Candididin Biosynthesis Gene Cluster Is Widely Distributed among Streptomyces spp. Isolated from the Sediments and the Neuston Layer of the Trondheim Fjord, Norway

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A large number of Streptomyces bacteria with antifungal activity isolated from samples collected in the Trondheim fjord (Norway) were found to produce polyene compounds. Investigation of polyene-containing extracts revealed that most of the isolates produced the same compound, which had an atomic mass and UV spectrum corresponding to those of candididin D. The morphological diversity of these isolates prompted us to speculate about the involvement of a mobile genetic element in dissemination of the candididin biosynthesis gene cluster (can). Eight candididin-producing isolates were analyzed by performing a 16S rRNA gene-based taxonomic analysis, pulsed-field gel electrophoresis, PCR, and Southern blot hybridization with can-specific probes. These analyses revealed that most of the isolates were related, although they were morphologically diverse, and that all of them contained can genes. The majority of the isolates studied contained large plasmids, and two can-specific probes hybridized to a 250-kb plasmid in one isolate. Incubation of the latter isolate at a high temperature resulted in loss of the can genes and candididin production, while mating of the “cured” strain with a plasmid-containing donor restored candididin production. The latter result suggested that the 250-kb plasmid contains the complete can gene cluster and could be responsible for conjugative transfer of this cluster to other streptomycetes.

Actinomycete bacteria, especially those belonging to the family Streptomycetaceae, are well-known producers of secondary metabolites with diverse biological activities. Representatives of the genus Streptomyces produce a variety of antibiotics with antibacterial, antifungal, and antitumor activities. The majority of antibiotic-producing streptomycetes have been isolated from terrestrial environments, while antibiotic-producing streptomycetes from the marine sources remain largely unexplored. Therefore, studies of streptomycetes from the marine environment are important for unraveling their potential for antibiotic production. In addition, such studies might reveal the means by which antibiotic biosynthesis and resistance genes are spread in nature.

It is widely acknowledged that plasmids play an important role in genetic exchange between bacterial species. Conjugative plasmids are quite common in Streptomyces strains (13), and a number of these mobile genetic elements have been characterized in detail. The characterized mobile genetic elements include both circular plasmids, such as pIJ101 from Streptomyces lividans (14) and SCP2 from Streptomyces coelicolor (2, 35), and linear plasmids, such as SLP2 from S. lividans (6) and SCP1 from S. coelicolor (38, 39). The presence of a linear plasmid in Streptomyces was first reported in 1979, and the plasmid was the 17-kb pSLA2 plasmid of Streptomyces rochei (11). SCP1 of S. coelicolor was discovered in the early 1970s (38, 39), but because of its large size (356 kb), isolation of this plasmid with conventional techniques was not possible and therefore it was not recognized as a linear plasmid until pulsed-field gel electrophoresis (PFGE) was invented. Later, SCP1 was shown to harbor a complete set of genes for biosynthesis of the antibiotic methylenomycin (21); K. F. Chater, C. J. Bruton, S. J. O’Rouke, and A. W. Wietzorrek, 5 July 2001, Patent Cooperation Treaty international application WO/2001/048232), while another linear plasmid, found in S. rochei, has been shown to contain genes for biosynthesis of both lankamycin and lankacidin (16, 19, 28, 36). Other examples of plasmids include pPZG103 carrying oxytetracycline biosynthesis genes acquired from the chromosome of Streptomyces rimosus (10) and pKSL from Streptomyces lasaliiensis, which might be involved in the production of lasalocid and/or echinomycin (17, 20).

Linear plasmids can be transferred between Streptomyces strains by means of conjugation, and SCP1 is an example of a conjugative linear plasmid as it is easily transferred from an SCP1+ strain to an SCP1− strain (39). Interspecific transfer to S. lividans and Streptomyces parvulus has also been reported for this plasmid, and it was demonstrated that the recipient strains had acquired the ability to produce and be resistant to methylenomycin (12, 21). Transfer of intact linear plasmids containing mercury resistance genes from two Streptomyces strains isolated from the marine environment to S. lividans, conferring mercury resistance to the initially mercury-sensitive recipient,
has been reported by Ravel et al. (32). It has also been shown that interspecific transfer of linear plasmids is possible in sterile amended soil microcosms, suggesting that mercury resistance might be spread by plasmid transfer in polluted environments (31).

We report here isolation and screening of several thousand actinobacterial strains from the Trondheim fjord (Norway), which resulted in identification of producers of both known and potentially new polyene macrolides with antifungal activity. The ability to produce the polyene macrolide candidicidin D was found to be widespread among the Trondheim fjord Streptomyces isolates. We also report that the candidicidin biosynthetic genes (can) are present on a linear plasmid identified in one of these isolates, suggesting that the can genes might be spread by means of conjugation.

### MATERIALS AND METHODS

**Strain isolation and handling.** Sediment samples were collected from different sites in the Trondheim fjord (Table 1). Sediments from depths of 1 to 28 m were collected by scuba divers (site 1 [63°34′N, 10°37′E] and site 2 [63°26′N, 10°21′E]), while sediments from a depth of 450 m (site 3 [63°26′N, 10°37′E]) were obtained by a box corer. The upper 5-cm portions of the sediments were collected in ziplock bags (scuba diver) or with a sterile spade (box corer) and transferred to 1-liter sterile plastic containers. Approximately 10% of the container volume was filled with 60% sediment and 40% seawater from the sampling site. This was done in order to ensure aerobic conditions in storage during processing. Samples were processed on the day of sampling or on the day after sampling. Neuston layer samples were obtained from one site (63°58′N, 10°21′E) and site 2 (63°26′N, 10°37′E). Actinomycetes were isolated from the sediment and neuston layer using different types of pretreatment and selective media (details are provided with the MEGA software using 2,000 bootstrap replicates.

**Screening for antifungal activity.** The use of 96-well plates for isolation of actinomycetes and for testing of antifungal metabolite production, as well as the automatic handling of extract plates using a robotic liquid handling system, made it possible to screen a large number of isolates. All technical details related to the antifungal screening procedure are described in the supplemental material. Two fungal strains were used in the screen, strains of Candida albicans and Candida glabrata (Table 2). Including the amphotericin B-resistant organism C. glabrata provided an opportunity to identify potential polyene producers based on differences in inhibition of the two fungal strains. Growth inhibition of the fungal strains was evaluated by measuring the optical density at 660 nm.

**LC-TOF MS analysis of bacterial extracts.** Liquid chromatography (LC)-mass spectrometry (MS) analysis was performed using an Agilent 1100 series LC with diode array detector (DAD) connected to an Agilent 6210 time of flight (TOF) MS. LC was performed using a Zorbax SB C18 column (2.1 by 150 mm) with a flow rate of 0.25 ml/min and the following mobile phases: 10 mM ammonium acetate in water (pH 4.0) and then an acetonitrile gradient from 0 to 2 min, 30% acetonitrile from 2 to 10 min, and 30 to 60% acetonitrile for 20 min. The TOF MS was operated in either negative or positive electron spray ionization mode with a gas temperature of 350°C, drying gas at a rate of 10 liters/min, and a nebulizer pressure of 50 psi. A reference solution for correction of mass axes was continuously injected using a second nebulizer needle. The mass accuracy of the TOF MS is 3 ppm, and a compound database search based on accurate mass determination was used with a range of ±5 ppm.

**16S rRNA gene analysis.** Total DNA of the bacteria was isolated using a DNeasy blood and tissue kit (Qiagen). Primers 5′-AGAGTTTGTATCMTGGCTCAG-3′ and 5′-TACGGYTACCTTGTTACGACTT-3′ (26) were used to amplify 1,490 bp of the 16S rRNA gene. The 50-μl PCR mixture contained total DNA isolated from the different isolates (10 to 20 ng), 1× Expand high-fidelity buffer with MgCl2 (Roche), 400 nM of each primer, 200 μM of each deoxynucleoside triphosphate, and 2.6 U of Expand high-fidelity enzyme mixture (Roche). The reaction was performed using the following conditions: 94°C for 4 min, followed by 35 cycles of 45 s at 94°C, 20 s at 55°C, and 2 min at 66°C and then a final 5-min extension at 70°C. The 50-μl reaction mixture was subjected to gel electrophoresis, and the resulting DNA fragment (about 1,400 bp) was purified and then cloned in the pDrive vector (Qiagen). Purified plasmids were sent to MWG Biotech for sequencing using the pDrive-specific primers M13 forward (5′-GTAAAACGACGGCCAGT-3′) and M13 reverse (5′-AAGCTATGACCATG-3′) described in the Qiagen PCR cloning handbook and primer 1100R (5′-AGGTGTCGCTGTTG-3′). Sequences were aligned with each other and with the most homologous actinomycete 16S rRNA gene sequences from the GenBank database using the Molecular Evolutionary Genes Analysis (MEGA) software, version 4.0.2 (37). As not all sequences retrieved from the GenBank database were the same length, the ends of the sequences were trimmed, leaving 1,476 bp as the basis for construction of a phylogenetic tree. The tree was constructed by the neighbor-joining method provided with the MEGA software using 2,000 bootstrap replicates.

### TABLE 1. Sample description

<table>
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<tr>
<th>Sample no.</th>
<th>Sampling location</th>
<th>Sample characteristics</th>
<th>No. of actinomycetes isolated</th>
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<tr>
<td>1</td>
<td>63°34′N, 10°37′E</td>
<td>1-m-deep sediment</td>
<td>179</td>
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<td>2</td>
<td>63°34′N, 10°37′E</td>
<td>7.5-m-deep sediment</td>
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<td>63°26′N, 10°21′E</td>
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<td>353</td>
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<td>6-m-deep sediment</td>
<td>1,213</td>
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<td>63°26′N, 10°21′E</td>
<td>27-m-deep sediment</td>
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<td>6</td>
<td>63°29′N, 10°18′E</td>
<td>450-m-deep sediment</td>
<td>696</td>
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<td>7</td>
<td>63°58′N, 10°81′E</td>
<td>Neuston layer</td>
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<td>Total</td>
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### TABLE 2. Bacterial strains used in this study

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<th>Phenotypea</th>
<th>Source or reference</th>
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<td>Candida albicans</td>
<td>5FC, FLC, ITRA, AMB</td>
<td>CCUG (strain 39343)</td>
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<tr>
<td>Candida glabrata</td>
<td>FLC, ITRA, AMB, 5FC</td>
<td>CCUG (strain 39342)</td>
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<td>Streptomyces sp.</td>
<td>Candidin producer</td>
<td>This study</td>
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<td>Streptomyces sp.</td>
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<td>Streptomyces sp.</td>
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a 5FC, fluocytosine; FLC, fluc(parser, ITRA, itraconazole; AMB, amphotericin B; Am, apramycin; Rif, rifampin. b CCUG, Culture Collection, Göteborg University.
Southern blot analyses. Enzymatic manipulations and agarose gel electrophoresis were performed as described by Sambrook and Russell (34). Total DNA was isolated with a DNeasy blood and tissue kit (Qiagen). DNA fragments were isolated from agarose gels using the QIAEX II suspension (Qiagen). PCR primers were designed for the candidicidin gene cluster of Streptomyces griseus IMRU 3570 (4) [primers: pabAB1 [5'-GTCGACAGACCGCTCTGAC-3'], pabAB2 [5'-TGGTCCCTCGGCCGACGTTG-3'], canRA1 [5'-ACCGGCTGGCGGAGCACATAC-3'], canRA2 [5'-GGCCCGCTTCTGGAGCTTCC-3'], canP3_1 [5'-CGCCAGACCGGACATCAT-3'], and canP3_2 [5'-GACCGCAGCAGCGTGAATC-3'] and were purchased from MWG Biotech.

RESULTS AND DISCUSSION

Isolation and antifungal activity screening of actinomyctones from the Trondheim fjord reveal a large number of polyene producers. Actinomycete bacteria were isolated from marine sediments collected at different locations and different depths (range, 1 m to 450 m [Table 1 shows characteristics of the samples]), as well as from the neuston layer in the Trondheim fjord (Norway) using different selective media (see Materials and Methods and the supplemental material). A total of 3,708 isolates obtained from the samples were cultivated on different solid production media in 96-well plates, and extracts were screened for antifungal activity against C. albicans and/or C. glabrata. This screen revealed 1,044 isolates with antifungal activity against one or both of the test organisms, and extracts of these isolates were characterized by UV/visible spectrophotometry in order to identify polyene macrolide-like spectra. Polyene macrolide compounds have a characteristic three-peak UV absorbance spectrum, and the wavelengths of the peak maxima are determined by the number of conjugated double bonds (29). The UV absorbance spectrum can therefore be used to identify potential polyene macrolide-producing isolates. The UV/visible scans indicated that there was polyene isolation in 655 of the 1,044 isolates. The isolates capable of producing a putative heptaene compound(s) with UV absorption maxima of 366, 388, and 412 nm clearly dominated the group of polyene producers, accounting for more than 70% of the polyene-like spectra. Extracts from 62 polyene-producing actinomycetes, representing the isolates from different sources in the Trondheim fjord, were analyzed using LC-DAD-TOF MS. DAD isolops of three of the extracts are shown in Fig. 1. The isoflot in Fig. 1A represents the putative heptaene compound(s) with UV absorption maxima of 366, 388, and 412 nm and shows three putative heptaene compounds eluting at 11.5 to 14.0 min. These three compounds have almost identical UV spectra, and only the profile of the second compound is shown in the inset in Fig. 1A. Accurate masses of these three compounds were determined by TOF MS using negative electron spray ionization, and the molecular ions are indicated in Fig. 1A. A search of the Dictionary of Natural Products database (http://dnp.chemnetbase.com/) with the corresponding accurate mass [M-H-] 1107.5630, corresponding to an accurate molecular mass of 1,108,5703 Da] using a range of ±5 ppm resulted in a match with candidicidin D (Fig. 1D), a known heptaene macrolide with antifungal activity produced by S. griseus (27). The first peak in Fig. 1A with molecular ion 1109.5803 may correspond to a candidicidin derivative with an hydroxyl group at the C-3 position instead of a keto group. The last peak in the chromatogram (accurate molecular mass, 1,091.5668 Da) most probably corresponds to a candidicidin analogue with one less oxygen due to the absence of an hydroxyl group at C-9 (7). The accurate mass determination coupled with the heptaene-like UV spectrum strongly suggested that the compounds identified were indeed candidicidin deriva-
Of the 62 extracts analyzed by LC-DAD-TOF MS, 52 produced this UV and mass chromatography profile. The DAD plot in Fig. 1B shows the results for a putative pentaene, as the UV absorption maxima are between 320 and 350 nm (29). TOF MS analysis of the extract showed that the accurate molecular mass of this possible pentane compound is 925.5035 Da. The Dictionary of Natural Products database search resulted in only one hit, nystatin A1, which is a tetraene and not a pentaene. The compound in question might therefore be an unknown pentaene with the same accurate molecular mass as nystatin A1. The DAD plot in Fig. 1C shows the results for a putative pentaene compound, and TOF MS analysis detected accurate molecular masses of 911.6168, 947.5951, and 1025.6118 Da associated with this compound. None of...
these molecular masses had any hits in the Dictionary of Natural Products database search using a range of ±5 ppm. In conclusion, the extracts described in Fig. 1B and 1C probably contained potentially new pentaene macrolides.

A group of streptomycetes isolated from the Trondheim fjord harbors the candicidin biosynthesis gene cluster. The morphological diversity of the actinomycetes isolated from different locations in the Trondheim fjord and found to synthesize candicidin prompted us to investigate the molecular taxonomy of a limited number of these isolates using 16S rRNA gene analysis. PCR amplification, cloning, and sequencing of the 16S rRNA gene fragments from eight candicidin producers (Streptomyces sp. strains MP47-06, MP47-91, MP18-04, MP15-36, MPS08-73, MPS08-39, MPS05-43, and MPS05-34) exhibiting different morphological characteristics were performed. The known candidin producer S. griseus IMRU 3570 (4) was also included in the analysis. The 16S rRNA gene sequence analysis demonstrated that seven of the eight isolates and S. griseus IMRU 3570 shared 16S rRNA gene sequence identities of 99.4 to 100%, corresponding to 0 to 9 nucleotide differences at 1,490 locations. Only one strain (MPS05-43) differed significantly from the other strains, and the levels of 16S rRNA gene sequence identity of this strain with the other seven isolates ranged from 95.4 to 95.6%. A phylogenetic tree was constructed for all the sequences and the most homologous isolate, S. griseus IMRU 3570, was confirmed to be 99% identical to the corresponding S. griseus IMRU 3570 sequences. Next, total DNA was isolated from the other six candidin producers (Streptomyces sp. strains MP47-06 and MP47-91) of the eight candidin-producing strains were used as templates in this experiment. PCRs resulted in successful amplification of the expected fragments with both templates, confirming that at least two of the isolates chosen contain the candidin biosynthesis gene cluster. The PCR fragments of pabAB and canP3 from Streptomyces sp. strain MP47-91 were sequenced, and the sequences were confirmed to be 99% identical to the corresponding S. griseus IMRU 3570 sequences. A series of Southern blot analyses of BamHI-digested total DNAs with putative producer of a different polyene macrolide (Streptomyces sp. strain MPS05-73), one putative producer of a different polyene macrolide (Streptomyces sp. strain MPS07-67), and S. griseus IMRU 3570. A series of Southern blot analyses of BamHI-digested total DNAs with two different can probes (pabAB and canP3) clearly showed that all of the candidin-producing strains tested harbor the candidin biosynthesis gene cluster (Fig. 2A and B).

Seven of the candidin producers (Streptomyces sp. strains MP47-06, MP47-91, MP18-04, MP15-36, MPS08-73, MPS08-39, and MPS05-34) displayed a hybridization pattern with pabAB resembling that of S. griseus, which indicated that the pabAB-containing region is highly conserved among these isolates as the restriction fragment size is the same. However, one isolate, Streptomyces sp. strain MPS05-43, displayed a totally different hybridization pattern (Fig. 2, lanes 9 and 10), suggesting that the organization of the pabAB-containing region in this strain is different. Interestingly, this strain was also the strain shown to be the strain most phylogenetically distinct from all the other strains and from S. griseus IMRU 3570. DNAs from the non-candidin-producing strain (Streptomyces sp. MPS07-63) and from the putative producer of a different
polyene macrolide (Streptomyces sp. strain MPS07-67) did not show any hybridization with the pabAB probe, confirming that this analysis was specific enough for the can cluster. The canP3 probe was found to be less specific, which is not surprising considering that streptomycetes often have more than one polyketide synthase cluster and that polyketide synthase genes in general share at least some homology. Hybridization of the canP3 probe to the total DNA of the putative producer of a different polyene macrolide (Streptomyces sp. strain MPS07-67) proves this point.

Candidin biosynthesis gene cluster is located on a linear plasmid in one of the isolates. So far, the ability to produce candidin has been described for S. griseus (27), Streptomyces acrimum (1), Streptomyces lividans (23), Streptomyces sp. strain FR-008 (7), S. coelicolor JI1259 (8), S. coelicolor JI1157 (8), S. griseus JI2212 (8), and Streptomyces albus G (8), which seem to have originated from different sources. The candidin producers identified in this study, however, all originated from the Trondheim fjord sediments and neuston layer. The eight strains selected for further study differed morphologically, but sequencing of the 16S rRNA genes showed that only one strain (MPS05-43) was phylogenetically distinct from all the other strains. It is therefore possible that the other seven strains are closely related and that the can cluster might either have originated from a common ancestor or have been transferred among the strains by a mobile genetic element.

Considering the size of the can gene cluster (ca. 140 kb), a linear plasmid seemed to be a plausible candidate for such a mobile element. To investigate the presence of linear plasmids in the eight isolates that harbor the can gene cluster, their total DNAs were subjected to PFGE. Also, S. griseus IMRU 3570, the non-candidin-producing strain (Streptomyces sp. strain MPS07-63), and the putative producer of a different polyene macrolide (Streptomyces sp. strain MPS07-67) were included in the analysis. PFGE revealed the presence of presumably linear plasmids ranging in size from ca. 50 kb to 820 kb in six of the candidin-producing strains (Fig. 3A). No linear plasmids were detected in S. griseus IMRU 3570 and isolates MP18-04, MPS08-73, MPS07-63, and MPS07-67. The gel from the PFGE was used for a Southern blot analysis with the pabAB and canP3 probes. Both probes hybridized to the same ca. 250-kb canP3 plasmid present in Streptomyces sp. strain MP15-36 (Fig. 3B and C). In addition, the probes hybridized to the chromosomal DNA of the other seven candidin producers, as well as to that of S. griseus. Integration into chromosomal DNA has been reported for at least two linear plasmids. SCPI is able to integrate into the chromosome of S. coelicolor (18), and pPZG101 can integrate into the chromosome of S. rimosus (10). It is therefore possible that the can plasmid might be integrated into the chromosomes of the candidin-producing strains that did not harbor this plasmid or that these strains are strains with chromosomal can genes.

Mating experiments with S. lividans and the non-candidin-producing Trondheim fjord isolate failed to demonstrate can plasmid transfer. To test the can plasmid’s interspecific transfer ability, a mating experiment was conducted. S. lividans TK64 carrying a streptomycin resistance marker has been used successfully in the past for mating experiments. The can plasmid-containing strain (Streptomyces sp. strain MP15-36) was tested for sensitivity to streptomycin and apramycin. As MP15-36 was resistant to streptomycin but not apramycin, S. lividans TK64 could not be used. Instead, S. lividans TK64(pSET152) was used as the recipient in the mating experiments (see Materials and Methods). As it was not possible to select the transconjugants directly, the plates were replicated on CP-6–apramycin plates on a layer of Streptomyces sp. strain FR-008 (7), Streptomyces sp. strain MP18-04; lane 5, Streptomyces sp. strain MP47-91; lane 6, Streptomyces sp. strain MP18-04; lane 7, Streptomyces sp. strain MP15-36; lane 8, Streptomyces sp. strain MPS08-73; lane 9, Streptomyces sp. strain MPS08-39; lanes 10 and 11, Streptomyces sp. strain MPS05-43; lane 12, Streptomyces sp. strain MPS07-63; lane 13, Streptomyces sp. strain MPS07-67; lane 14, Streptomyces sp. strain MPS05-34.

FIG. 3. (A) PFGE. (B) Southern hybridization with the pabAB probe, and (C) Southern hybridization with the canP3 probe for candidin-producing strains. Lane 1, MidRange II PFG marker; lanes 2 and 15, yeast chromosome PFG marker (markers are not shown for Southern hybridization); lane 3, S. griseus; lane 4, Streptomyces sp. strain MP47-06; lane 5, Streptomyces sp. strain MP47-91; lane 6, Streptomyces sp. strain MP18-04; lane 7, Streptomyces sp. strain MP15-36; lane 8, Streptomyces sp. strain MPS08-73; lane 9, Streptomyces sp. strain MPS08-39; lanes 10 and 11, Streptomyces sp. strain MPS05-43; lane 12, Streptomyces sp. strain MPS07-63; lane 13, Streptomyces sp. strain MPS07-67; lane 14, Streptomyces sp. strain MPS05-34.
a Southern blot analysis. DMSO extracts of these 10 candidates were also prepared to assess whether they were able to produce candicidin. UV/visible scans of the extracts did not show any trace of candicidin, and the pabAB probe did not hybridize to the total DNA from any of the candidates (data not shown). The mating experiments were repeated using ISP2 and SFM, but no positive results were obtained.

Next, we decided to try mating with one of the can-negative Trondheim fjord isolates (Streptomyces sp. strain MPS07-63), assuming that the “native” strain might be able to accept and stabilize the plasmid. The strain chosen was the strain that was negative with both the pabAB and canP3 probes in the Southern blot analysis described above. Also, the strain did not carry any linear plasmids, which was confirmed by PFGE, and comparison of 16S rRNA gene sequences from MP15-36 and MPS07-63 showed that these two strains are closely related, as the sequences were identical. To have a means of selecting for transconjugants after mating, rifampin-resistant mutants were obtained (see Materials and Methods). Two of the mutants were chosen for mating with the can plasmid strain on both ISP2 and SFM agar. After selection for the Rifr strain, 100 random colonies were screened by extraction with DMSO, followed by UV/visible scanning. Two putative transconjugants were chosen for Southern blot analyses with the pabAB and canP3 probes as the UV/visible scans indicated that a heptaene was present in the extracts, but again the results were negative (data not shown).

There are several possible explanations for why these mating experiments were unsuccessful. Assuming that the can plasmid is responsible for the abundance of candicidin producers in our strain collection, one would expect the transfer frequency of the plasmid to be quite high. However, the transfer frequency might be much lower than anticipated, and without a selectable marker, detecting the transconjugants is quite difficult. Also, for the Rifr strain the initial screening of putative transconjugants was dependent on actual production of candicidin (see Materials and Methods). It is possible that the plasmid might transfer at a higher rate but the transconjugants are not able to produce candicidin and are therefore not recognized. Also, we cannot exclude the possibility that the plasmid is not stable in the recipient hosts chosen and that it is therefore easily lost. can plasmid can be reintroduced into the “cured” strain, restoring candicidin production. In order to demonstrate transfer of the can plasmid, a “curing” experiment with the can plasmid strain was performed in order to obtain a suitable recipient for mating. Loss of linear plasmids as a result of growth at a higher temperature has been demonstrated for Streptomyces (30). A “curing” experiment with Streptomyces sp. strain MP15-36 was carried out (see Materials and Methods) by incubating the strain at 37°C. Candidates that did not produce candicidin were checked for loss of the plasmid by a Southern blot analysis using the pabAB probe, and one putative plasmid-free candidate was obtained. A Rifr mutant of the potential plasmid-free strain was obtained for easier selection of transconjugants (see Materials and Methods), and mating between the original plasmid-containing strain and the Rifr plasmid-free strain was performed on SFM agar. Twelve rifampin-resistant colonies were selected, and a Southern blot analysis of their total DNAs was performed using the pabAB probe. This probe hybridized to the DNAs from all candidates (data not shown), and three of them were subsequently tested for candicidin production by LS-TOF MS. A molecule with the mass of candicidin was shown to be present in the extracts of all three candidates tested, indicating that the plasmid was successfully transferred to the plasmid-free strain at a high frequency, restoring the ability to produce candicidin.

Conclusion. In this study we investigated the antibiotic production potential of 3,708 isolates derived from marine sediment and neuston layer samples. We showed that many of these isolates produce compounds with antifungal activity and that a high proportion of Streptomyces bacteria isolated from different sources in the Trondheim fjord produce the antifungal polyene macrolide candicidin. Genes involved in the biosynthesis of candicidin were shown to be present on a linear plasmid in one of the Streptomyces strains isolated from the Trondheim fjord sediment. This strain could be cured of the plasmid, resulting in loss of candicidin production, while reintroduction of the plasmid by means of conjugation restored production. Although the can plasmid could not be transferred to other Streptomyces strains in the laboratory, it is possible that this plasmid is responsible for spreading the candicidin biosynthesis gene cluster to other strains in its natural environment.

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