang H, Lu D, Wang M, Liu Y, Liu Z.** 2010. Degradation of hexadecane by Enterobacter cloacae strain TU that secretes an exopolysaccharide as a bioemulsifier. Chemosphere **8**:951–956.
16. **Peng F, Liu Z, Wang L, Shao Z.** 2007. An oil-degrading bacterium: *Rhodococcus erythropolis* strain 3C-9 and its biosurfactants. J. Appl. Microbiol. **6**:1603–1611.
17. **Puntus IF, Sakharovsky VG, Filonov AE, Boronin AM.** 2005. Surface activity and metabolism of hydrocarbon-degrading microorganisms growing on hexadecane and naphthalene. Proc. Biochem. **40**:2643–2648.
18. **Zheng C, Li S, Yu L, Huang L, Wu Q.** 2009. Study of the biosurfactant-producing profile in a newly isolated *Rhodococcus ruber* strain. Ann. Microbiol. **4**:771–776.
19. **Nie Y, Liang JL, Fang H, Tang YQ, Wu XL.** 2011. Two novel alkane hydroxylase-rubredoxin fusion genes isolated from a *Dietzia* bacterium and the functions of fused rubredoxin domains in long-chain n-alkane degradation. Appl. Environ. Microbiol. **77**:7279–7288.
20. **Wang XB, Chi CQ, Nie Y, Tang YQ, Tan Y, Wu G, Wu XL.** 2011. Degradation of petroleum hydrocarbons (C6-C40) and crude oil by a novel *Dietzia* strain. Bioresour. Technol. **102**:7755–7761.
21. **Rizzo ACL, da Cunha CD, Santos RLC, Santos RM, Magalhaes HM, Leite SGF, Soriano AU.** 2008. Preliminary identification of the bioremediation limiting factors of a clay bearing soil contaminated with crude oil. J. Braz. Chem. Soc. **19**:169–174.
22. **Sabirova JS, Ferrer M, Regenhardt D, Timmis KN, Golyshin PN.** 2006. Proteomic insights into metabolic adaptations in *Alcanivorax borkumensis* induced by alkane utilization. J. Bacteriol. **188**:3763–3773.
23. **Nakano MM, Corbell N, Besson J, Zuber P.** 1992. Isolation and characterization of *sfp*: a gene that functions in the production of the lipopeptide biosurfactant, surfactin, in *Bacillus subtilis*. Mol. Gen. Genet. **2**:313–321.
24. **Liu Y, Yang S, Liu Q, Tay J.** 2003. The role of cell hydrophobicity in the formation of aerobic granules. Curr. Microbiol. **46**:270–274.
25. **Edmonds P, Cooney JJ.** 1969. Lipids of *Pseudomonas aeruginosa* cells grown on hydrocarbons and on Trypticase soybean broth. J. Bacteriol. **98**:16–22.
26. **Finnerty WR, Singer ME.** 1985. A microbial biosurfactant—physiology, biochemistry and applications. Dev. Ind. Microbiol. **25**:31–46.
27. **Neidleman SL, Geigert J.** 1984. Biotechnology and oleochemicals: changing patterns. J. Am. Oil Chem. Soc. **61**:290–297.
28. **Syldatk C, Lang S, Wagner F, Wray V, Witte L.** 1985. Chemical and physical characterization of four interfacial-active rhamnolipids from *Pseudomonas* sp. DSM 2874 grown on *n*-alkanes. Z. Naturforsch. **40**:51–60.