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ORIGINAL PAPER

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Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench

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Abstract Thirty-eight actinomycetes were isolated from sediment collected from the Mariana Trench (10,898 m) using marine agar and media selective for actinomycetes, notably raffinose-histidine agar. The isolates were assigned to the class *Actinobacteria* using primers specific for members of this taxon. The phylogenetic analysis based on 16S rRNA gene sequencing showed that the isolates belonged to the genera *Dermacoccus*, *Kocuria*, *Micromonospora*, *Streptomyces*, *Tsukamurella* and *Williamsia*. All of the isolates were screened for genes encoding nonribosomal peptide and polyketide synthetases. Nonribosomal peptide synthetase sequences were detected in more than half of the isolates and polyketide synthases type I (PKS-I) were identified in five out of 38 strains. The *Streptomyces* isolates produced several unusual secondary metabolites, including a PKS-I associated product. In initial testing for piezotolerance, the *Dermacoccus* strain MT1.1 grew at elevated hydrostatic pressures.

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Introduction

Much of our research efforts over the past decade or so have been directed at assessing the diversity of actinomycetes in the marine environment. Actinomycetes are very widely distributed in the world's oceans and, moreover, truly indigenous marine actinomycetes have now been described (Bull et al. 2005). Although many questions remain to be answered regarding the ecological function of actinobacteria in the marine environment as well as their biogeographic distribution and evolutionary history, it is becoming increasingly clear that marine actinomycetes, in particular, present a major resource for biotechnological search and discovery (Bull 2004; Fiedler et al. 2005; Jensen et al. 2005). Using both culture dependent and culture independent survey methods, we have shown that actinomycetes can be recovered from abyssal (2,000–6,000 m) and hadal (>6,000 m) sediments (Colquhoun et al. 1998; Brandão et al. 2002; Stach et al. 2003a) and that some of the culturable strains of *Rhodococcus* can be grown at in situ pressures in the laboratory (Heald et al. 2001).

The microbiological exploration of the very deep-seas presents considerable technological and logistical difficulties and the deployment of the JAMSTEC submersible fleet has been prominent in this context. In particular, the unmanned submersible *Kaiko*, introduced in 1995 (Kyo et al. 1995), has enabled the ultimate ocean depths to be accessed and sediments to be collected aseptically. To date, the principal emphasis has been on the study of Gram-negative piezophilic bacteria in these sediments (Kato et al. 1998). We have had the opportunity to study actinomycetes in sediments from the deepest region of the world's oceans, namely the Mariana Trench in the western Pacific Ocean, within which

the Challenger Deep, at its the southernmost end, is the deepest point on Earth (its depths are variously reported to be 10,915 m (Taira et al. 2004) to 10,920 m (Gvirtzman and Stern 2004). Two samples provided by *Kaiko* dives, numbers 10K-21-1 (11°21.11′N; 142°25.95′E; 1996) and 74 (1998), have been subject to culture-dependent screens for actinomycetes, the first of which (Colquhoun et al. 1998) failed to reveal the presence of such organisms. The second sample was obtained from a closely related site (see below) and, using a much more extensive portfolio of selective isolation media, actinomycetes were successfully recovered. The sediment sample available to us for this study was small and, consequently, we have refrained from drawing any conclusions regarding the representative nature of our data for the Mariana Trench per se. However, because ROV *Kaiko* was lost at sea in 2003 (http://www.jamstec.go.jp/jamstec-e/index-e.html), even this small, uncontaminated sediment sample from 10,898 m constituted a precious research resource. In this baseline paper, we make the first report on the diversity of culturable actinomycetes in Challenger Deep sediment, and also provide preliminary evidence of their biotechnological potential and indigenicity in the marine environment.

Materials and methods

Sample site and sample collection

Sediment was collected from the Mariana Trench (Challenger Deep; 11°19′911″ N; 142°12′372″ E) at a depth of 10,898 m (maximum bottom current speed 8.1 cm/s; Taira et al. 2004) by the remotely operated submarine *Kaiko*, using sterilized mud samplers (Kato et al. 1997), on 21/05/1998, during dive number 74. The sample (approximately 2 ml) was transported to the UK in an insulated container at 4°C and then stored at −20°C until analysed for actinomycetes.

Selective isolation and enumeration of culturable actinomycetes

Actinobacteria were isolated from the wet sediment using the dilution plate technique. Approximately 1 ml of sediment was added to 9 ml of 0.25 strength Ringer's solution (Oxoid), held in universal bottles and agitated on a shaker (Orbital incubator, Sanyo, UK) for 30 min at 150 rpm, to disperse bacterial propagules. Serial dilutions were prepared using 0.25 strength Ringer's solution and aliquots (100 µl) of appropriate dilutions were used to inoculate a range of media designed for the selective isolation of specific actinomycete taxa. The isolation plates were incubated at 28°C for up to 12 weeks. The viable bacterial count was determined using Difco marine agar 2216. Thermoactinomycetes were sought using half-strength nutrient agar (Oxoid) supplemented with 25 µg/ml novobiocin (Cross 1968), and the plates were incubated at 55°C for 3 days. The presence of thermoactinomycetes in marine environments is a reliable and established indicator of terrestrial wash-in; spore germination and growth do not occur at the in situ temperatures of the deep-seas. In all the cases, three plates were prepared for each dilution. The actinomycete and total bacterial counts were calculated as the mean number of colony forming units (cfu) per ml of wet sediment.

Identification of actinomycetes with specific primers

The total genomic DNA from all the marine isolates was used as a template for the diagnostic PCR identification of actinobacteria, using the specific primers S-C-Act-0235-a-S-20 and S-C-Act-0878-a-A-18 (Stach et al. 2003b). The reactions were carried out in a final volume of 50 µl containing 0.125 mM of each of the four dNTPs (Bioline, UK), 0.2 µM of each primer, 0.5 µl DNA template, 1.5 mM $MgCl_2$ and 1.25 units of Bio*Taq* DNA polymerase (Bioline). Amplifications were made in a Perkin Elmer ThermoCycler 480 under the following conditions: hot start of 5 min at 95°C, 35 cycles of 1 min at 95°C, 1 min at 62°C and a final extension step at 72°C for 1 min.

Dereplication by *rep*-PCR and colour grouping

Repetitive DNA fingerprinting was performed on all the isolates following the method of Versalovic et al. (1994). The PCR primer derived from the repetitive sequence BOX A1R (5′-CTACGGCAAGGCGACGCTGACG-3′) was used to amplify the DNA samples. The PCR mixtures contained 2.5 units of Bio*Taq* DNA polymerase (Bioline), 2 µM BOX A1R primer, 6 mM $MgCl_2$ and 10% DMSO in a 25 µl reaction volume. Control reactions, without bacterial DNA templates, were included in all the amplifications. The following PCR temperature profile was performed: hot start of 5 min at 95°C, 30 cycles of 1 min at 95°C, 1 min at 52°C, 8 min at 65°C and a final extension step at 65°C for 16 min. The amplifications were made in a Perkin Elmer ThermoCycler 480. The gels were analysed using BioNumerics Version 2.0 (Applied Maths, Kortrijk, Belgium). The similarity matrices of densitometric curves of strains were calculated using the Pearson Product Moment Correlation Coefficient (Pearson 1926), followed by a dendrogram construction using Ward's algorithm (Ward 1963).

Colour grouping was done by growing all the isolates, except MT5.1–MT5.5, on glucose yeast extract agar. Colony morphology and the ability to produce pigments were observed after 5 days incubation at 28°C. Strains MT5.1–MT5.5 were inoculated onto oatmeal (ISP medium 3; Shirling and Gottlieb 1966) and peptone-yeast extract-iron (ISP medium 6; Shirling and Gottlieb

1966) agar plates, which were incubated at 28°C for 21 and 4 days, respectively. The oatmeal agar plates were examined by eye after 7, 14 and 21 days and aerial spore-mass colour, substrate mycelium pigmentation and the colour of any soluble pigments recorded using the National Bureau of Standards (NBS) Colour Name Charts (Kelly 1958; NBS 1964). The peptone-yeast extract-iron agar plates were examined to determine whether the test strains had produced their characteristic dark coloured melanin pigments. The isolates were assigned to colour groups on the basis of the recorded properties.

Amplification of 16S rRNA gene sequences and phylogenetic analysis

The extraction of total genomic DNA, PCR amplification and the direct sequencing of 16S rRNA genes of representative isolates were carried out as described by Kim et al. (1998). The resultant 16S rRNA gene sequences were aligned manually, using the PHYDIT program (available at http://plaza.snu.ac.kr/~jchun/phydit/), against corresponding sequences of representatives of the suborder *Corynebacterineae* and *Micrococcineae* retrieved from the DDBJ/EMBL/GenBank databases. Phylogenetic trees were inferred using the neighbour-joining algorithm (Saitou and Nei 1987) and evolution distance matrices generated after Jukes and Cantor (1969). The robustness of the resultant trees were evaluated by performing a bootstrap analysis (Felsenstein 1985) of neighbour-joining data based on 1,000 resamplings, using the TREECON program (Van der Peer and de Wachter 1994)

Screening and analysis of nonribosomal peptide synthetase (NRPS), type I polyketide synthases (PKS I) and type II polyketide synthase (PKS II)

The PCR reactions for the amplification of NRPS and PKS-I from the tested isolates were carried out as recommended by Ayuso-Sacido and Genilloud (2005) and Courtois et al. (2003), respectively. The amplification reactions were made in a final volume of 50 µl on a Perkin Elmer ThermoCycler 480. The amplification of PKS II was made according to Ketela et al. (1999), with some modifications. The PCR mixtures contained 2.5 units of Bio*Taq* DNA polymerase (Bioline), 2 µM of each primer, 1 mM $MgCl_2$, 0.4 mM dNTPs and 5% DMSO in a 50 µl reaction volume. Control reactions, without the bacterial DNA template, were included in all amplifications. The PCR temperature profile was: hot start of 2 min at 95°C, 30 cycles of 1 min at 96°C, 1 min at 64°C, 1.5 min at 73°C and a final extension step at 73°C for 8.5 min. The amplifications were carried out in a Biometra Tgradient (Whatman). The nucleotide sequences of amplified products were aligned using the Clustal X Version 1.81 program (Jeanmougin et al. 1998), and trees were constructed using the neighbour-joining algorithm (Saitou and Nei 1987). The nucleotide sequences, derived from the NRPS genes of the marine strains MT6.1 and MT8, were translated and submitted to protein–protein database searching using the blastp function of the BLAST program (Altschul et al. 1990). Protein–protein similarities were calculated using the PHYLIP suite of software packages (Felsenstein 1989).

Growth of representative strains at elevated pressure

Cultures (1 ml) of *Dermacoccus* isolate MT1.1 and *Dermacoccus nishinomiyaensis* DSM 20448^T were suspended in a glucose-yeast extract broth to approximately $OD_{600} = 0.1$, then incubated at an elevated pressure in sterile Hungate tubes (Glasgerätebau OCHS GmbH, Germany) containing 0.5 ml of Fluorinert FC-77 (Sigma, Deisenhofen, Germany) as an oxygen source (Heald et al. 2001). The tubes were filled completely with the medium so that no gaseous headspace remained. Prior to use, the Fluorinert was prepared by degassing under vacuum, oxygenated for 2 h before use, then filter sterilized and stored at −20°C. Triplicate cultures were incubated at 30°C in hydraulically pressurized vessels (Bomb No. 4740, Parr Instrument Co., USA) constructed from Inconel 625, a high strength nickel–chromium alloy. The growth was measured as the change in the total viable count on glucose-yeast extract agar, after incubation for 4 days at 40 MPa. The control cultures were incubated at atmospheric pressure.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences of representative isolates were deposited in the DDBJ, EMBL and GenBank nucleotide sequence databases under the accession numbers AY894323–AY894337 (see Fig. 2).

Results and discussion

Selective isolation and enumeration of culturable actinobacteria

The present study was focused on the analysis of culturable actinomycetes that grew aerobically at atmospheric pressure. The small volume of sediment available for this study was insufficient for the generation of physico-chemical data. However, the temperature, salinity and water pressure of the Challenger Deep have been previously reported to be 2.6°C, 34.7% and 116.86 MPa, respectively (Akimoto et al. 2001).

The total culturable bacterial count was $2.56 \pm 0.57 \times 10^3$ cfu/ml of wet sediment in the sample recovered from the Mariana Trench, a result similar to those reported for other deep-sea trench sediments (Colquhoun et al. 1998); although these latter authors

did not isolate any actinomycetes from a Mariana Trench sample. An analysis of the mud sample taken from the same site in the Mariana Trench revealed the presence of various extremophile and nonextremophile bacteria, which accounted for more than 10^5 cfu/g of the dried sediment (Takami et al. 1997). It is difficult to compare counts made per ml of the wet sediment with those based per gram of the dried weight; but even so, the counts recorded in this study are consistent with those reported by other workers (Goodfellow and Haynes 1984; Jensen et al. 1991; Takizawa et al. 1993; Colquhoun et al. 1998).

The total actinomycete count on marine agar was $3.8 \pm 1.7 \times 10^2$ cfu/ml, which represents 15% of the total viable bacteria count on this medium. Higher numbers of actinomycetes were isolated on humic acid-vitamin agar ($1.7 \pm 1.5 \times 10^3$ cfu/ml). Actinomycetes usually account only for a small fraction of bacterial communities (0–9%) in marine sediments (Bull et al. 2005). However, the proportion can be as high as 14%, as reported, for example, in sediments taken from the Northeastern Atlantic Ocean between the Faroe and the Shetland Islands (Weyland 1981). Weyland (loc cit.) found that actinomycetes constituted 60% of the total bacterial counts in sediments taken from sites between the British Isles and the North Atlantic ridge. Again, it is difficult to compare data obtained in the present study with those given in previous reports because of the different selective media, incubation conditions and pretreatment regimes used by different researchers. Given the very small quantity of sediment available, we were unable to estimate the proportion of actinomycetes within the total bacterial community; moreover, we have also found that sediment samples of 10 g or more are required for DNA extraction in a culture independent studies of deep-sea actinomycetes (Stach et al. 2003a).

Members of the genus *Thermoactinomyces* are useful markers of terrestrial wash-in and for yielding information about the distribution and survival of microorganisms in marine environments (Attwell and Colwell 1984; Goodfellow and Haynes 1984). Thermoactinomycetes were not detected in this study, a result that suggests the absence of terrestrial contamination at the sampling site.

Thirty-eight presumptive actinomycetes were isolated from the Mariana Trench sediment (Table 1). A specific 640 bp amplification product (Stach et al. 2003b) was obtained with all these isolates, thereby confirming their identity as actinobacteria. Members of the suborders *Corynebacterineae* (genus *Williamsia*) and *Micrococcineae* (genus *Dermacoccus*) were isolated whereas a previous investigation of the same site revealed the presence of members of the genera *Dietzia* (suborder *Corynebacterineae*) and *Aureobacterium* (suborder *Micrococcineae*) strains (Takami et al. 1997). Actinomycetes were isolated on four out of the 20 selective isolation media, namely Brain Heart Infusion (Schaal 1977), humic acid-vitamins (Hayakawa and Nonomura 1987), marine (Han et al. 2003) and raffinose–histidine agars. Most strains (58%) were isolated from raffinose–histidine agar plates, a medium designed for the isolation of rare streptomycetes (Vickers et al. 1984).

It is interesting that, in the present study, most of the isolates were recovered using relatively simple nutrient media, an approach that has been used successfully by other investigators (Janssen et al. 2002). Similarly, simple nutrient media and extended incubation conditions have been used to isolate taxonomically diverse soil bacteria, including novel members of families belonging to the subclass *Actinobacteridae* (Sait et al. 2002; Joseph et al. 2003). In addition, cultivation independent procedures have been used to guide strategies that promote the isolation of novel actinomycetes from marine habitats (Maldonado et al. 2005). Selective agents drawn from extensive taxonomic phenotypic databases can also be used to formulate media designed to isolate members of specific actinomycete taxa (Vickers et al. 1984; Duangmal et al. 2005).

Dereplication of isolates

Dereplication procedures are used to assign taxonomically similar isolates to groups in order to select representative strains for more exacting taxonomic studies and to prevent redundant screening efforts (Bull et al. 1992). Repetitive DNA fingerprinting or *rep*-PCR (Versalovic et al. 1994) was used to determine the diversity of actinomycetes isolated from the Mariana Trench sediment as this procedure is known to be reproducible, sensitive and applicable to the analyses of large numbers of strains (Sadowsky et al. 1996; Olive and Bean 1999; Yamamura et al. 2004). Good congruence has been found between groups of streptomycetes based on *rep*-PCR and DNA:DNA relatedness data (Lanoot et al. 2004). In the present study, representatives of the genera *Dietzia* and *Williamsia* and the isolates were assigned to two major groups A and B, and three minor groups C–E (Fig. 1); isolate MT4.1 did not give any bands. The integrity of the BOX-PCR groups was underpinned by the assignment of the isolates to groups based on their ability to produce a range of pigments on glucose yeast extract and oatmeal agars (Table 1).

Phylogenetic analysis

Isolates taken to represent the BOX-PCR groups were the subject of 16S rRNA gene sequencing studies. The phylogenetic relationships of the isolates are shown in Fig. 2a–d. It is apparent that the isolates can be assigned to the genera *Dermacoccus*, *Kocuria*, *Micromonospora*, *Streptomyces*, *Tsukamurella* and *Williamsia*. The taxonomic relationships of branches composed of the individual isolates are supported by high-bootstrap values (99–100%) that are based on neighbour-joining analyses. A scrutiny of the 16S rRNA phylogenetic trees

Table 1 Preliminary classification of culturable actinomycetes isolated from the Mariana Trench sediment and the presence of NRPS, PKS types I and II genes

Isolate codes	Selective medium	Colour group	*Rep*-PCR group	NRPS	PKS-I	PKS-II	Genus assignment
MT1.1	RH	1	A	−	−	−	*Dermacoccus*
MT1.2	RH	1	A	+	−	−	*Dermacoccus*
MT1.3	RH	1	A	+	−	−	*Dermacoccus*
MT1.4	RH	1	A	−	−	−	*Dermacoccus*
MT1.5	RH	1	A	−	−	−	*Dermacoccus*
MT1.6	RH	1	A	−	−	−	*Dermacoccus*
MT1.7	RH	1	A	+	−	−	*Dermacoccus*
MT1.8	RH	1	A	+	−	−	*Dermacoccus*
MT1.9	RH	1	A	+	−	−	*Dermacoccus*
MT1.10	RH	1	A	+	−	−	*Dermacoccus*
MT1.11	RH	1	A	+	−	−	*Dermacoccus*
MT1.12	RH	1	A	−	−	−	*Dermacoccus*
MT1.13	RH	1	A	−	−	−	*Dermacoccus*
MT1.14	RH	1	A	−	−	−	*Dermacoccus*
MT1.15	RH	1	A	−	−	−	*Dermacoccus*
MT1.16	RH	1	A	+	−	−	*Dermacoccus*
MT2.1	RH	2	E	−	−	−	*Dermacoccus*
MT2.2	RH	2	E	−	−	−	*Dermacoccus*
MT3.1	RH	3	E	−	−	−	*Dermacoccus*
MT4.1	RH	4	ND	−	−	−	*Kocuria*
MT5.1	HV	5	C	+	+	−	*Streptomyces*
MT5.2	HV	5	C	+	+	−	*Streptomyces*
MT5.3	HV	5	C	+	+	−	*Streptomyces*
MT5.4	HV	5	C	+	+	−	*Streptomyces*
MT5.5	HV	5	C	+	+	−	*Streptomyces*
MT6.1	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.2	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.3	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.4	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.5	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.6	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.7	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.8	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.9	Marine agar	6	B	+	−	−	*Tsukamurella*
MT6.10	BHI	6	B	+	−	−	*Tsukamurella*
MT6.11	BHI	6	B	+	−	−	*Tsukamurella*
MT8	RH	7	D	+	−	−	*Williamsia*
MT25	RH	8	D	+	−	+	*Micromonospora*

BHI brain heart infusion, *HV* humic acid-vitamins agar, *RH* raffinose-histidine agar
1 *Dermacoccus* sp., 2 *Dermacoccus* sp., 3 *Dermacoccus* sp., 4 *Kocuria* sp., 5 *Streptomyces* spp., 6 *Tsukamurella* spp., 7 *Williamsia* sp., 8 *Micromonospora* sp., ND not determined

suggests that some of the isolates may represent novel species. This is the first report on the isolation of members of the genera *Dermacoccus* and *Tsukamurella* from marine habitats; taxonomic descriptions of the putative novel taxa will be published elsewhere.

Detection, distribution and analysis of NRPS, PKS-I and PKS-II sequences

As part of our bioprospecting programme we screened the Mariana Trench isolates in order to assess their biosynthetic potential, using sets of degenerate primers to detect the presence of NRPS, PKS types I and II sequences in the corresponding genomes (Table 1). Nonribosomal peptide synthetase sequences were detected in most of the isolates (68%) whereas PKS-I sequences were detected in only five out of the 38 strains (13%). The single PKS-II sequence was associated with the *Micromonospora* strain MT25. The *Streptomyces* isolates gave positive amplification products with both the NRPS and PKS-I primers. A preliminary analysis of secondary metabolite production by these streptomycete isolates in submerged cultivation revealed a heptaene polyene antibiotic (PKS-I) in cell extracts and several unusual metabolites in culture filtrate extracts (H.-P. Fiedler, personal communication). The results are consistent with the findings from the PCR screening.

The absence of amplification products from some of the isolates may reflect the lack of NRPS, PKS-I and PKS-II genes, though it is possible that less conserved domain sequences shared low homology with the primers. Furthermore, not all NRPS genes are involved in the biosynthesis of bioactive secondary metabolites; indeed, the products of such genes may be involved in functions such as iron metabolism or quorum sensing (Finking and Marahiel 2004). It is also possible that the genes detected by the PCR are nonfunctional. Nevertheless,

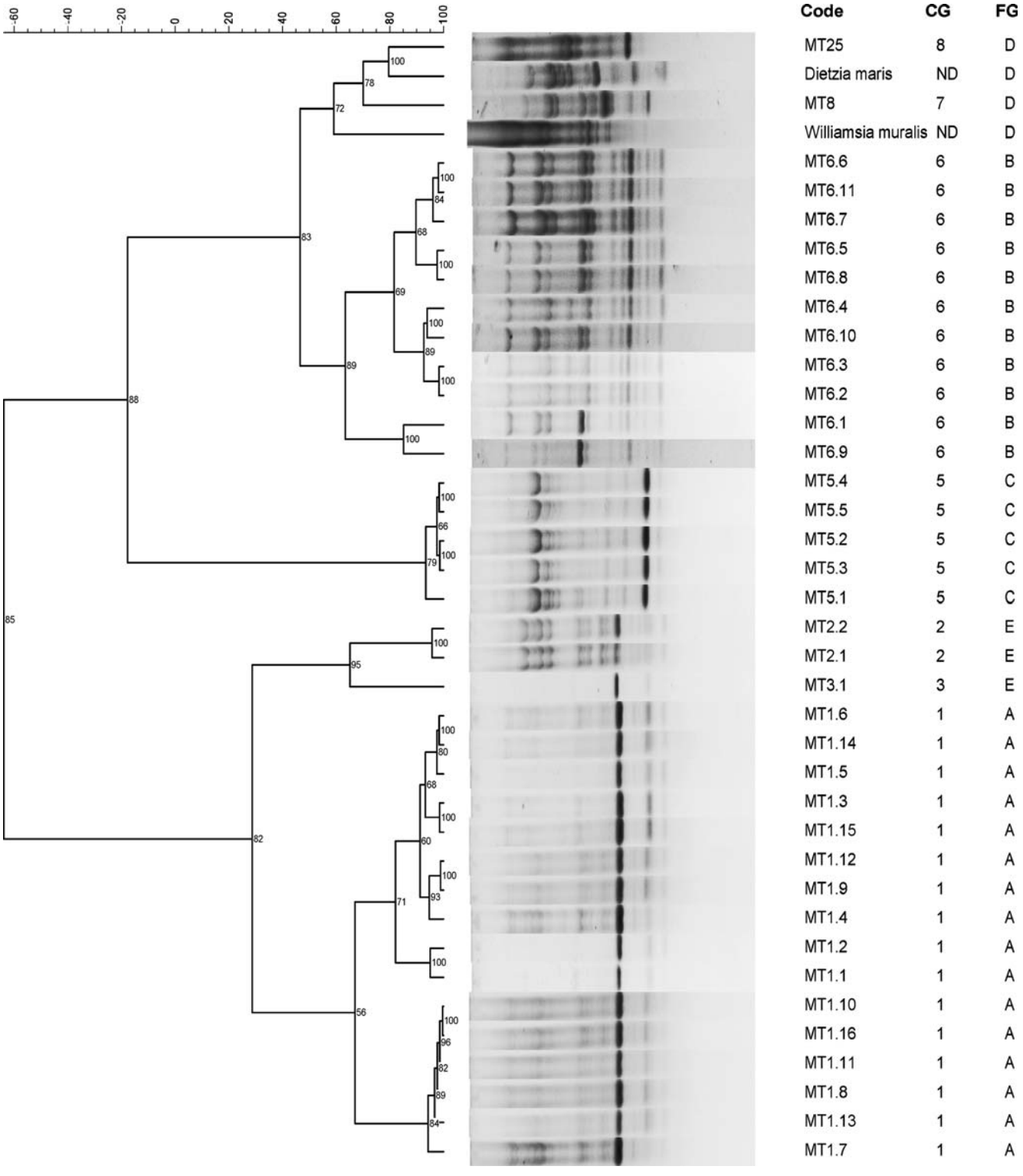

Fig. 1 Dendrogram showing BOX-PCR fingerprints of the Mariana Trench isolates, *Dietzia maris* DSM43672^T and *Williamsia muralis* DSM44343^T, *CG* colour group, *FG* BOX-PCR group

the strategy of prescreening with PCR primers, which target genes encoding for bioactive compounds, is an effective approach for detecting novel and useful secondary metabolites (Ketela et al. 1999, 2002; Courtois et al. 2003; Liu et al. 2003; Ginolhac et al. 2004).

Nonribosomal peptide synthetase gene fragments, about 700 bp in length, retrieved from *Tsukamurella* strain MT6.1 and *Wiliamsia* strain MT8 were sequenced. The analysis of the deduced amino acid sequences by BLAST indicated that these sequences are peptide syn-

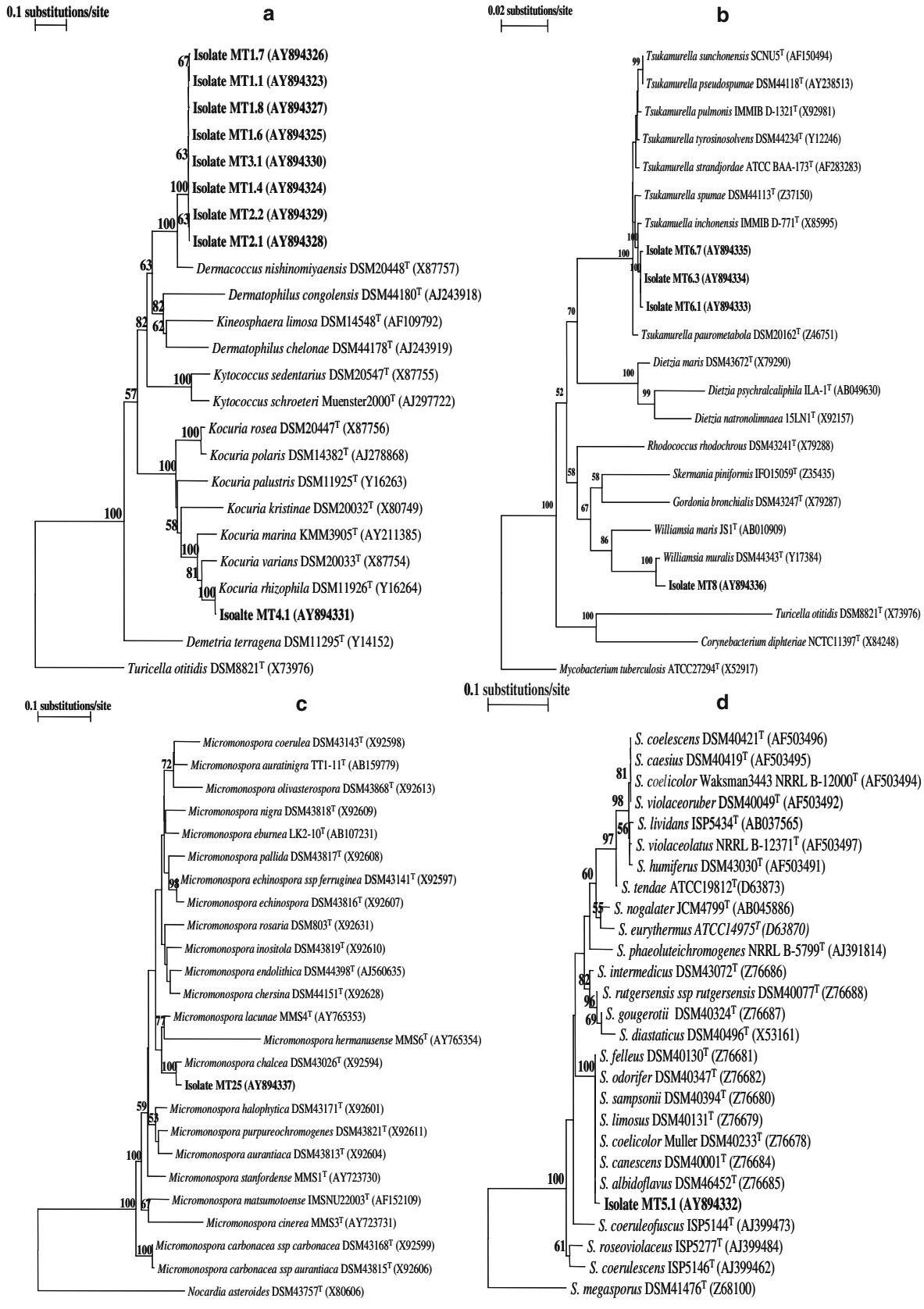

Fig. 2 Neighbour-joining tree (Saitou and Nei 1987) based on almost complete 16S rRNA gene sequences, showing relationships between the Mariana Trench isolates and representatives of the suborder *Micrococcineae* (**a**); suborder *Corynebacterineae* (**b**); family *Micromonosporaceae* (**c**) and the genus *Streptomyces* (**d**). The numbers at the nodes indicate the level of bootstrap support (%) based on a neighbour-joining analysis of 1,000 resampled datasets; only values above 50% are given. The *scale bar* indicates 0.02 or 0.1 substitutions per nucleotide position

thetase products that shared 53% (MT6.1) and 55% (MT8) identity with the NRPS from the actinomycete, *Nocardia farcinica* (Genbank accession NC006361). Phylogenetic analysis indicated that the NRPS products from both Mariana Trench strains formed a distinct cluster together, which were separated from other known NRPS sequences (data not shown), a result suggesting that they represent novel and as yet uncharacterized natural products that merit further investigation. Gene analyses of this type previously have revealed novel catalytic activities (e.g., nitrile hydratase) in bathyal actinomycetes (Brandão and Bull 2003; Bull et al. 2005).

Growth at elevated pressure

The effect of elevated hydrostatic pressure on the growth of the tested strains was determined in a glucose-yeast extract broth. It was particularly interesting that the *Dermacoccus* strain MT1.1 grew well at 40 Mpa, giving a viable count ($3.55 \pm 0.4 \times 10^6$ cfu/ml) that was nearly 60% higher than that of the control culture ($1.46 \pm 0.7 \times 10^6$ cfu/ml) grown at atmospheric pressure. In contrast, the terrestrial type species *Dermacoccus nishinomiyaensis* DSM 20448^T showed a decrease (36%) in viable counts, from $1.27 \pm 0.4 \times 10^6$ cfu/ml at atmospheric pressure to $8.1 \pm 1.3 \times 10^5$ cfu/ml at 40 MPa. Although we have not attempted to explore the presence of piezophilic actinomycetes in the marine environment, the response of the *Dermacoccus* strain MT1.1 to deep-sea pressures suggests that it is part of the indigenous deep-sea microbiota. We have yet to establish whether or not the other actinomycetes isolated during this study may be indigenous to the deep-sea.

Conclusions

The results of the present study provide further evidence that deep-sea sediments are a rich source of taxonomically diverse and indigenous marine actinobacteria (Mincer et al. 2002; Stach et al. 2003a, b; Maldonado et al. 2005). The diversity of actinobacteria in extreme habitats and the presence of gene sequences implicated in secondary metabolite synthesis support the view that this poorly studied habitat is a rich source of novel actinomycetes for bioprospecting.

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