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Bioactivity of chitinolytic actinomycetes of marine origin

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Summary. Of 116 chitinolytic actinomycetes, previously isolated from marine sediments, 85 were found to possess antimicrobial activity. A high correlation was noted between chitinolysis and bioactivity. A majority of them suppressed the growth of Gram-positive bacteria but only a few inhibited Gram-negative strains and almost one-half of them exhibited antimycotic activity. Several of the last group yielded polyenes of which heptaenes were the most commonly occurring ones. Other strains synthesized detectable amounts of β-lactam antibiotics. It is suggested that chitinolysis may serve as an indicator of potential bioactivity among marine actinomycetes.

Introduction

Actinomycetes have long been recognized as prime sources of antibiotics, enzymes and of other important metabolites. For example, over 4000 of the naturally occurring antibiotics discovered are synthesized by this group of microorganisms (Okami and Hotta 1988). The search for novel antimicrobial substances has, in recent years, included actinomycetes obtained from sources other than soils. Included among these have been actinomycetes isolated from marine environments (Okami 1986).

A variety of pretreatments and selective media are available to enhance the isolation of specific groups of actinomycetes (Cross 1982). The application of pretreatments and selective media to microbial populations found in marine sediments has led to the successful isolation of bioactive actinomycetes. For example, heat, phenol, the use of membrane filters and plating on chitin agar has contributed to the recovery of antibiotic-producing strains from marine sediments (Pisano et al. 1986).

With respect to chitin agar, the selective nature of this substrate is attributed to a widespread ability of actinomycetes to hydrolyze chitin (Hsu and Lockwood 1975). Based on this observation, we recently surveyed our collection of marine actinomycetes for chitinolytic activity and found that many of the strains share this property. Consequently, part of our collection has been divided into an easily definable cluster based on a common secretory property, namely the elaboration of chitinases.

In the present investigation, we have attempted to ascertain if chitinolytic activity may be related to the synthesis of antimicrobial substances. Toward this end, 116 chitinolytic strains were grown in submerged culture and analyzed for the production of antibacterial and/or antifungal compounds.

Materials and methods

Microorganisms. The actinomycetes used in this investigation have been isolated from marine sediments and deposited in the St. John's University culture collection.

Chitinolytic activity. Cultures were transferred to chitin agar (Hsu and Lockwood 1975) and incubated at 28° C for up to 4 weeks. Plates were examined at 48-h intervals for evidence of chitinolysis expressed as clear zones around individual colonies. Two separate trials were made to confirm the activity.

Cell wall analysis. Analyses of whole-cell hydrolysates of the actinomycetes were made for the determination of cell-wall chemotypes (Becker et al. 1964).

Fermentations. Each actinomycete selected was grown in test tubes (150 × 25 mm) containing 12 ml of two fermentation media. One was based on the seed medium used by Tunac et al. (1985) except that Proflo (Traders Protein, Ft. Worth, Tex., USA) was substituted for cotton seed meal. The other was the PS medium reported by Okazaki and Okami (1972), which contains 3% starch, 2% corn steep liquor, 1.5% Pharmamedia (Traders Protein) and 1% beef extract. The fermentation tubes were inoculated with one loopful of the desired actinomycete and incubated for 72–96 h on a rotary shaker (Psycrotherm, New Brunswick Scientific, Edison, NJ, USA) operated at 250 rpm at 28° C. The contents of each tube were then centrifuged at 2000 *g* for 20 min and both the cleared fermentation broths and mycelial pellets collected.

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Determination of bioactivity. Fermentation broths were examined for antimicrobial activity against Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi. The bacteria used were *Bacillus subtilis, Escherichia coli, Mycobacterium smegmatis, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The yeasts tested were *Candida albicans* and *C. krusei.* The filamentous fungi included *Aspergillus niger* ATCC 12846, *Fusarium moniliforme* ATCC 10052 and *Trichoderma viride* ATCC 8678. The preparation of fungal assay plates was described in an earlier report (Pisano et al. 1987). All test microorganisms were grown on glucose-peptone agar (Difco, Detroit, Mich., USA) to which paper discs (12.5 mm, Schleicher and Schuell, Keene, NH, USA), charged with 0.1 ml of a specific fermentation broth, were added. The plates were incubated at 37° C for the detection of antibacterial and at 28° C of antifungal activity. Growth inhibition of the test microorganisms was assessed daily for up to 72 h.

Nature of antimicrobial activity. The nature of the antimycotic activity exhibited by some of the actinomycetes was determined spectrophotometrically (Pisano et al. 1987). Both the fermentation broths and mycelial pellets were examined. β-Lactam antibiotics were detected by the induction of β-lactamase activity in *Bacillus licheniformis* (Sykes and Scott 1985) using the chromogenic substrate 2-azo-*p*-dimethylaniline cephalosporin (PADAC) according to Jones et al. (1982).

Results and discussion

Of the 116 marine actinomycetes capable of degrading chitin, 85 (73%) were bioactive. The activity was primarily directed against Gram-positive bacteria, which were inhibited by 68% of the actinomycetes (Fig. 1). In contrast, only 11% were active against Gram-negative strains. Figure 1 also reveals that 47% of the actinomycetes exhibited antimycotic activity. Both yeasts and filamentous fungi were also inhibited. Antibacterial and antifungal activity was produced by 37% of the actinomycetes. Analysis of the antimicrobial spectrum provided specific information on the inhibitory patterns obtained. *B. subtilis,* for example, was inhibited by 58% of the producer strains (Fig. 2). Similarly, another Gram-positive test organisms, *S. aureus,* was also susceptible to inhibition by a large proportion (50%) of the

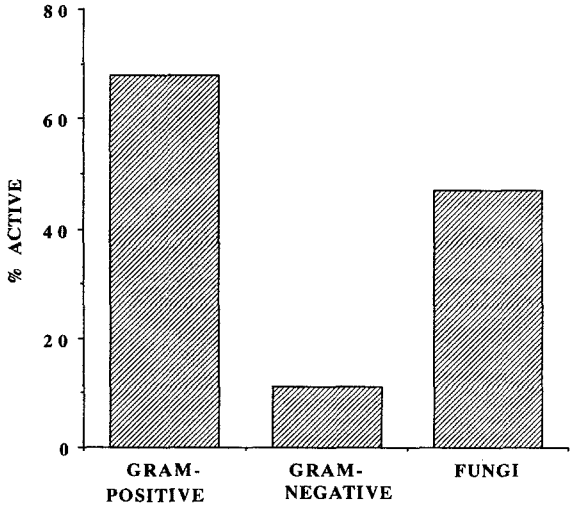

Fig. 1. Groups of microorganisms inhibited by bioactive chitinolytic marine actinomycetes

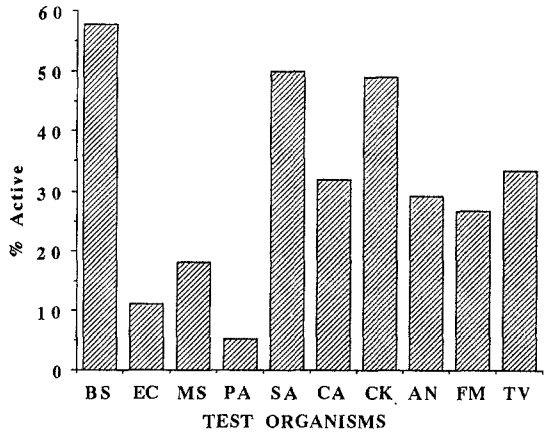

Fig. 2. Bacterial and fungal species inhibited by bioactive chitinolytic marine actinomycetes. *Abbreviations* refer to test organisms listed in Materials and methods

Table 1. Nature of inhibitory substances produced by some strains of actinomycetes isolated from marine sediments

Active component	Strain number
Antifungal	
Heptaenes	124, 427, 441, 899, 942, 584, 1234, 1318, 1892
Hexaenes	869, 1894, 1904, 1908
Methylpentaenes	940
Pentaenes	1112
Antibacterial	
β-Lactams	587B, 619, 1049, 1050

actinomycetes. *M. smegmatis,* the only acid-fast bacterium utilized, was inhibited by 18% of the bioactive actinomycetes, *E. coli* was inhibited by 11%, whereas *P. aeruginosa* was suppressed by only 5%. Among the fungi, *C. krusei* was susceptible to the action of 49% of the actinomycetes, whereas *C. albicans* was inhibited by 32%. With respect to the filamentous fungi, *T. viride* was slightly more prone to inhibition than either *A. niger* and *F. moniliforme.*

The nature of the antimicrobial substances produced by selected isolates was also determined. Of 53 of those that exhibited antifungal activity, 15 were identified as producing polyene antibiotics (Table 1). Heptaenes were produced by nine strains and hexaenes by four strains. A methylpentaene and a pentaene were each synthesized by only one actinomycete. In a previous investigation, heptaenes were also found to be the major polyene synthesized by marine actinomycetes (Pisano et al. 1987), but in a later report hexaenes predominated (Pisano et al. 1989). The only antibacterial activity identified was attributable to the production of β-lactams by four of the marine actinomycetes (Table 1). The amounts detected, as determined by the use of PADAC, appeared to be minimal. Consequently, activity was not always evident in repeat trials. This is in agreement with the observation that secondary metabolites are usually produced in minor amounts by wild-type strains (Hütter 1982). Although screening for β-lactam

antibiotics in nature has been successful (Sykes and Wells 1984), no reports are available concerning the production of this class of antibiotics by actinomycetes isolated from marine sediments.

The importance of the composition of the medium employed for the production of antibiotic activity by marine microorganisms was demonstrated previously (Okazaki and Okami 1972). In the present investigation, the nature of the fermentation medium directly influenced the elaboration of antimicrobial substances. This was demonstrated by the fact that 60% of the actinomycetes produced active broths when grown in the medium of Tunac et al. (1985) as compared to 40% in the PS medium of Okazaki and Okami (1972).

The determination of cell wall chemotypes revealed that only 16% of the 116 chitinolytic actinomycetes provided cell extracts that contained *meso*-2,6-diaminopimelic acid (DAP) whereas the remainder yielded cell extracts containing LL-DAP. This proportion of *meso*-DAP- to LL-DAP-containing strains is significantly lower than that obtained in an earlier investigation in which rifampicin was employed for the isolation of actinomycetes from marine sediments (Pisano et al. 1989). The proportion of *meso*-DAP-containing actinomycetes rose with the rifampicin concentrations utilized. In the present report, 12 of the 18 (67%) actinomycetes displaying the *meso*-DAP chemotype were bioactive. In comparison, 73 of 98 (75%) strains containing LL-DAP were active.

These observations suggest that a good correlation exists between chitinolytic activity and the production of antimicrobial substances by marine actinomycetes. Based on these results it is tempting to suggest that chitinolysis might be employed as an indicator of potential bioactivity in this group of microorganisms. Tests for chitin-degrading activity are both inexpensive and easy to perform. In cases where actinomycetes are collected from marine environments, initial isolations might be effected through the use of chitin agar. In the present investigation, pre-selection of chitinolytic strains segregated a group of marine actinomycetes in which approximately 75% were bioactive. This proportion of active strains significantly exceeds the proportions obtained in previous studies in which chitinolysis was not used a selective criterion. In those investigations the percentages of bioactive strains obtained were 16% (Pisano et al. 1986); 13% (Pisano et al. 1987); and 46% (Pisano et al. 1989).

References

Becker B, Lechevalier MP, Gordon RE, Lechevalier HA (1964) Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. Appl Microbiol 12:421–423

Cross T (1982) Actinomycetes: a continuing source of new metabolites. Dev Ind Microbiol 23:1–18

Hsu SC, Lockwood JL (1975) Powdered chitin agar as a selective medium for the enumeration of actinomycetes in water and soil. Appl Microbiol 29:422–426

Hütter R (1982) Design of culture media capable of provoking wide gene expression. In: Bu'Lock JD, Nisbet LJ, Winstanley DJ (eds) Bioactive microbial products: search and discovery. Academic Press, New York, pp 37–50

Jones RN, Wilson HW, Novick WJ Jr (1982) In vitro evaluation of pyridine-2-azo-*p*-dimethylaniline cephalosporin, a new diagnostic chromogenic reagent, and comparison with nitrocefin, cephacetrile, and other beta-lactam compounds. J Clin Microbiol 15:677–683

Okami Y (1986) Marine microorganisms as a source of bioactive agents. Microb Ecol 12:65–78

Okami Y, Hotta K (1988) Search and discovery of new antibiotics. In: Goodfellow M, Williams ST, Modarski M (eds) Actinomycetes in biotechnology. Academic Press, New York, pp 33–67

Okazaki T, Okami Y (1972) Studies on marine microorganisms. II. Actinomycetes in Sagami Bay and their antibiotic substances. J Antibiot 25:461–466

Pisano MA, Sommer MJ, Lopez MM (1986) Application of pretreatments for the isolation of bioactive actinomycetes from marine sediments. Appl Microbiol Biotechnol 25:285–288

Pisano MA, Sommer MJ, Brett BP (1987) Hudson River sediments as a source of actinomycetes exhibiting antifungal activity. Appl Microbiol Biotechnol 27:214–217

Pisano MA, Sommer MJ, Brancaccio L (1989) Isolation of bioactive actinomycetes from marine sediments using rifampicin. Appl Microbiol Biotechnol 31:609–612

Sykes RB, Wells JS (1985) Screening for β-lactam antibiotics in nature. J Antibiot 38:119–121

Tunac JB, Graham BD, Dobson WE, Lenzini MD (1985) Fermentation by a new daunomycin-producing organism, *Streptomyces insignis* ATCC 31913. Appl Environ Microbiol 49:265–268