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Biotransformations of (+/−)-geosmin by terpene-degrading bacteria

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Abstract Two terpene-degrading bacteria able to transform (+/−)-geosmin have been identified. *Pseudomonas* sp. SBR3-tpnb, following growth on γ-terpinene, converts (+/−)-geosmin to several products; the major products are ketogeosmins. *Rhodococcus wratislaviensis* DLC-cam, isolated on D-camphor, also converts (+/−)-geosmin to several oxidation products, primarily ketogeosmins identical to those produced by strain SBR3-tpnb as well as hydroxygeosmins. This conversion appears to be inducible by (+/−)-geosmin and not by D-camphor.

Keywords Geosmin · Biotransformation · Terpene · *Pseudomonas* · *Rhodococcus wratislaviensis*

Introduction

(−)-Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) is produced by various microorganisms in the environment, including *Streptomyces* sp. (Gerber 1968; Gerber and Lechevalier 1965; Pollak and Berger 1996), cyanobacteria (Medsker et al. 1968; Safferman et al. 1967; Tabachek and Yurkowski 1976), myxobacteria (Trowitzsch et al. 1981), and fungi (Mattheis and Roberts 1992). It is one of a few chemicals that separately or together are responsible for the characteristic earthy smell of soil (Gerber 1979). Although geosmin has some value in formulating fragrances (Escher and Morris 1981) and as a natural component of some foods (Maga 1987), in many circumstances its presence is not desirable such as when it occurs in farm-raised catfish and other aquaculture products (Howgate 2004; Tucker 2000) and drinking water (Jüttner and Watson 2007). While it can sometimes be eliminated from catfish prior to harvesting by moving the fish to clean water, this often results in harvest delays and increased costs with significant reductions in profits for farmers (Engle et al. 1995). Cyanobacteria appear to be the major producers of (−)-geosmin in catfish ponds (Tucker 2000). Since it is not currently possible to prevent (−)-geosmin-producing cyanobacterial blooms from occurring in ponds and reservoirs, a practical solution to the geosmin problem might be to remove the chemical by treatment with geosmin-degrading bacteria.

There have only been a few studies of the biodegradation of geosmin and there is little information on intermediates and products. Several studies have shown geosmin removal by biofilms or filters. For

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example, granulated activated carbon biofilters fed with biodegradable organic matter could remove up to 60% of influent geosmin (initially 100 ng l^{-1}) (Elhadi et al. 2006). A crushed expanded clay based biofilter removed 90% of 20 ng influent geosmin l^{-1} (Persson et al. 2007). A peat fulvic acid-grown biofilm reactor removed 55% of 100 μg influent geosmin l^{-1} (Namkung and Rittman 1987). Sand filters and sand filter biofilm-containing bioreactors could degrade 200 ng geosmin l^{-1} (Ho et al. 2007). From one of these biofilm-containing sand filters, a consortium of three bacteria identified as *Sphingopyxis alaskensis*, *Novosphingobium stygiae*, and *Pseudomonas veronii* was isolated (Hoefel et al. 2006). The three strains were able to degrade geosmin only when all three were present; none was able to initiate attack on geosmin without the other two. The consortium was able to degrade 131 ng l^{-1} geosmin in 20 days with a 14-fold increase in active cell numbers. No products or intermediates were identified in any of these studies.

An activated sludge suspension in mineral salts medium containing ethanol and 0.1% (−)-geosmin gave small amounts of four products (together less than 10% of the final geosmin concentration) after a 45 day incubation (Saito et al. 1999). Two of these, identified by comparisons of their mass spectra to those of authentic chemicals, were formed by dehydration: (1,10-dimethyldecal-9,1-ene = argosmin C, Gerber 1979) and a subsequent oxidation (1,10-dimethyldecal-8,9-en-7-one).

Despite many attempts using a variety of environmental sources, it has not been possible to isolate bacteria by enrichment culture using (+/−)-geosmin as sole carbon and energy source (Eaton, unpublished). As an alternative, bacteria that grow with potential (+/−)-geosmin substrate analogs including D-camphor, γ-terpinene, and other terpenes were isolated by enrichment culture. Although most of these were unable to act on (+/−)-geosmin, a few had some activity and two strains were able to rapidly transform (+/−)-geosmin. These will be described here.

Materials and methods

Bacterial strains

Rhodococcus wratislaviensis DLC-cam was isolated by enrichment culture with D-camphor as sole carbon and energy source (Eaton and Sandusky 2009) from water obtained from Donner Lake, CA (DLC) while *Pseudomonas* sp. strain SBR3-tpnb was obtained by enrichment culture with γ-terpinene as sole carbon and energy source from activated sewage sludge from the South Baton Rouge, LA (SBR) sewage treatment plant. Bacterial strains were identified by sequencing 16S ribosomal RNA genes as previously described (Eaton and Sandusky 2009). DNA sequence searches of the GenBank database were carried out using BLASTN (Altschul et al. 1990). *Streptomyces citreus* CBS 109.60 (NRRL ISP 5369) (Pollak and Berger 1996) was obtained from the USDA ARS Northern Regional Research Laboratory Collection, Peoria, IL.

Chemicals and media

Neat, 95+% (+/−)-geosmin was obtained from Dalton Pharma Services, Toronto, ON, Canada; D-camphor ([1R]-[+]-camphor) and γ-terpinene (1-isopropyl-4-methyl-1,4-cyclohexadiene) were from Aldrich Chemical Company, Milwaukee, WI. All chemicals were of the highest purity available. The minimal medium was R medium (Eaton 2001), solidified when needed with 1.6% Noble Agar (Difco). Cultures were usually maintained on carbon-free minimal medium to which a carbon source, either D-camphor crystals or γ-terpinene in a small glass tube was added in the lid of the petri dish.

Screening for geosmin biotransformation

For the initial screening of geosmin biotransformation, bacteria were inoculated into 2 ml minimal medium supplemented with 0.1% succinate and 0.025% yeast extract to which about 1 mg D-camphor or 1 μl γ-terpinene was added in 20 ml glass scintillation vials with PTFE-lined caps. After overnight growth, 0.5 μl (+/−)-geosmin was added to each vial and the incubations continued for 2–3 days. The cultures were extracted with 1 ml methylene chloride which was dried over sodium sulfate and analyzed by GC-MS.

Time series transformations of (+/−)-geosmin

Minimal medium (50 ml) containing 10 mM succinate and 0.025% yeast extract in 2 × 250 ml bottles

was inoculated with *R. wratislaviensis* DLC-cam. To each bottle was added 50 µl ethanol containing either 37.5 mg D-camphor or no inducer and the cultures were incubated at 30°C for 23 h with shaking. The cultures were harvested by centrifugation at 6,000 rpm for 10 min in a Sorvall SS-34 rotor and resuspended in a total of 25 ml of the same medium without D-camphor. A volume of 2 ml of culture was added to each of 12 new vials to which 2 µl (+/−)-geosmin in ethanol (1/100, v/v) was subsequently added (to 9.36 µg (+/−)-geosmin ml^{-1}; 51 µM) Vials were incubated at 30°C with shaking at 250 rpm. At intervals, individual vials were removed and extracted with 2 ml methylene chloride which was subsequently dried over sodium sulfate. A volume of 1 µl was analyzed by GC-MS.

To investigate the possible requirement for enzyme induction during the incubation of strain DLC-cam with (+/−) geosmin, duplicate incubations were carried out in which the protein synthesis inhibitor, chloramphenicol (100 µg ml^{-1}), was added to one series of vials prior to the addition of geosmin.

To test whether the solvent for (+/−) geosmin, ethanol, might be the inducer of (+/−)-geosmin transforming enzymes, three parallel incubations were carried out. Strain DLC-cam was grown as before in 100 ml, harvested, then resuspended in 25 ml (cell density $A_{600} = 4.7$), and divided into 12 vials. Eight of the vials received 2 µl ethanol, while four vials received 2 µl geosmin in ethanol (1/100 v/v). Vials were incubated as before. After 1 h, 2 µl geosmin in ethanol was added to four of the ethanol vials (2 µl ethanol was added to the geosmin vials to keep the ethanol concentrations identical). At 2 h, 2 µl geosmin in ethanol was added to the remaining four ethanol vials. Vials were extracted at hourly intervals after these additions.

Transformation experiments with *Pseudomonas* sp. SBR3-tpnb were identical to the time-series biotransformation of (+/−)-geosmin by strain DLC-cam except that a single 50 ml culture was used. To this was added either 25 µl γ-terpinene or no inducer. Incubations were for 20 h with shaking.

Culture densities at the beginning of time series incubations with geosmin were recorded at 600 nm in 1 cm cuvettes with a Perkin–Elmer Lambda 35 spectrophotometer.

Analysis of metabolites

GC-MS analyses were carried out using an Agilent 6890 gas chromatograph in the splitless mode with an HP5-ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness) coupled to an Agilent 5973 mass selective detector. Helium (1 ml min^{-1}) was the carrier gas, the inlet was set at 250°C, and the oven was programmed as follows: 50°C for 2 min then increasing 20°C per minute to 250°C. The mass spectrometer operated in the electron ionization mode at 70 eV and a source temperature of 230°C. Mass spectra were acquired over a 50–300 amu range at 2.94 scans s^{-1}.

For preparation of metabolites for NMR spectroscopy, multiple incubations were carried out in 50 ml minimal medium containing a growth substrate (0.1% succinate, 0.05% camphor or γ-terpinene, and 0.025% yeast extract) in 250 ml bottles. After 1 day, 5 µl (4.68 mg) (+/−)-geosmin was added and after a further 2–4 days incubation, cultures were extracted with methylene chloride which was dried over sodium sulfate. Products were purified by flash chromatography using a Combiflash Companion (Teledyne Isco, Lincoln, NE) with a silica gel column and toluene:ethyl acetate 20:80 as solvent. Collected fractions were analyzed by GC-MS; peak fractions were pooled and the solvent evaporated.

NMR spectra of major metabolites including 1D proton, 1D carbon, multiplicity-edited HSQC (heteronuclear single quantum correlation), HMBC (heteronuclear multiple bond correlation), COSY (correlation spectroscopy), and NOESY (nuclear Overhauser effect spectroscopy) spectra were acquired at 25°C in "vial quality" 99.96% D_6-dimethylsulfoxide (Cambridge Isotope Laboratories, Andover, MA) in 3 mm tubes using 400 and 700 MHz Varian INOVA Plus instruments equipped with 5 mm inverse probes as previously described (Eaton and Sandusky 2009). Carbon chemical shifts were back-calculated using advanced chemical development (ACD) CNMR Predictor 8.08 accessed on line via ACD I-Lab.

Results and discussion

A collection of bacteria, obtained by selective enrichment using various terpenes as sole carbon

and energy sources, was screened for the ability to transform (+/−)-geosmin. Two strains were able to completely remove (+/−)-geosmin within a day or two. One of these, *Rhodococcus wratislaviensis* DLC-cam (16S ribosomal RNA-encoding DNA sequence in GenBank # EU043327) has been previously described for its ability to transform 2-methylisoborneol (Eaton and Sandusky 2009). The second, *Pseudomonas* sp. SBR3-tpnb (16S ribosomal RNA-encoding DNA sequence in GenBank # EU043324), was isolated using γ-terpinene. Neither strain could grow with (+/−)-geosmin a sole carbon source.

R. wratislaviensis DLC-cam, incubated with (+/−)-geosmin, converted it into a mixture of products. The product mixtures and the times required for their formation were essentially identical for cells grown in the presence or absence of D-camphor. The transformation of (+/−)-geosmin by strain DLC-cam grown in the absence of D-camphor is shown in Fig. 1a. Although there is a sharp, major peak at

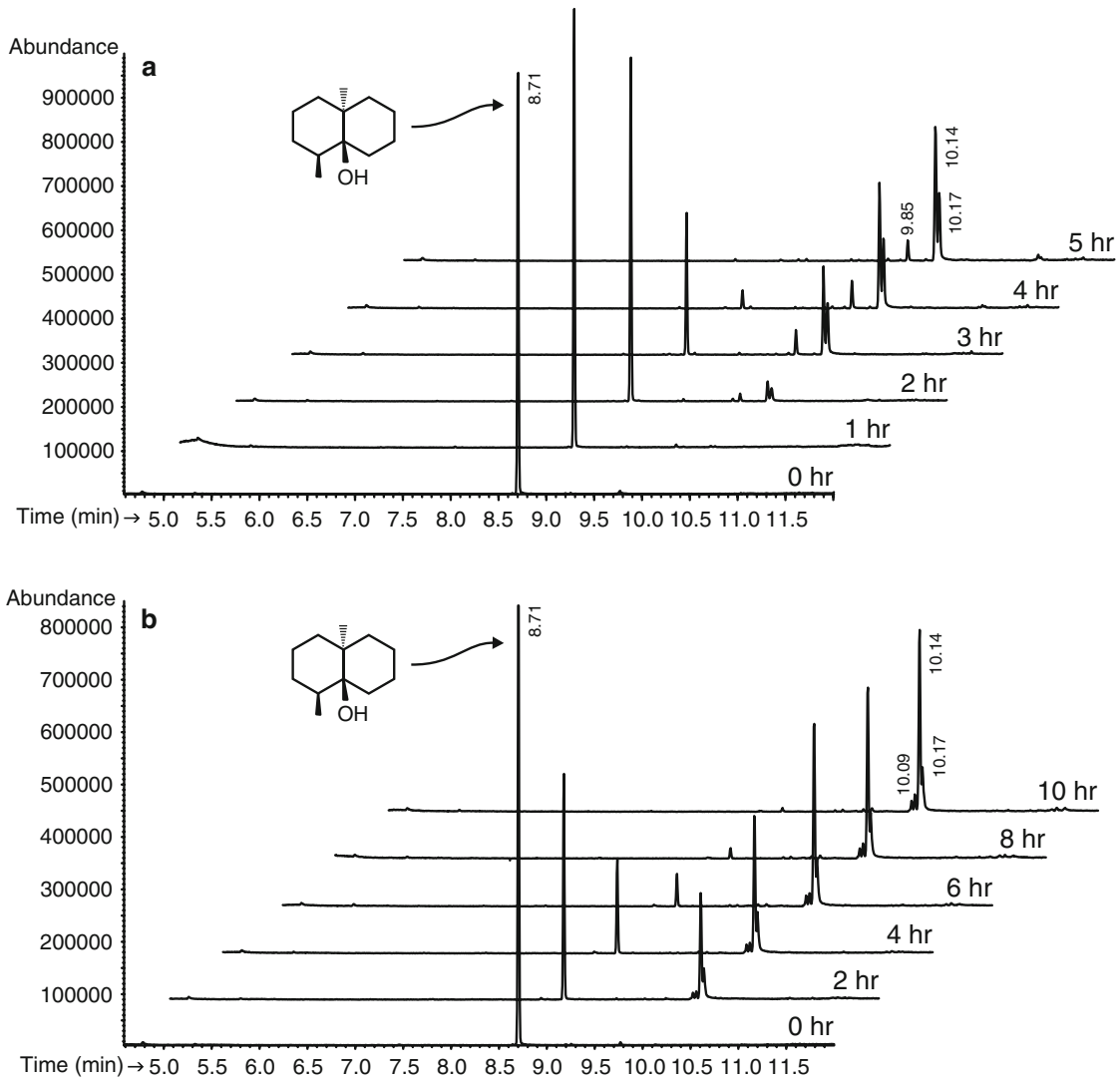

Fig. 1 Incubations of terpene-degrading bacteria with (+/−)-geosmin. The time series were carried out as described in the "Materials and Methods". Samples were taken at the beginning and at intervals, extracted with methylene chloride and analyzed by GC-MS. **a** *R. wratislaviensis* DLC-cam grown in the absence of D-camphor. **b** γ-Terpinene-grown *Pseudomonas* sp. SBR3-tpnb. Although the starting substrate was a mixture of (−)-geosmin and (+)-geosmin, only the structure of (−)-geosmin is shown

10.14 min, mass spectra taken at early and late times in the peak indicate that it is composed of at least two chemicals (Fig. 2a, c). The molecular ion (196) and mass spectra of both products suggests that they are monoketogeosmins. The base peaks in the mass spectra of the two metabolites differ significantly: that of the 10.135 min slice is *m/z* 112 while that of the 10.147 min slice is *m/z* 126. Some minor products are also evident. These appear to include a hydroxygeosmin (9.85 min, mwt = 198, Fig. 2b) and a product of the dehydration of a hydroxygeosmin (10.17 min, mwt = 180, Fig. 2d). Although not directly comparable, the total peak area of products is about 69% that of starting geosmin, suggesting a significant conversion (9.85 min peak [5%], 10.14 min [41%], and 10.17 min [23%].

When γ-terpinene-grown *Pseudomonas* sp. SBR3-tpnb was incubated with (+/−)-geosmin (Fig. 1b) it converted it into a mixture of products within 10 h. The mass spectrum of the major product eluting at 10.14 min (Fig. 2e) suggests that it is a monoketogeosmin (mwt = 196), while mass spectra of minor products indicate that they are probably products of hydroxygeosmin dehydration (mwt = 180, 10.17 min, Fig. 2f and 10.09 min, Fig. 2h). The 10.14 min peak is not completely homogeneous and there is evidence of a less abundant metabolite with an increase in the *m/z* = 126 ion at 10.153 min (mass spectrum not shown). The total peak areas of products are about 53% that of starting geosmin, suggesting a significant conversion (10.14 min peak [42%] and 10.17 min [11%]).

In the geosmin mass spectrum (Fig. 2g), the $C_7H_{12}O^+$ fragment that includes the right ring of (−)-geosmin (or left ring of (+)-geosmin, as shown in Fig. 3) is probably a major contributor to the *m/z* 112 ion that is the base peak. The $C_8H_{14}O^+$ fragment that includes the left ring of (−) geosmin (or right ring of (+)-geosmin) is probably a major contributor to the *m/z* 126 ion. A reduction in the relative proportion of one of these ions should indicate a substitution into the corresponding ring. Thus, in the ketogeosmin mass spectrum in Fig. 2a, the increase in *m/z* 126 suggests that the keto substitution is in the right ring as in 7-ketogeosmin, while in the ketogeosmin mass spectrum in Fig. 2c, the *m/z* 112 base peak suggests that the keto group is in the left ring as in 2-ketogeosmin.

Regulation of geosmin biotransformation in *R. wratislaviensis* DLC-cam

In the time series incubations with strain DLC-cam (Fig. 1a), the concentration of (+/−)-geosmin remained the same for 1 h, decreased slightly by 2 h and then rapidly fell. This suggested that the enzymes acting on (+/−)-geosmin might be induced during the incubation. This was confirmed by an experiment in which two series of incubations were carried out, one including the protein synthesis inhibitor chloramphenicol, one without. While the untreated vials gave typical geosmin transformation results as in Fig. 1a, (+/−)-geosmin in the chloramphenicol-treated vials remained unchanged (data not shown). This confirmed that (+/−)-geosmin-transforming enzymes were induced during the incubation and suggested three possible inducers: the solvent, ethanol, (+)-geosmin, or (−)-geosmin. An experiment in which cells were preincubated with ethanol prior to addition of (+/−)-geosmin demonstrated that ethanol has no effect on induction of (+/−)-geosmin-transforming enzymes. If ethanol were the inducer of the geosmin-transforming activity, bacteria in vials preincubated with ethanol should have transformed geosmin more quickly after geosmin addition than the control. This did not occur. All incubations resembled the standard incubation in Fig. 1a (data not shown). It appears, therefore, that one or both enantiomers of (+/−)-geosmin induce their own transformation in *R. wratislaviensis* DLC-cam.

NMR spectroscopy

The major peak eluting at about 10.14 min was purified from each strain (about 5 mg) away from other metabolite peaks by flash chromatography and analyzed by NMR spectroscopy. A sample of (+/−)-geosmin was also analyzed as a reference. Although the products purified from both strains gave a single 10.14 min peak with minor impurities when analyzed by GC-MS, both contained several components, primarily two ketogeosmins, the products A(+/−) and B(+/−) (Fig. 3). Application of 2D NMR spectroscopy, particularly multiplicity-edited HSQC, HMBC, and COSY, produced resolution of cross-peaks for all the positions of the carbonyl-containing rings in the product A and B ketogeosmins from both strains and confirmed the molecular structures.

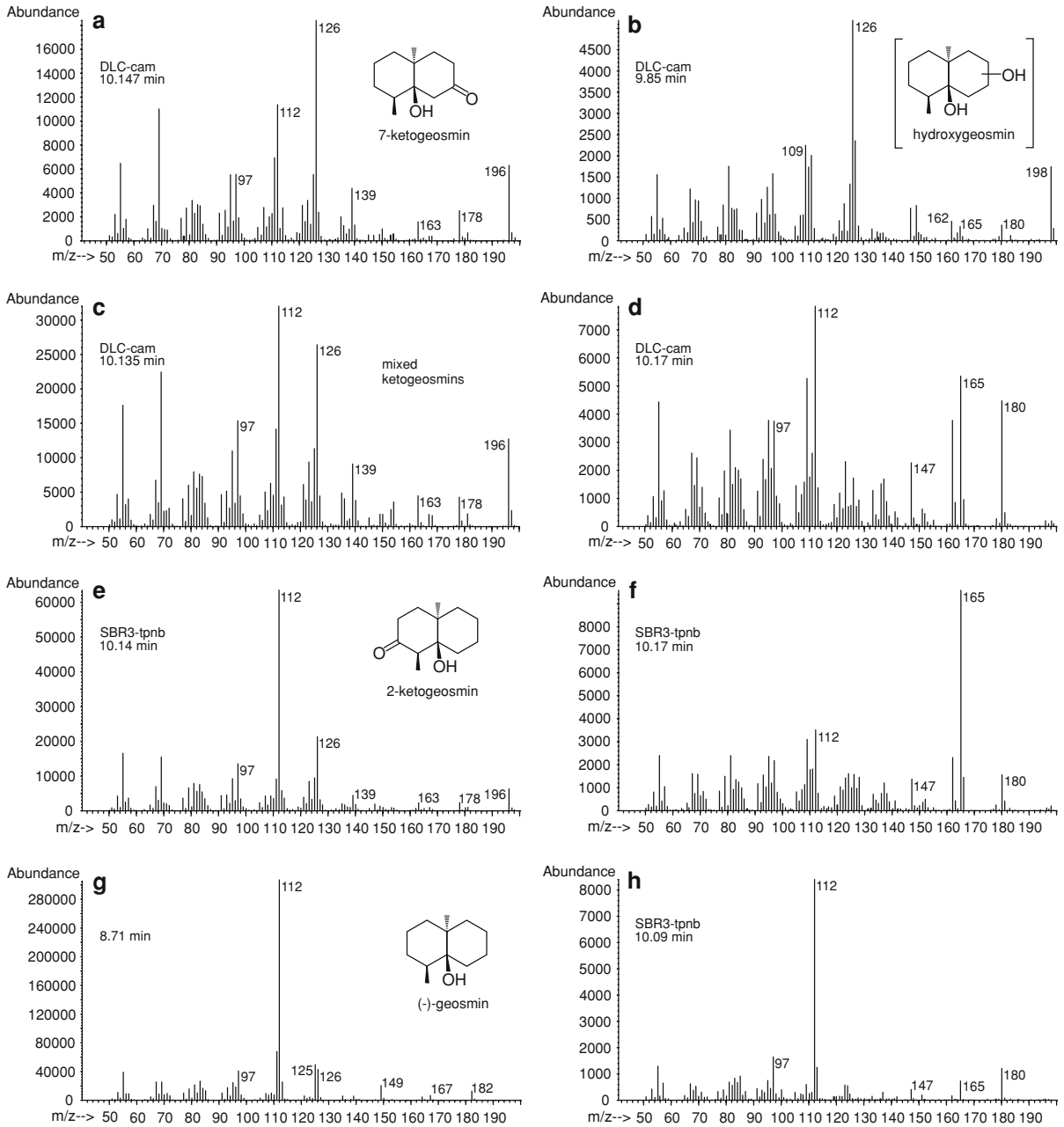

Fig. 2 Mass spectra of products of (+/−)-geosmin (**g**) transformation by *R. wratislaviensis* DLC-cam (**a**, **b**, **c**, **d**), and *Pseudomonas* sp. SBR3-tpnb (**e**, **f**, **g**) analyzed by GC-MS (Fig. 1). Although the starting substrate was a mixture of (−)-geosmin and (+)-geosmin, only the structures of (−)-geosmin and products derived from it are shown

Carbon and proton assignments are given in Table 1. Note that products A(−) and A(+), as a mirror-image pair, are indistinguishable in standard NMR spectroscopy, as are products B(−) and B(+).

The carbonyl region of the 1D carbon NMR spectra of products from both strains has two peaks at 210.9 and 211.5 ppm. For product A, HMBC showed the 210.9 ppm carbonyl to be coupled to an isolated methylene peak (δ ^{13}C = 47.6 ppm and δ ^{1}H = 2.33, 2.22 ppm) as well as a coupled CH_2–CH_2 unit (δ ^{13}C = 37.6 and 35.1 ppm and δ ^{1}H = 2.48, 2.08 and 1.93, 1.23 ppm). The location of the isolated

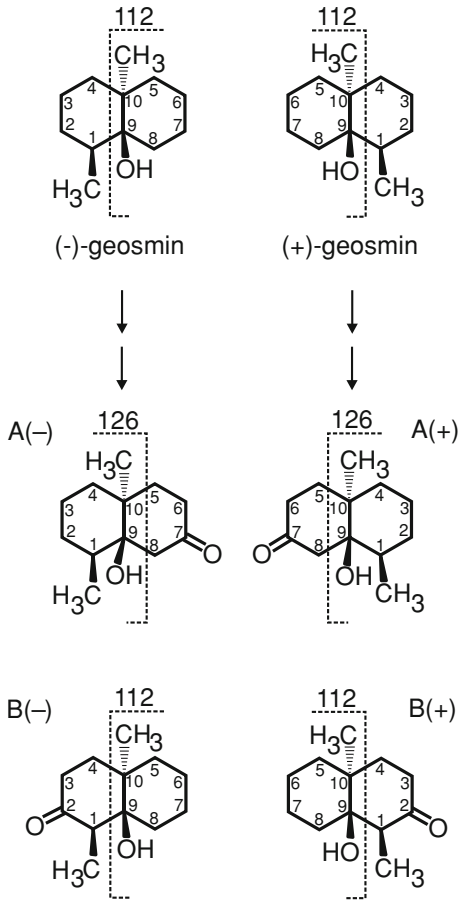

Fig. 3 Biotransformation of (+/−)-geosmin by strains DLC-cam and SBR3-tpnb. (+/−)-Geosmin is converted to ketogeosmins (mwt = 196) having mass spectra with base ions of *m/z* 112 or *m/z* 126. These are consistent with the structures A(+), A(−), B(+), and B(−) which are indicated by NMR spectroscopy data

methylene at C-8 and not C-5 was determined from the NOESY spectra (not shown) which indicate short through-space distances between protons. Specifically, the NOESY spectra show that the proton of the isolated methylene that is down in product A(+/−) is close in space to the methyl group attached to C-10 and the proton of the isolated methylene group that is up in product A(+/−) is close in space to the methyl group attached to C-1. Since protons attached to the methylene at C-5 are too distant from protons of the C-1-attached methyl group to produce NOESY cross peaks with them, the isolated methylene group could only be at position 8 and not at position 5, which places the carbonyl at ring position 7.

For product B, examination of the HSQC, HMBC, and COSY spectra showed the 211.5 ppm carbonyl to be coupled to an isolated methyl-coupled methine peak (δ $^{13}C = 49.3$ ppm and δ $^{1}H = 2.52$ ppm) which was assigned position 1 in product B(+/−), and to a coupled CH_2–CH_2 unit (δ $^{13}C = 37.6$ and 35.1 ppm and δ $^{1}H = 2.48$, 2.08 and 1.93, 1.23 ppm) assigned to product B(+/−) positions 3 and 4. The coupling of the carbonyl to the sole methine in the molecule clearly places the carbonyl at position 2.

Quaternary carbons at positions 9 and 10 were assigned from HMBC spectra. The position 1 and 10 methyls were assigned from HMBC and NOESY spectra. There is a high level of agreement between observed and back-calculated chemical shifts for the ketogeosmin metabolite carbon assignments (Table 1).

The substrate for these incubations was a racemic commercial mixture of the enantiomers (−)-geosmin and (+)-geosmin (Fig. 3, top). The starting mixture disappeared (Fig. 1) with the formation of products that were primarily the 7-ketogeosmins A+ and/or A− and the 2-ketogeosmins B+ and/or B−. It is not possible to tell these apart by normal GC-MS or NMR spectroscopy.

It seemed possible that one metabolite, A+ or A−, might be produced from one enantiomer while the other product, B+ or B−, was produced from the other enantiomer. To test this, a source of a single enantiomer, natural, (−)-geosmin, was needed. That source was *Streptomyces citreus* CBS 109.60 (NRRL ISP 5369) which produces a small amount of (−)-geosmin as one of 56 volatile compounds when grown in V1 medium (Pollak and Berger 1996). A 2 day old 100 ml culture of strain CBS-109.60 was extracted with methylene chloride which was dried and then evaporated in scintillation vials. Four 2 ml cultures of each of strains SBR3-tpnb and DLC-cam were grown overnight, pooled, and resuspended in 2 ml of media which was added to a (−)-geosmin-containing vial. After overnight incubation, cultures were extracted with methylene chloride and analyzed by GC-MS as usual. In both cases, the (−)-geosmin was completely removed, with the appearance of a peak composed of chemicals having mass spectra consistent with both compound A(−) and compound B(−) (data not shown). These data suggest that the enzymes acting on geosmin enantiomers are not very

Table 1 NMR spectroscopy data for (+/−)-geosmin and (+/−)-geosmin transformation products

C/H	Product A				Product B				(+/−)-Geosmin			
	Type	δ ^{1}H	δ ^{13}C		Type	δ ^{1}H	δ ^{13}C		Type	δ ^{1}H	δ ^{13}C	
			Observed	Calculated			Observed	Calculated			Observed	Calculated
1	CH	1.73	34.2	39.4	CH	2.52	49.3	49.6	CH	1.56	34.3	34.3
2	–	–	–	–	CO	–	211.5	211.9	CH_2	1.44, 1.22	30.3	29.9
3	–	–	–	–	CH_2[a]	2.48, 2.08	37.6	37.9	CH_2	1.57, 1.33	20.9	20.7
4	–	–	–	–	CH_2[a]	1.93, 1.23	35.1	35.6	CH_2	1.64, 0.78	34.6	35.7
5	CH_2[a]	1.93, 1.23	35.1	34.1	–	–	–	–	CH_2	1.70, 0.82	35.4	35.1
6	CH_2[a]	2.48, 2.08	37.6	37.1	–	–	–	–	CH_2	1.50, 1.35	20.7	21.4
7	CO	–	210.9	212.4	–	–	–	–	CH_2	1.66, 1.34	21.1	20.8
8	CH_2	2.33, 2.22	47.6	49.6	–	–	–	–	CH_2	1.44, 1.25	29.6	30.5
9	Q	–	77.0	79.7	Q	–	77.3	78.7	Q	–	73.3	74.5
10	Q	–	35.4	40.7	Q	–	36.6	37.9	Q	–	37.1	37.4
1-CH_3	CH_3	0.73 (d)	15.0	15.5	CH_3	0.83 (d)	7.1	6.7	CH_3	0.70	15.2	14.9
10-CH_3	CH_3	1.15 (s)	19.9	20.2	CH_3	1.19 (s)	20.6	20.8	CH_3	0.95	20.2	20.3
9-OH		–				–				3.37		

Molecules with numbered carbons are shown in Fig. 3

[a] NMR spectral features of positions 5 and 6 in product A were unresolved from those of positions 4 and 3, respectively, in product B

site-specific and that all four compounds (Fig. 3) are likely to be produced from (+/−)-geosmin.

Two terpene-degrading bacteria, one Gram positive, the other Gram negative have been shown here to remove (+/−)-geosmin yielding primarily 2-ketogeosmin and 7-ketogeosmin as well as several minor products. The normal functions of the enzymes involved have not been determined. *Pseudomonas* sp. SBR3-tpnb employs enzymes inducible by γ-terpinene and which are likely to be involved in γ-terpinene metabolism for which a pathway has not yet been established. *R. wratislaviensis* DLC-cam uses enzymes that appear to be inducible by (+/−)-geosmin and which are not the D-camphor pathway enzymes that it uses to transform 2-methylisoborneol (Eaton and Sandusky 2009). Whether these bacteria are useful for eliminating geosmin in actual aquaculture or drinking water situations remains to be determined.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Eaton RW (2001) Plasmid-encoded phthalate catabolic pathway in *Arthrobacter keyseri* 12B. J Bacteriol 183:3689–3703

Eaton RW, Sandusky P (2009) Biotransformations of 2-methylisoborneol by camphor-degrading bacteria. Appl Environ Microbiol 75:583–588

Elhadi SLN, Huck PM, Slawson RM (2006) Factors affecting the removal of geosmin and MIB in drinking water biofilters. J AWWA 98(8):108–119

Engle CE, Pounds GL, van der Ploeg M (1995) The cost of off-flavor. J World Aquac Soc 26:297–306

Escher SD, Morris AF (1981) Use of a hydroxylic bicyclic derivative as perfuming ingredient. US Patent 4,248,742 issued to Firmenich SA, Geneva, CH

Gerber NN (1968) Geosmin, from microorganisms, is *trans*-1, 10-dimethyl-*trans*-9-decalol. Tetrahedron Lett 9:2971–2974

Gerber NN (1979) Volatile substances from actinomycetes: their role in the odor pollution of water. CRC Crit Rev Microbiol 7:191–214

Gerber NN, Lechevalier HA (1965) Geosmin, an earthy-smelling substance isolated from actinomycetes. Appl Microbiol 13:935–938

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

Hoefel D, Ho L, Aunkofer W, Monis PT, Keegan A, Newcombe G, Saint CP (2006) Cooperative biodegradation of geosmin by a consortium comprising three gram-negative bacteria isolated from the biofilm of a sand filter column. Lett Appl Microbiol 43:417–423

Howgate P (2004) Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: a review of sensory aspects and of uptake/depuration. Aquaculture 234:155–181

Jüttner F, Watson SB (2007) Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. Appl Environ Microbiol 73:4395–4406

Maga JA (1987) Musty/earthy aromas. Food Rev Int 3:269–284

Mattheis JP, Roberts RG (1992) Identification of geosmin as a volatile metabolite of *Penicillium expansum*. Appl Environ Microbiol 58:3170–3173

Medsker LL, Jenkins D, Thomas JF (1968) Odorous compounds in natural waters: an earthy-smelling compound associated with blue-green algae and actinomycetes. Environ Sci Technol 2:461–464

Namkung E, Rittman BE (1987) Removal of taste- and odor-causing compounds by biofilms grown on humic substances. J AWWA 79(7):107–112

Persson F, Heinecke G, Hedberg T, Hermansson M, Uhl W (2007) Removal of geosmin and MIB by biofiltration—an investigation discriminating between adsorption and biodegradation. Environ Technol 28:95–104

Pollak FC, Berger RG (1996) Geosmin and related volatiles in bioreactor-cultured *Streptomyces citreus* CBS 109.60. Appl Environ Microbiol 62:1295–1299

Safferman RS, Rosen AA, Mashni CI, Morris ME (1967) Earthy-smelling substance from a blue-green alga. Environ Sci Technol 1:429–430

Saito A, Tokuyama T, Tanaka A, Oritani T, Fuchigami K (1999) Microbiological degradation of (−)-geosmin. Water Res 33:3033–3036

Tabachek JL, Yurkowski M (1976) Isolation and identification of blue-green algae producing muddy odor metabolites, geosmin, and 2-methylisoborneol, in saline lakes in Manitoba. J Fish Res Board Can 33:25–35

Trowitzsch W, Witte L, Reichenbach H (1981) Geosmin from earthy smelling cultures of *Nannocystis exedens* (Myxobacterales). FEMS Microbiol Lett 12:257–260

Tucker CS (2000) Off-flavor problems in aquaculture. Rev Fish Sci 8:45–88