Isolation of Two Novel Marine Ethylene-Assimilating Bacteria, *Haliea*
Species ETY-M and ETY-NAG, Containing Particulate Methane Monooxygenase-like Genes

TOSHIHIRO SUZUKI1, TAKAMICHI NAKAMURA2, and HIROYUKI FUSE1*

1Graduate School of Regional Environment Systems, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama, Saitama 337–8570, Japan; 2Chugai Technos Corp., 9–12 Yokogawa Shinmachi, Nishi-ku, Hiroshima, Hiroshima 733–0013, Japan; and 3College of Systems Engineering and Science, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama, Saitama 337–8570, Japan

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Two novel ethylene-assimilating bacteria, strains ETY-M and ETY-NAG, were isolated from seawater around Japan. The characteristics of both strains were investigated, and phylogenetic analyses of their 16S rRNA gene sequences showed that they belonged to the genus *Haliea*. In C1–4 gaseous hydrocarbons, both strains grew only on ethylene, but degraded ethane, propylene, and propane in addition to ethylene. Methane, n-butane, and i-butane were not utilized or degraded by either strain. Soluble methane monooxygenase-type genes, which are ubiquitous in alkene-assimilating bacteria for initial oxidation of alkenes, were not detected in these strains, although genes similar to particulate methane monooxygenases (pMMO)/ammonia monooxygenases (AMO) were observed. The phylogenetic tree of the deduced amino acid sequences formed a new clade near the monooxygenases of ethene-assimilating bacteria similar to other clades of pMMOs in type I, type II, and Verrucomicrobia methanotrophs and AMOs in alpha and beta proteobacteria.

Key words: short-chain alkene, *Haliea*, particulate methane monooxygenase (pmm)

Ethylene occurs in the atmosphere at approximately 0.1–10 ppbv (41) and affects atmospheric chemistry and the global climate. It provides a sink for hydroxyl radicals and plays a key role in the production and destruction of ozone in the troposphere (10). Ethylene acts as a hormone and plays a key role in the production and destruction of the global climate. It provides a sink for hydroxyl radicals (41) and affects atmospheric chemistry and the global climate. Ethylene is produced in the sea, and ethylene concentrations in rock pools were recorded to range between 47.2 and 856.4 pmol L⁻¹ (3). A seasonal cycle was observed with a summer maximum and concentrations varied from 17 to 951 pmol L⁻¹ in coastal waters. Ethylene concentrations in surface water vary in the range of 1.8–39.2 nL L⁻¹ and generally show a vertical maximum at the pycnocline (approximately 100 m depth), where elevated concentrations of chlorophyll-α, dissolved oxygen, and nutrients were also found in the western Atlantic (36). Photochemical transformation of dissolved organic matter in surface water results in the production of ethylene (34). Some micro- and macroalgae, photosynthetic bacteria, and cyanobacteria produce ethylene, probably via ACC or acrylate from methionine (3, 27, 32). Few reports have examined the physiological effects of ethylene on algae, which is surprising given that ethylene may play a multifaceted role in algae, having driven the loss of chlorophyll-α (32) and having contributed to mastoparan-induced cell death in green algae (48). Nevertheless, no reports on ethylene-degrading marine microorganisms currently exist.

We isolated ethylene-assimilating bacteria from seawater and investigated their characteristics, particularly those related to ethylene assimilation, to clarify the role of bacteria in ethylene circulation in the sea.

Materials and Methods

Growth conditions

A 5V medium containing 100 mg NH₄NO₃, 10 mg KH₂PO₄, 2.5 mg Fe(III)EDTA, 2.75 mg vitamin B₁₂, 2.5 mg biotin, 500 mg...
thiamine–HCl, 372 mg Na2EDTA, 0.25 mg CuSO4·5H2O, 5.75 mg ZnSO4·7H2O, 4.55 mg MnCl2·4H2O, 0.6 mg of CoCl2·6H2O, 0.27 mg (NH4)2MoO4·2H2O, and 5 mg yeast extract (Difco, Detroit, MI, USA) in 1 L filtered seawater (pH 8.1) was used to isolate and culture ethylene-assimilating bacteria. Ethylene was supplied by replacing 50% of the air in a culture vessel. Solid medium used for culturing 5VM media and 1% gellan gum. The culture was incubated at 25°C.

Escherichia coli strains were grown in Luria–Bertani (LB) broth containing 1% polypeptone, 0.5% yeast extract, and 1% NaCl medium supplemented with ampicillin (100 μg/mL) when necessary.

Sampling and isolation of bacteria

Surface seawater was collected in 120-mL glass vials from several Japanese coasts during July 1998 and May 1999, and cultured with 5VM medium and ethylene. After growth was observed in the liquid medium, a portion of the broth was streaked onto solid medium and cultured with ethylene, and any visible colonies were transferred to liquid medium. This isolation procedure was repeated at least five times, at which point the purity of the isolated strain was confirmed by microscopic observation and by the lack of growth of other bacteria on Marine Agar 2216 (Difco) plates.

Results and Discussion

Isolation, phenotypic characterization, and phylogenetic analyses of two ethylene-assimilating bacteria, strains ETY-M and ETY-NAG

Two strains of ethylene-assimilating bacteria, strain ETY-M and ETY-NAG, were isolated from surface seawater from the inner wall of the culture vessel in the liquid medium but did not grow well on Marine Agar 2216 plates.

The 16S rRNA gene sequences were analyzed to examine the phylogeny of the strains. The analyses showed that the most closely related bacteria belonged to genus Haliea (Gammaproteobacteria). A BLAST search of strains ETY-M and ETY-NAG revealed similarities to the bacteria Haliea sp. MOLA 104 and Haliea rubra strain CM41_15a (43, 44), which were 97% and 96% identical, respectively. The phylogenetic tree showed that the two isolated strains were in the clade comprising Haliea spp. (Fig. 1).

Strains ETY-M and ETY-NAG were Gram-negative.
Characteristics of both strains are shown in Table 1 with three type strains of *Haliea* spp. Strains ETY-M and ETY-NAG and *Haliea* spp. were isolated from marine samples and all strains needed NaCl for growth. All strains produced pigments although the colors were different. The DNA G+C contents of strains ETY-M and ETY-NAG were 65.2 and 58.8 mol%, respectively, which is in accordance with the ranges reported for other *Haliea* species (Table 1). In addition, ETY-M and ETY-NAG exhibit ubiquinone Q-8, as do other *Haliea* species. The cellular fatty acid profile of strain ETY-M was determined, and five components of fatty acids were detected at concentrations greater than 1%: C18:1 ω7c, 37.5%; (C16:1 ω7c or C15:0 iso 2OH), 28.0%; C16:0, 21.0%; C14:0, 6.5%; C10:0 3OH, 3.2%. This profile is most similar to *H. rubra* in *Haliea* spp.

Additional characteristics of both strains are compared with *Haliea* strains in Table 1.

With respect to carbon sources except for hydrocarbons, both strains showed good growth with the addition of acetate and pyruvate, as did the *H. mediterranea* strain 7SM29 (26), whereas strain ETY-NAG also assimilated maltose, sucrose, aspartate, and glutamate, and strain ETY-M also assimilated serine and alanine. As a nitrogen source, both strains utilized ammonium sulfate but not potassium nitrate. Furthermore, strain ETY-M utilized aspartate and arginine and strain ETY-NAG utilized arginine as nitrogen sources in addition to the amino acids they used for carbon sources. Therefore, on the basis of 16S rRNA gene sequence analyses as well as their physiological and biochemical characteristics, both strains were identified as *Haliea* spp.

**Fig. 1.** Phylogenetic tree of 16S rRNA genes constructed using a neighbor-joining dendrogram. *Methylcystis echinoides* served as an outgroup. Numbers to the right are accession numbers in the database. Scale bar indicates 0.02 substitutions per 100 base positions. Numbers at tree nodes are bootstrap values from 100 trials.

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**Strains E TY-M and E TY-NAG specifically assimilate ethylene**

No strains of the genus *Haliea* have been reported to assimilate gaseous hydrocarbons, including ethylene. The assimilation of various gas hydrocarbons by strains E TY-M and E TY-NAG was investigated, and both strains were found to assimilate only ethylene, but not methane, ethane, propene, or propylene (Table 1). Only two strains, *Rhodochrobacter* B-276 and *Mycobacterium* E20, have been reported to grow well on both alkanes and alkenes (37). *Mycobacterium* E20 utilized ethylene, but grew poorly on propylene and butene as well as ethene and higher alkanes (7), although ethylene was oxidized by the monoxygenases differently than for alkane oxidation (8). *Rhodochrobacter* B-276 grew well on ethylene, propylene, propane, 1-butene, butane, and butadiene, but not on ethane or methane (14). *X. autotrophicus* Py2, as well as some other isolates, were able to use ethylene and propylene but not ethene, propene, or butene (45). Of the seven ethylene- or propylene-utilizing *Mycobacterium* strains tested, only one strain used both ethylene and propylene (9).

To test the conversion of gaseous hydrocarbons in both strains, the degradation of gaseous hydrocarbons by resting cells was performed with methane, ethene, ethane, propene, n-butene, ethylene, and propylene (Table 2). Methene, i-butane, and n-butane were hardly degraded by both strains, while ethane, ethylene, and propylene were markedly degraded by both strains. Ethene, ethylene, and propylene were degraded to 0.48%, 0.02%, and 1.0%, respectively, by strain E TY-M, and to 0.06%, 0.15%, and 4.6%, respectively, by strain E TY-NAG. Propene was degraded a little more
Ethene-utilizing Haliea with pmo-like genes

slowly than ethane, ethylene, and propylene by both strains, and was degraded to 9.3–58% by strain ETY-M and to 26% by strain ETY-NAG. Thus, C1 or C4 gases, such as methane, i-butane, and n-butane, were hardly degraded, while C2 or C3 gases, such as ethane, ethylene propane, and propylene, were degraded well by both strains. This indicated that ETY-M and ETY-NAG are able to specifically degrade C2 and C3 gas hydrocarbons, but not to assimilate them, except for ethylene.

Both strains grew on ethanol but not on 2-propanol. Strain ETY-M grew weakly on 1-propanol whereas strain ETY-NAG did not (Table 1). X. autotrophicus Py2 and Mycobacterium E20 were also able to assimilate ethanol. To clarify the ethylene degradation pathway in strains ETY-M and ETY-NAG, their metabolites and utilization should be investigated, along with other alkene-assimilating bacteria that epoxidize alkene for their growth.

Strains ETY-M and ETY-NAG were isolated from seawater using enrichment culturing with 50% ethylene. Both strains specifically assimilated ethylene in C1–4 gaseous hydrocarbons even though the ethylene concentration in seawater is very low. Ethylene appears to be produced by seaweed as well as some marine microorganisms. These ethylene-assimilating bacteria may coexist with ethylene producers such as members of the methylotrophic genus Methylobacterium, which are ubiquitous on plant surfaces and potentially dominate the phyllosphere population (6).

The genus Haliea genus reportedly comprises several percent of the bacterial abundance in mangrove sediments and these species are sensitive to oil contamination (11). Haliea bacteria may interact with mangrove plants via ethylene, which is produced in abundance when plants are wounded; however, no information exists on the utilization of short-chain hydrocarbons by other Haliea spp.

Table 1. Characterization of ETY-M, ET-NAG, and other Haliea species

| Characteristic | ETY-M | ETY-NAG | 3X/A02/235 | CM41_15a | 7SM29T |
|---------------|------|--------|------------|----------|-------|
| Cell morphology | Short Rods | Short Rods | Straight Rods | Straight Rods | Short Rods |
| Cell dimensions (µm) | 0.4–0.45 × 1.2–1.3 | 0.75–1.0 × 0.5–0.6 | 0.3–0.7 × 1.3–1.9 | 0.5 × 2.7 | 0.4–0.5 × 1.1–1.3 |
| Colony color (agar medium) | Purple (5VM) | Yellow (5VM) | Cream (MA) | Red (MA) | Yellow (MA) |
| Growth of nutrient agar (MA) | – | + | + | – | + |
| Flagella | – | – | – | – | – |
| DNA G+C content (mol%) | 65.2 | 58.8 | 61.4 | 64.8 | 62.1 |
| Growth temperature range (°C) | 20–37 | 20–30 | 10–37 | 15–44 | 15–40 |
| Optimum | 30 | 30 | 25–30 | 30 | 28 |
| Salinity range (g L⁻¹) | 13.2–52.7 | 26.4–52.7 | 7–70 | 7–42 | 3.5–150 |
| Optimum | 13.2 | 26.4 | 40 | 35 | unknown |
| Growth substrate | | | | | |
| Glucose | – | – | – | + | – |
| Maltose | – | + | – | (+) | – |
| Sucrose | – | + | – | – | – |
| Arabinose | – | – | – | – | – |
| Xylose | – | – | N.A. | N.A. | – |
| Fructose | – | + | – | (+) | – |
| Mannose | – | – | (+) | (+) | – |
| Cellobiose | – | – | – | – | (+) |
| Mannitol | – | – | – | N.A. | – |
| Citrate | – | – | – | + | – |
| Succinate | – | – | – | – | – |
| Gluconate | – | – | N.A. | N.A. | – |
| Pyruvate | + | + | – | + | – |
| Acetate | + | + | – | + | – |
| Glycerol | – | (+) | – | – | + |
| Alanine | + | – | – | – | + |
| Glutamate | – | + | (+) | – | + |
| Aspartate | – | + | + | – | + |
| Serine | – | – | – | – | – |
| Gas hydrocarbon utilization of | | | | | |
| Methane | – | – | N.A. | N.A. | N.A. |
| Ethane | – | – | N.A. | N.A. | N.A. |
| Propane | – | – | N.A. | N.A. | N.A. |
| Ethylene | + | + | N.A. | N.A. | N.A. |
| Propylene | – | – | N.A. | N.A. | N.A. |
| Methanol | – | – | N.A. | N.A. | N.A. |
| Ethanol | – | – | N.A. | N.A. | N.A. |
| 1-Propanol | (+) | – | N.A. | N.A. | N.A. |
| 2-Propanol | – | – | N.A. | N.A. | N.A. |

MA, marine agar 2216; +, positive; –, negative; (+), weakly positive; N.A., data not available.
A particulate methane monooxygenase (pmoA)-like gene exists in ETY-M and ETY-NAG

Almost all alkene-assimilating bacteria, including ethylene-assimilating bacteria, carry soluble methane monooxygenase (sMMO)-like genes. To examine the existence of sMMO in both strains, we attempted to amplify the putative mmoX as a target gene, which encodes an α-subunit of the hydroxylase of the sMMO-like gene. The gene was amplified by all combinations of the four primers, i.e., mmoX206F or mmoX1 as a forward primer and mmoX886R or mmoXr901 as a reverse primer; however, no mmoX-like genes were detected in either strain (data not shown). These results suggest that the strains do not carry sMMO-like genes, but rather possess particulate methane monooxygenase (pMMO)-like genes because some pMMO-like enzymes related to short-chain alkane degradation were detected (16, 33). pMMO is a membrane-bound enzyme that requires copper for its activity. The pMMO gene cluster is composed of pmoC, pmoA, and pmoB, and pMMO is analogous to ammonia monooxygenase (AMO), whose gene cluster is composed of amoC, amoA, and amoB (19).

PCR amplification of the pmoA-like gene revealed the presence of the pmoA-like gene in both strains ETY-M and ETY-NAG. The BLAST search results for amplified pmoA-like gene sequences revealed similarities to pmoA (<54% similarity) and ammonia monooxygenase gene (amoA) (<63% similarity). Phylogenetic analysis of these genes indicated that pmoA-like genes of strain ETY-M and ETY-NAG were distant from other pmo-like genes, including methane-, ethane-, and ammonia monooxygenase; however, the genes of strains ETY-M and ETY-NAG clustered nearest to the genes of putative ethane oxidizers that were retrieved from marine sediment by the SIP technique (33) as well as the genes of ethane-assimilating strains ET-HIRO and ET-SHO in GenBank (Fig. 2). The pmoA-like genes of strains ETY-M and ETY-NAG were particularly analogous to those of Nitrosospina and Nitrosomonas, which are beta-proteobacteria (31), compared to those of methanotrophs. In the phylogenetic tree, the pmoA-like genes of both strains formed a new branch of pmoA-like genes of ethylene-
assimilating bacteria. Some environmental pmoa/amoA-like clones, which were reported as the RA21 cluster/group (24, 40), were placed near this clade (Fig. 2). Clone RA21 was retrieved from a beech forest soil sample in Denmark and was described as not being placed in any known group of pmo or amo sequences (20). Clone MR1 was also retrieved from forest soil near Marburg, Germany, and was described as a putative ammonium oxidizer (18). These genes could be specific to bacteria that assimilate short-chain hydrocarbons, particularly ethylene or ethane.

This is the first report to demonstrate that some strains of Halicea have the ability to degrade gaseous hydrocarbons and that pmoa-like genes are found in isolated bacteria besides methanotrophs and ammonia oxidizers. The genes encoding pMMO or AMO clusters are in the order ‘CAB’ with the exception of archaean AMO and pXMO, which are putatively involved in ammonium oxidation in some methanotrophs, although their specific functions are not clear (40). The gene clusters of pmoa-like genes in strains ETY-M and ETY-NAG and their relation to ethylene degradation and these genes will be clarified in future studies.

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References
1. Ali, H., J. Scanlan, M.G. Dumont, and J.C. Murrell. 2006. Duplication of the pmoaA gene in Methylosinus sporium: Cloning, sequencing and mutational analysis. Microbiology 152:2931–2942.
2. Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. Anal. Biochem. 70:241–250.
3. Broadgate, W.J., G. Malin, F.C. Küpper, A. Thompson, and P.S. Liss. 2004. Isoprene and other non-methane hydrocarbons from seaweeds: A source of reactive hydrocarbons to the atmosphere. Mar. Chem. 88:61–73.
4. Brosius, T.J., D.T. Dult, D.D. Sleeter, and H.F. Noller. 1981. Gene organization and primary structure of a ribosomal RNA operon from Escherichia coli. J. Mol. Biol. 148:107–127.
5. Coleman, N.V., and J.C. Spain. 2003. Epoxycalanke: Coenzyme M transferase in the ethene and vinyl chloride biodegradation pathways of Mycobacterium strain JS60. J. Bacteriol. 185:5536–5545.
6. Corpe, W.A., and S. Rheem. 1989. Ecology of the methlyotrophic bacteria on living leaf surfaces. FEMS Microbiol. Ecol. 6:243–250.
7. De Bont, J.A., and R.A. Albers. 1976. Microbial metabolism of ethylene. Antonie van Leeuwenhoek 42:73–80.
8. De Bont, J.A., M.M. Attwood, S.B. Primrose, and W. Harder. 1979. Epoxidation of short chain alkenes in Mycobacterium E20: The involvement of a specific mono-oxygenase. FEMS Microbiol. Lett. 6:183–188.
9. De Bont, J.A., S.B. Primrose, M.D. Collins, and D. Jones. 1980. Chemical studies on some bacteria which utilize gaseous unsaturated hydrocarbons. J. Gen. Microbiol. 117:97–102.
10. Donahue, N.M., and R.G. Prinn. 1990. Nonmethane hydrocarbon chemistry in the remote marine boundary layer. J. Geophys. Res. 95:18387–18417.
11. Dos Santos, H.F., J.C. Cary, F.L. do Carmo, A.L. dos Santos, J. Tiedje, J.D. van Elsas, A.S. Rosado, and R.S. Peixoto. 2011. Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: Bacterial proxies for oil pollution. PLoS One 6:e16943.
12. Fosdike, W.L., J.T. Smith, and H. Dalton. 2005. Adventitious reactions of alkene monoxygenase reveal common reaction pathways and component interactions among bacterial hydrocarbon oxygenases. FEBS J. 272:2661–2669.
13. Fukuda, H., T. Ogawa, and S. Tanase. 1993. Ethylene production by micro-organisms. Adv. Microbiol. Physiol. 35:275–306.
14. Furuhashi, K., A. Taoka, S. Uchida, L. Karube, and S. Suzuki. 1981. Production of 1,2-epoxyalkanes from 1-alkenes by Nocardia corallina B-276. Eur. J. Appl. Microbiol. Biotechnol. 12:39–45.
15. Habets-Grünz, A.Q.H., and J.A. de Bont. 1985. Inactivation of alkane oxidation by epoxides in alkene- and alkane-grown bacteria. Appl. Microbiol. Biotechnol. 22:428–433.
16. Hamamura, N., C.M. Yeager, and D.J. Arp. 2000. Two distinct monoxygenases for alkane oxidation in Nocardioideae sp. strain C8F. Appl. Environ. Microbiol. 67:4992–4998.
17. Hamstra, R.S., M.R. Murris, and J. Tramper. 1987. The influence of immobilization and reduced water activity on gaseous-alkane oxidation by Mycobacterium PY1 and Xanthobacter PY2 in a gas-solid bioreactor. Biotechnol. Bioeng. 29:884–891.
18. Henkel, T., U. Jäckel, S. Schnell, and R. Conrad. 2000. Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. Appl. Environ. Microbiol. 66:1801–1808.
19. Holmes, A.J., A.M. Costello, M.E. Lidstrom, and J.C. Murrell. 1995. Evidence that particulate methane monoxygenase and ammonia monoxygenase may be evolutionarily related. FEMS Microbiol. Lett. 132:203–208.
20. Holmes, A.J., P. Roslev, J.R. McDonald, N. Iversen, K. Henrika, and J.C. Murrell. 1999. Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. Appl. Environ. Microbiol. 65:3312–3318.
21. Holmes, A.J., and N.V. Coleman. 2008. Evolutionary ecology and multidisciplinary approaches to prospecting for monoxygenases as biocatalysts. Antonie van Leeuwenhoek 94:75–84.
22. Jin, Y.O., and T.E. Mattes. 2008. Adaptation of aerobic, ethene-assimilating Mycobacterium strains to vinyl chloride as a growth substrate. Environ. Sci. Technol. 42:4784–4789.
23. Krum, J.G., and S.A. Ensign. 2000. Heterologous expression of bacterial Epoxycalanke: Coenzyme M transferase and inducible coenzyme M biosynthesis in Xanthobacter strain Py2 and Rhodococcus rhodochrous B276. J. Bacteriol. 182:2629–2634.
24. Lau, E., A. Ahmad, P.A. Steudler, and C.M. Cavanaugh. 2007. Molecular characterization of methanotrophic communities in forest soils that consume atmospheric methane. FEMS Microbiol. Ecol. 60:490–500.
25. Leathy, J.G., P.J. Batchelor, and S.M. Morcom. 2003. Evolution of the soluble diiron monoxygenases. FEMS Microbiol. Rev. 27:449–479.
26. Lucena, T., J. Pascual, E. Garay, D.R. Arahal, M.C. Macían, and M.J. Pujalte. 2010. Haloferax mediterranea sp. nov., a marine gammaproteobacterium. Int. J. Syst. Evol. Microbiol. 60:1844–1848.
27. Maillard, P., C. Thepenier, and C. Gudin. 1993. Ethylene production by photosynthetic bacteria, cyanobacteria, and algae. J. Mar. Biotechnol. 1:97–100.
28. Mattes, T.E., N.V. Coleman, J.C. Spain, and J.M. Gossett. 2003. Physiological and molecular genetic analyses of vinyl chloride and ethene biodegradation in Nocardioideae sp. strain JS614. Arch. Microbiol. 183:95–106.
29. Miguez, C.B., D. Bourque, J.A. Sealy, C.W. Greer, and D. Groele. 1997. Detection and isolation of methanotrophic bacteria possessing soluble methane monoxygenase (SMO) genes using the polymerase chain reaction (PCR). Microb. Ecol. 33:21–31.
30. Nakamura, T., T. Hoaki, S. Hanada, A. Maruyama, Y. Kamagata, and H. Fuse. 2007. Soluble and particulate methane monoxygenase gene clusters in the marine methanotroph Methylomonas sp. strain NI. FEMS Microbiol. Lett. 277:157–164.
31. Norton, J.M., J.J. Alzerraca, Y. Suwa, and M.G. Klotz. 2002. Diversity of ammonia monoxygenase operon in autotrophic ammonia-oxidizing bacteria. Arch. Microbiol. 177:139–149.
32. Plettner, I., M. Steinke, and G. Malin. 2005. Ethene (ethylene) production in the marine macroalgae Ulva (Enteromorpha) intestinalis L. (Chlorophyta, Ulvophyceae): Effect of light-stress and co-production with dimethyl sulphide. Plant Cell Environ. 28:1136–1145.
33. Redmond, M.C., D.L. Valentine, and A.L. Sessions. 2010. Identification of novel methane-, ethane-, and propane-oxidizing bacteria at marine hydrocarbon seeps by stable isotope probing. Appl. Environ. Microbiol. 76:6412–6422.
34. Riemer, D.D., P.J. Milne, R.G. Zika, and W.H. Pos. 2000. Photoproduction of nonmethane hydrocarbons (NMHCs) in seawater. Mar. Chem. 71:177–198.
35. Sambrook, J., and D.W. Russell. 2001. Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, NY.
36. Seifert, R., N. Delling, H. Richnow, S. Kempe, J. Heftier, and W. Michaelis. 1999. Ethylene and methane in the upper water column of the subtropical Atlantic. Biogeochemistry 44:73–91.
37. Sherman, J.J. 2006. Utilisation of C2–C4 gaseous hydrocarbons and isoprene by microorganisms. J. Chem. Technol. Biotechnol. 81:237–256.
38. Shigematsu, T., S. Hanada, M. Eguchi, Y. Kamagata, T. Kanagawa, and R. Kurane. 1999. Soluble methane monooxygenase gene clusters from trichloroethylene-degrading Methylomonas sp. strains and detection of methanotrophs during in situ bioremediation. Appl. Environ. Microbiol. 65:5198–5206.
39. Smith, T.J., J.S. Lloyd, S.C. Gallagher, W.L. Fosdike, J.C. Murrell, and H. Dalton. 1999. Heterologous expression of alkene monooxygenase from Rhodococcus rhodochrous B-276. Eur. J. Biochem. 260:446–452.
40. Tavormina, P.L., V.J. Orphan, M.G. Kalyuzhnaya, M.S.M. Jetten, and M.G. Klotz. 2011. A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. Environ. Microbiol. Rep. 3:91–100.
41. Tsunogai, U., N. Yoshida, and T. Gamo. 1999. Carbon isotopic compositions of C2–C4 hydrocarbons and methyl chloride in urban, coastal, and maritime atmospheres over the western North Pacific. J. Geophys. Res. 104:16033–16039.
42. Tsunogai, U., N. Yoshida, J. Ishibashi, and T. Gamo. 2000. Carbon isotopic distribution of methane in deep-sea hydrothermal plume, Myojin Knoll Caldera, Izu-Bonin arc: Implications for microbial methane oxidation in the oceans and applications to heat flux estimation. Geochim. Cosmochim. Acta 64:2439–2452.
43. Urios, L., L. Intertaglia, F. Lesongeur, and P. Lebaron. 2008. Haliea salexigens gen. nov., sp. nov., a member of the Gammaproteobacteria from the Mediterranean Sea. Int. J. Syst. Evol. Microbiol. 58:1233–1237.
44. Urios, L., L. Intertaglia, F. Lesongeur, and P. Lebaron. 2009. Haliea rubra sp. nov., a member of the Gammaproteobacteria from the Mediterranean Sea. Int. J. Syst. Evol. Microbiol. 59:1188–1192.
45. Van Ginkel C.G., and J.A. de Bont. 1986. Isolation and characterization of alkene-utilizing Xanthobacter spp. Arch. Microbiol. 145:403–407.
46. Weisburg, W.G., S.M. Barns, D.A. Pelletier, and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173:697–703.
47. Xu, X., and K. Inubushi. 2009. Ethylene oxidation, atmospheric methane consumption, and ammonium oxidation in temperate forest soils. Biol. Fertil. Soils 45:265–271.
48. Yordanova, Z.P., E.T. Iakimova, S.M. Cristescu, F.J. Harren, V.M. Kapchina-Toteva, and E.J. Woltering. 2010. Involvement of ethylene and nitric oxide in cell death in mastoparan-treated unicellular alga Chlamydomonas reinhardtii. Cell Biol. Int. 34:301–308.
49. Zhou, N.Y., A. Jenkins, C.K. Chan Kwo Chion, and D.J. Leah. 1999. The alkene monooxygenase from Xanthobacter strain Py2 is closely related to aromatic monooxygenases and catalyzes aromatic mono- hydroxylation of benzene, toluene, and phenol. Appl. Environ. Microbiol. 65:1589–1595.