Published in final edited form as:

J Inorg Biochem. 2012 February ; 107(1): 90–95. doi:10.1016/j.jinorgbio.2011.10.013.

A suite of citrate-derived siderophores from a marine *Vibrio* species isolated following the Deepwater Horizon oil spill

Julia M. Gauglitz^{a,b,1}, Hongjun Zhou^b, and Alison Butler^{b,*}

^aGraduate Program in Marine Science, University of California, Santa Barbara, CA 93106, United States

^bDepartment of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, United States

Abstract

Nearly all microbes require iron for growth. The low concentration of iron found in the ocean makes iron acquisition a particularly difficult task. In response to these low iron conditions, many bacteria produce low-molecular-weight iron-binding molecules called siderophores to aid in iron uptake. We report herein the isolation and structural characterization of a suite of amphiphilic siderophores called the ochrobactins-OH, which are produced by a *Vibrio* species isolated from the Gulf of Mexico after the 2010 Deepwater Horizon oil spill. The citrate-based ochrobactins-OH are derivatives of aerobactin, replacing the acetyl groups with fatty acid appendages ranging in size from C8 to C12, and are distinctly different from the ochrobactins in that the fatty acid appendages are hydroxylated rather than unsaturated. The discovery of the marine amphiphilic ochrobactin-OH suite of siderophores increases the geographic and phylogenetic diversity of siderophore-producing bacteria.

Keywords

Marine siderophore; Amphiphilic siderophore; Citrate-based; Ochrobactin; Gulf of Mexico

1. Introduction

The explosion of the Deepwater Horizon oil well in April 2010 marked the largest accidental marine oil spill recorded to date. The ecological effects of this large-scale perturbation are under investigation [1–5], with particular focus on the impacts of released oil and gas on the community structure and metabolic activity of the microbial community.

Recent observations indicate that the microbial community shifts following a large input of oil and that hydrocarbon degrading bacteria or closely related bacteria flourish [5]. The rapid increase and decrease of different populations of bacteria may imply differential utilization of hydrocarbons or other nutrients. A number of metalloenzymes used in hydrocarbon degradation require iron, notably the non-heme iron enzyme AlkB and the iron-heme enzyme CYP153 which oxidize nalkanes [6–8]. Soluble and particulate methane monooxygenase (sMMO and pMMO) also contain iron and pMMO is found in all known

© 2011 Published by Elsevier Inc.

*Corresponding author. Tel.: +1 805 893 8178; fax: +1 805 893 4120. butler@chem.ucsb.edu (A. Butler).

¹Previously published under Julia M. Vraspir.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jinorgbio.2011.10.013.

methanotrophs, with the exception of species of the *Methylocella* 9]. To meet the metabolic iron demand of these organisms following an oil spill, when labile, small chain hydrocarbons are most prevalent, bacteria may produce siderophores. Siderophore-producing bacteria in the vicinity of an oil spill have not been studied previously.

Siderophores are low-molecular-weight iron chelators produced by bacteria. Marine bacterial siderophores are often produced in amphiphilic suites, where each member within the suite has a conserved Fe(III)-binding polar head group appended by one or two fatty acids. The chemical structure of the polar head group subdivides marine amphiphilic siderophores into two groups: peptide-based amphiphilic siderophores and citrate-based amphiphilic siderophores [10]. The citrate-based siderophores (Fig. 1) are photoreactive when coordinated to Fe(III), resulting in the production of an oxidized siderophore and Fe (II), which rapidly reoxidizes to Fe(III) [11–13].

The amphiphilic and photoreactive characteristics seen among marine siderophores may be unique adaptations for the surface waters of the oceans. Amphiphilic siderophores are most commonly documented among marine bacteria, with the exceptions falling among pathogenic strains of bacteria, such as *Mycobacteria* [19,20] and *Acinetobacteria* 21]. The hydrophobic nature of the fatty acid appendages found in amphiphilic siderophores confers the ability to partition into the bacterial membrane [13,21,22], which is likely advantageous in an aqueous environment, such as pathogenic bacteria encounter in their hosts or marine bacteria encounter in the ocean.

Marine bacteria, in addition to the challenges of an aqueous environment, are also exposed to UV irradiation in surface waters. Marine amphiphilic siderophores which are also photoreactive may therefore function to both efficiently sequester iron for uptake and contribute to iron cycling. Examples of photoreactive amphiphilic siderophores include the synechobactins (*Synechococcus* PCC7002) [18], the membrane-associated ochrobactins (*Ochrobactrum* sp. SP18, *Vibrio* sp. DS40M5) [13], and the ochrobactins-OH (*Vibrio* sp. S4BW, this work).

We report herein the identification and isolation of the ochrobactins-OH, a new suite of amphiphilic citrate-based siderophores produced by *Vibrio* sp. S4BW isolated from the Gulf of Mexico.

2. Materials and methods

2.1. Bacterial strain

Vibrio sp. S4BW was isolated from surface seawater collected in the Gulf of Mexico after the April 20th, 2010 Deepwater Horizon oil spill [GenBank ID: JN701478]. The water sample was collected at 10 pm on June 4th, 2010 at 28°43.615'N, 88°23.067'W, in an area with visible oil at the ocean surface. Water was collected in sterile 50 mL falcon tubes and kept refrigerated until plating onto agar plates on June 11th, 2010. *Vibrio* sp. S4BW and several other bacterial strains were isolated and screened for siderophore production as described in Vraspir et al. [23]. The bacteria were identified by sequencing of the 16S small subunit rRNA gene as previously described [23] with the following modifications: 16S rRNA genes were amplified by PCR using variations of commonly used bacterial primers, 8F (5'-AGR GTT YGA TYM TGG CTC AG-3') and 1492R (5'-GGY TAC CTT GTT ACG ACT T-3'). A single band of the predicted length was observed by agarose gel electrophoresis. The DNA was extracted and purified using a gel extraction kit (Qiagen) prior to sequencing. Samples were sequenced using the following primers: 8F, 519F (5'-CAG CMG CCG CGG TAA TWC-3'), 519R (5'-GWA TTA CCG CGG CKG CTG-3'), and 1492R, to ensure adequate sequence overlap between runs (UC Berkeley DNA

Sequencing Facility). Sequences were aligned using a biological sequence alignment editor (BioEdit).

2.2. Siderophore isolation

For siderophore isolation, *Vibrio* sp. S4BW was cultured in 2 L natural seawater medium (NSW) in 4 L acid-washed Erlenmeyer flasks. NSW medium consists of 1 g ammonium chloride, 2 g bacto-casamino acids, and 0.1 g glycerol phosphate per liter of aged natural seawater. Cultures were grown on a rotary shaker (180 rpm) at room temperature for 24–30 h before harvesting. The cultures were harvested by centrifugation at 6000 rpm for 30 min at 4 °C. Amberlite XAD-2 resin (Aldrich) was added to the cell-free supernatant to adsorb the siderophores. The supernatant and resin mixture was shaken for approximately 3.5 h at 110 rpm, after which the mixture of XAD-2 and supernatant was washed with doubly deionized water to remove salts. The XAD-2 resin was then packed in a filter column and the siderophores were eluted with 100% methanol through a 0.22- μ m filter into glass vials. Siderophore-containing fractions were pooled and concentrated via rotary vacuum evaporation.

The bacterial cell pellet of *Vibrio* sp. S4BW was also extracted by shaking for 24 h in 100% EtOH and analyzed for siderophore production. The ethanol extract was filtered through a 0.22- μ m filter and concentrated via rotary evaporation. The concentrate was brought to a volume of about 1 L with water and purified by application to a C₁₈ Sep-Pak column (Waters), rinsed with doubly deionized water and eluted in 100% methanol.

The siderophores were purified by reversed-phase high-performance liquid chromatography (RP-HPLC) on a preparative C₄ column (250 mm length $\times$ 20 mm diameter, Higgins) with a gradient from 50% solvent A (0.05% trifluoroacetic acid (TFA) in doubly deionized water) to 100% solvent B (0.05% TFA in 80% methanol, 20% doubly deionized water) over 15 min, holding at 100% solvent B for an additional 10 min using a dual-pump Waters HPLC system. The eluent was continuously monitored at 215 nm and peaks were collected by hand and stored on dry ice. If necessary, fractions were ultrapurified on the same preparative C₄ column using the same program as before. Purified siderophores were lyophilized and stored at –20 °C.

2.3. Structure determination

The masses of the siderophores and the siderophore fragments were analyzed by electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry on a Micromass Q-TOF2 (Waters Corp.).

The amino acid composition was determined using the chiral derivatizing agent 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide (FDAA; Marfey's reagent) after hydrolysis of the siderophore with 55% hydroiodic acid (Spectrum Chemicals and Laboratory Products) at 110 °C for 48 h[24]. The hydrolyzed and derivatized amino acid was identified by co-injection with derivatized authentic amino acid standards and by comparison of the retention times when resolved by RP-HPLC.

UV–vis spectroscopy of the ferric ochrobactin-OH B complex was performed on a Cary 300 Bio UV–Visible Spectrophotometer (Varian). ¹H, ¹³C, heteronuclear single quantum correlation spectroscopy (HSQC), correlation spectroscopy (COSY), and heteronuclear multiple bond correlation (HMBC) NMR spectra of ochrobactin-OH B in d₆-dimethyl sulfoxide (d₆-DMSO, 99.9 % Cambridge Isotopes, Inc.) were recorded on a Bruker 800 MHz instrument; see Supplementary Information.

3. Results

Vibrio sp. S4BW was found to produce the ochrobactins-OH, a suite of at least three secreted siderophores. The ochrobactins-OH were isolated from the supernatant of the bacterial culture and ESI-MS gave molecular ion peaks of m/z 793, m/z 821 (dominant peak eluting at approximately 32 min), and m/z 849 for ochrobactins-OH A, B, and C, respectively (Fig. 2). No appreciable amounts of siderophore were isolated from the cell pellet extract. Chiral amino acid analysis on hydrolyzed ochrobactin-OH B coupled to Marfey's derivitization reveals L-lysine as the sole amino acid.

The tandem mass spectrometry fragmentation patterns of ochrobactins-OH A, B and C are consistent with a suite of citrate-based siderophores which vary in the identity of one of the fatty acid appendages (see Fig. 3 and Supplementary Information). The fragmentation pattern of ochrobactin-OH B presented in Figs. 3 and 4 indicates the connectivity of the molecule. The masses at 128, 145, and 163 are indicative of the fragmentation of lysine. The loss of 46 m/z from the parent ion 821 m/z (to 775 m/z) and two other fragments of 651 m/z (to 605 m/z) and 489 m/z (to 443 m/z) indicate multiple fragments of the molecule containing a citrate moiety. The fragment with m/z of 651 corresponds to a loss of one fatty acid appendage; the fragment at m/z 489 corresponds to the further loss of a lysine moiety. The dominant fragment at 333 m/z corresponds to the loss of one fatty acid appendage, one lysine and the citrate moiety. The 333 m/z fragment does not show a further loss of 46, as can be seen for the other major fragments, because the citrate moiety is no longer part of the fragment. For all of the major fragments the loss of 18, corresponding to the loss of water, is observed.

Chemical shift values from the ^1H and ^{13}C NMR spectra of ochrobactin-OH B in d_6 -DMSO (D , 99.9%) are summarized in Table 1 (see Supplementary Information for 1D and 2D NMR spectra). The spectral values have a high degree of similarity with those reported for other citrate-based siderophores, specifically with the ochrobactins [13], a similar amphiphilic derivative of aerobactin. The ^1H NMR spectrum reveals the presence of two amide protons at δ_{H} 8.08 and δ_{H} 8.17, two lysine C_α protons at δ_{H} 4.12, a cluster of peaks around δ_{H} 1.25 consistent with methylene protons, and a triplet at δ_{H} 0.84, consistent with a terminal methyl group. The ^{13}C spectrum reveals the presence of a quaternary carbon signal at δ_{C} 73.4 and five carbonyl signals at δ_{C} 169.4, δ_{C} 169.8, δ_{C} 171.4, δ_{C} 173.6, and δ_{C} 174.9. The carbon signals of C16/C16' at δ_{C} 173.6 and C10/C10' at δ_{C} 171.4 likely have identical chemical shifts due to the symmetry of the molecule.

Interestingly, although the structure of ochrobactin-OH B is symmetric, certain chemical shifts within citrate are asymmetric, as seen in the slight difference between the carbonyl resonances at C17 (δ_{C} 169.4) and C17' (δ_{C} 169.8), the carbon and proton resonances of C18 and C18' and the two distinct α -amide protons (δ_{H} 8.08 and δ_{H} 8.17) observed from the lysines. This asymmetric chemical environment suggests hydrogen-bonding between one of the amide protons and the carboxylic acid group at C20; this asymmetry is also apparent in the ^1H NMR of the ochrobactins in d_6 -DMSO [13] and the nannochelins in 1:1 d_6 -DMSO: CHCl_3 [26], and is attributed to the analogous hydrogen-bonding interaction.

The ^1H - ^1H COSY and ^1H - ^{13}C HMBC reveal an alkyl group starting with the triplet methyl signal, H1 (H1'), through to the methylene resonance H7 (H7'). The COSY spectrum also shows the connection of the methylene to H8 (H8') δ_{H} 3.83 (the tertiary carbon adjacent to the hydroxyl), which is also indicated by HMBC correlations from H9 (H9') to C8 (C8') at δ_{C} 66.8 and C10 (C10') at δ_{C} 171.4. In addition to these data, the number of carbon correlations to the alkyl chain seen in the ^1H - ^{13}C HSQC and ^{13}C NMR spectra indicate that the hydroxylated alkyl group is a 3-hydroxydecanoic acid.

2D NMR data and comparison with published values for aerobactin and lysine confirm the presence of lysine in ochrobactin-OHB. The presence of a citrate moiety is confirmed by HSQC and HMBC correlations from the methylene signals, H18 (H18') to the quaternary carbon signal (C19), and the carbonyl signals C17, C17' and C20. HMBC correlation from H11 (H11') in the lysine to the carbonyl carbon C10 (C10') further indicates that each lysine is N-acylated by 3-hydroxydecanoic acid. Hence, through examination of the NMR data the connectivity of the molecule has been established; ochrobactin-OH B has been found to have an acylated structure similar to the amphiphilic ochrobactins [13] and structurally derived from aerobactin [27–29].

The UV–visible spectrum of Fe(III)-ochrobactin-OH B (see Supplementary Information) shows the characteristic charge-transfer bands from the α -hydroxy carboxylate to Fe(III) at ~300 nm and the hydroxamate to Fe(III) charge-transfer band at ~400 nm, as expected for these siderophores based on their structural similarity to aerobactin (λ -max of 296 nm and 400 nm [30]).

The structures of ochrobactin-OH A (793 m/z) and ochrobactin-OH C (849 m/z) are deduced from tandem mass spectrometry (Supplementary Information) and are related compounds of ochrobactin-OH B. The difference between them is the composition of the fatty acid appendages attached to the N position of the lysine residues (Fig. 4). Whereas ochrobactin-OH B is appended by two 3-hydroxydecanoic acid moieties, ochrobactin-OH A has one hydroxylated C8 fatty acid appendage and one hydroxylated C10 fatty acid appendage, and ochrobactin-OH C has one hydroxylated C12 fatty acid appendage and one hydroxylated C10 fatty acid appendage.

Based on analysis of the tandem mass spectra of ochrobactins-OH A–C, chiral amino acid analysis, and NMR data, it follows that the suite of ochrobactins-OH has a citrate backbone, two L-lysine linkers and two fatty acid appendages ranging from C8 to C12 (Fig. 4). Unlike the structurally similar ochrobactins, the ochrobactins-OH are secreted into the medium and are not found associated with the cell membrane.

4. Discussion

Citrate-based amphiphilic siderophores are relatively rare in the marine environment. Previous studies have only reported two suites of such siderophores: the synechobactins are produced by a coastal marine cyanobacterium, *Synechococcus* PCC 7002 [18], and the ochrobactins are produced by the coastal α -proteobacterium *Ochrobactrum* sp. SP18 and the γ -proteobacterium *Vibrio* sp. DS40M5 [13]. To date, *Synechococcus* is the only known cyanobacterium producing a suite of siderophores and *Ochrobactrum* is the only known α -proteobacterium producing a suite of siderophores (Fig. 5).

The siderophores produced by *Vibrio* sp. S4BW are structurally similar to the ochrobactins, but include hydroxylation in contrast to desaturation in the fatty acid appendages, and have therefore been named the ochrobactins-OH. The suite consists of three members, A–C, where one fatty acid appendage remains constant and the length of the second is varied (Fig. 4). The production of the ochrobactins-OH by *Vibrio* sp. S4BW is the first detected case in which a citrate-based amphiphilic siderophore suite is produced and secreted into the medium by a γ -proteobacterium. The production of citrate-based amphiphilic siderophores from three distinct classes indicates that these genes for siderophore production may be quite widespread.

Given the structural similarity of the ochrobactins-OH to aerobactin, we expect the Fe(III) stability constant and photochemical experiments to yield similar results to aerobactin. Based on the similarity in the UV–visible spectrum of the ferric ochrobactin-OH B complex

(λ -max ~300 nm and ~400 nm) in comparison with that of ferric aerobactin, we further expect the Fe(III) coordination chemistry and the attendant photoreactivity to be very similar.

While the structures of the ochrobactins and ochrobactins-OH are similar, the bacteria which produce them are phylogenetically distinct and were isolated from different marine environments. *Ochrobactrum* sp. SP18 was isolated from near-shore water collected from the pier at Scripps Institution of Oceanography in California and *Vibrio* sp. DS40M5 was isolated off the west coast of Africa, while *Vibrio* sp. S4BW was isolated from seawater collected from the Gulf of Mexico after the Deepwater Horizon oil spill. The presence of similar siderophore-producing genes in bacteria from such remote bodies of water further indicates that siderophore production is widespread.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We obtained a water sample from the region near the Deepwater Horizon oil spill from Drs. Molly C. Redmond and David L. Valentine (UCSB). A.B. gratefully acknowledges NSFCHE1059067 and NIHGM38130. J.M.G. is supported by a National Science Foundation Graduate Research Fellowship. Any opinions, findings, conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

References

1. Kessler JD, Valentine DL, Redmond MC, Du M, Chan EW, Mendes SD, Quiroz EW, Villanueva CJ, Shusta SS, Werra LM, Yvon-Lewis SA, Weber TC. Science. 2011; 331:312–315. [PubMed: 21212320]
2. Kujawinski EB, Kido Soule MC, Valentine DL, Boysen AK, Longnecker K, Redmond MC. Environ. Sci. Technol. 2011; 45:1298–1306.
3. Valentine DL, Kessler JD, Redmond MC, Mendes SD, Heintz MB, Farwell C, Hu L, Kinnaman FS, Yvon-Lewis S, Du M, Chan EW, Tigreros FG, Villanueva CJ. Science. 2010; 330:208–211. [PubMed: 20847236]
4. Camilli R, Reddy CM, Yoerger DR, Van Mooy BAS, Jakuba MV, Kinsey JC, McIntyre CP, Sylva SP, Maloney JV. Science. 2010; 330:201–204. [PubMed: 20724584]
5. Hazen TC, Dubinsky EA, De Santis 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 H-YN, Osman S, Lu Z, Van Nostrand JD, Deng Y, Zhou J, Mason OU. Science. 2010; 330:204–208. [PubMed: 20736401]
6. Kloos K, Munch J, Schlöter M. J. Microbiol. Meth. 2006; 66:486–496.
7. Asperger O, Naumann A, Kleber H-P. FEMS Microbiol. Lett. 1981; 11:309–312.
8. van Beilen J, Wubbolts M, Witholt B. Biodegradation. 1994; 5:161–174. [PubMed: 7532480]
9. Dedysh S, Liesack W, Khmelenina V, Suzina N, Trotsenko Y, Semrau J, Bares A, Panikov N, Tiedje J. Int. J. Syst. Evol. Microbiol. 2000; 50:955–969. [PubMed: 10843033]
10. Vraspir JM, Butler A. Ann. Rev. Mar. Sci. 2009; 1:43–63.
11. Barbeau K, Rue EL, Bruland KW, Butler A. Nature. 2001; 413:409–413. [PubMed: 11574885]
12. Barbeau K, Zhang G, Live DH, Butler A. J. Am. Chem. Soc. 2002; 124:378–379. [PubMed: 11792199]
13. Martin JD, Ito Y, Homann VV, Haygood MG, Butler A. J. Biol. Inorg. Chem. 2006; 11:633–641. [PubMed: 16791646]

14. Kunze B, Trowitzsch-Kienast W, Hoefle G, Reichenbach H. J. Antibiot. 1992; 45:147–150. [PubMed: 1556005]
15. Amin SA, Kupper FC, Green DH, Harris WR, Carrano CJ. J. Am. Chem. Soc. 2007; 129:478–479. [PubMed: 17226996]
16. Homann V, Edwards K, Webb E, Butler A. Biometals. 2009; 22:565–571. [PubMed: 19357970]
17. Hickford SJH, Kuepper FC, Zhang G, Carrano CJ, Blunt JW, Butler A. J. Nat. Prod. 2004; 67:1897–1899. [PubMed: 15568785]
18. Ito Y, Butler A. Limnol. Oceanogr. 2005; 50:1918–1923.
19. Ratledge C. Tuberculosis. 2004; 84:110–130. [PubMed: 14670352]
20. Snow G, White A. Biochem. J. 1969; 115:1031–1045. [PubMed: 5360674]
21. Luo M, Fadeev EA, Groves JT. J. Am. Chem. Soc. 2005; 127:1726–1736. [PubMed: 15701007]
22. Martinez JS, Carter-Franklin JN, Mann EL, Martin JD, Haygood MG, Butler A. Proc. Natl. Acad. Sci. U. S. A. 2003; 100:3754–3759. [PubMed: 12651947]
23. Vraspir JM, Holt PD, Butler A. Biometals. 2011; 24:85–92. [PubMed: 20853137]
24. B'Hymer C, Montes-Bayon M, Caruso JA. J. Sep. Sci. 2003; 26:7–19.
25. Schwyn B, Neilands J. Anal. Biochem. 1987; 160:47–56. [PubMed: 2952030]
26. Bergeron RJ, Phanstiel O. J. Org. Chem. 1992; 57:7140–7143.
27. Haygood MG, Holt PD, Butler A. Limnol. Oceanogr. 1993; 38:1091–1097.
28. Crosa LM, Wolf MK, Actis LA, Sanders-Loehr J, Crosa JH. J. Bacteriol. 1988; 170:5539–5544. [PubMed: 3192510]
29. Gibson F, Magrath DI. Biochim. Biophys. Acta. 1969; 192:175–184. [PubMed: 4313071]
30. Harris WR, Carrano CJ, Raymond KN. J. Am. Chem. Soc. 1979; 101:2722–2727.
31. Castresana J. Mol. Biol. Evol. 2000; 17:540–552. [PubMed: 10742046]
32. Chevenet F, Brun C, Banuls A, Jacq B, Chisten R. BMC Bioinforma. 2006; 7:439.
33. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard J, Guindon S, Lefort V, Lescot M, Claverie J, Gascuel O. Nucleic Acids Res. 2008; 36:W465–W469. (Web Server). [PubMed: 18424797]
34. Edgar R. Nucleic Acids Res. 2004; 32:1792–1797. [PubMed: 15034147]
35. Guindon S, Gascuel O. Syst. Biol. 2003; 52:696–704. [PubMed: 14530136]
36. Huson D, Richter D, Rausch C, DeZulian T, Franz M, Rupp R. BMC Bioinforma. 2007; 8:460.
37. Anisimova M, Gascuel O. Syst. Biol. 2006; 55:539–552. [PubMed: 16785212]
38. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. Appl. Environ. Microbiol. 2006; 72:5069–5072. [PubMed: 16820507]
39. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. Nucleic Acids Res. 2011; 39:D32–D37. [PubMed: 21071399]

Fig. 1.

Selected citrate-based siderophores. Top panel includes aerobactin and its derivative siderophores, the ochrobactins [13], and the nannochelins [14]. The nannochelins are produced by *Nannocystis exedens* a terrestrial bacterium. Bottom panel includes the marine siderophores, vibrioferin [15], the petrobactins [12,16,17], and the synechobactins [18].

Fig. 2.
RP-HPLC chromatogram for total XAD extract using a preparative Higgins C₄ column. Sample is monitored at 215 nm. The peak at 32.5 min is a putative siderophore, based on the positive response to the CAS assay [25]. Based on mass spectrometric results the dominant mass in the peak is 821 m/z, while two other unique masses are found in the front shoulder and the small back side peak.

S4BW peak B, conc; MSMS 821.4

BUT031411MA1 10 (0.196) Cm (10:17)

TOF MSMS 821.40ES+
679

Fig. 3.
Fragmentation in the ESI-MSMS of ochrobactin-OH B.

Fig. 4.
Expected fragmentation of ochrobactins-OH A, B, and C.

Fig. 5. Phylogenetic tree of siderophore-producing bacteria and related species based on maximum likelihood analysis of SSU rDNA sequences [31–37]. Amphiphilic siderophore-producing bacteria are labeled in bold. All isolates are marine. Bootstrap values greater than 50% are included. Sequences of related bacteria and other known siderophore-producing bacteria were obtained from green genes [38] or GenBank [39].

Table 1

^{13}C and ^1H NMR chemical shift assignments for ochrobactin-OH B (5 mg ochrobactin-OH B in 300 μL d_6 -dimethyl sulfoxide, 800 MHz).

Position	^{13}C (ppm)	^1H (ppm) [m]
C1, C1'	14.0	0.84 [t, 6H, J=7.0 Hz]
C2, C2'	22.1	1.25 ^a
C3, C3'	31.3	1.21 ^a
C4, C4'	28.8	1.23 ^a
C5, C5'	29.0	1.19 ^a
C6, C6'	36.9	1.28 ^a ; 1.35 [m, 2H]
C7, C7'	25.2	1.23 ^a ; 1.35 [m, 2H]
C8, C8'	66.8	3.83 [dd, 2H, J=9.9, 5.7 Hz]
C9, C9'	39.8	2.40 [m, 2H]; 2.48 [m, 2H]
C10, C10'	171.4	– N _H OH 9.7 [s, 2H, br]
C11, C11'	46.8	3.44 [t, 4H, J=7.1 Hz]
C12, C12'	26.1	1.48 [m, 4H]
C13, C13'	22.5	1.25 ^a
C14, C14'	30.9	1.53 [m, 2H]; 1.66 [m, 2H]
C15, C15'	51.7	4.12 [m, 2H, J=7.2 Hz] N _H H 8.08 [d, 1H, J=7.9 Hz] N _H H 8.17 [d, 1H, J=7.8 Hz]
C16, C16'	173.6	– OH 12.5 [br]
C17	169.4	–
C17'	169.8	–
C18	43.5	2.61 [s, 2H]
C18'	43.3	2.55 [d, 1H, J=14.5 Hz] 2.64 [d, 1H, J=14.4 Hz]
C19	73.4	–
C20	174.9	– OH 12.5 [br]

^aSum of protons is a multiplet, which sums to 24 protons.