Microbiological Research **160** (2005) 119—126

Available online at www.sciencedirect.com

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Microbiological Research

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Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus*

P. Sujatha*, K.V.V.S.N. Bapi Raju, T. Ramana

Department of Biotechnology, Andhra University, Visakhapatnam 530003, India

Accepted 19 October 2004

KEYWORDS
Streptomyces;
Morphology;
Antibiotic against MRSA;
Nutritional requirements;
Cultural conditions;
Optimized media

Summary

A new actinomycete strain designated as BT-408 producing polyketide antibiotic SBR-22 and showing antibacterial activity against methicillin resistant *Staphylococcus aureus* has been characterized and found to be a novel strain of *Streptomyces psammoticus*. Nutritional and cultural conditions for the production of antibiotic by this organism under shake-flask conditions have been optimized. Glucose and ammonium nitrate were found to be best carbon and nitrogen sources respectively for growth and antibiotic production. Similarly initial medium pH of 7.2, incubation temperature of 30 °C and incubation time of 96 h were found to be optimal. Optimization of medium and cultural conditions resulted in 1.82-fold increase in antibiotic yield.

Introduction

Search for new antibiotics effective against multi-drug resistant pathogenic bacteria is presently an important area of antibiotic research. Natural products having novel structures have been observed to possess useful biological activities. Filamentous soil bacteria belonging to the genus *Streptomyces* are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics (Williams et al., 1983). Indeed, different *Streptomyces* species produce about 75% of commercially and medically useful antibiotics (Miyadoh, 1993). In the course of screening for new antibiotics, several studies are oriented towards isolation of new *Streptomyces* species from different habitats.

Several methods have been developed to identify *Streptomyces* species. These include culturing methods using the selective plating technique (Kuster and Williams, 1964), construction of genetic

*Corresponding author.
E-mail address: sujatha_peela@rediffmail.com (P. Sujatha).

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doi:10.1016/j.micres.2004.10.006

marker systems (Wipat et al., 1991), a combination of chemical markers, and the presence of LL-diaminopimelic acid and the absence of characteristic sugars in the cell wall (Lechevalier and Lechevalier, 1970b). Antibiotics are synthesized by pathways, which are often connected and influenced by primary metabolism; the intermediate metabolites from primary metabolism serve as precursors for biosynthesis of the antibiotics. In fact, the composition of the culture medium, closely connected with the metabolic capacities of the producing organism, greatly influences the biosynthesis of antibiotics (Fisher and Sonnenshein, 1991; Vilches et al., 1990). In this paper, the taxonomic characterization of the strain BT-408 and optimization of medium and cultural conditions for maximum production of antibiotic are reported.

Materials and methods

Isolation and maintenance

The strain BT-408 has been derived from the sediments of Bay of Bengal Ocean in India. It was isolated on Starch casein agar medium while incubating at 28 °C (Ellaiah et al., 2004). Plates containing the culture were stored at 4 °C. For long storage, it was grown in starch — casein broth for 7 days. To it glycerol was added to a final concentration of 15% (v/v) and stored at –20 °C (Maniatis et al., 1989).

Morphological characteristics

The micro morphology of strain BT-408 was observed by light microscopy and scanning electron microscopy (SEM). The samples used for SEM (JSM 5600, JEOL Ltd.) have been cultured for 14 days on agar media and then fixed overnight with osmium tetroxide vapors, freeze-dried and sputtering-coated with gold palladium.

Chemotaxonomic analysis

Cells used for chemotaxonomic analysis were obtained after incubation at 28 °C for 3 days in yeast extract-glucose broth (pH 7.0) containing 10 g/l of yeast extract and 10 g/l of glucose. Isomers of diaminopimelic acid in the whole-cell hydrolysates were determined by thin-layer chromatography according to the method of Hasegawa et al. (1983). Whole-cell sugars were analyzed according to the method of Becker et al. (1965).

Physiological, biochemical and cultural characteristics

Media used were those recommended by Shirling and Gottlieb (1966) in the International Streptomyces Project (ISP) and by Waksman (1961). Mycelium was observed after incubation at 28 °C for 2 weeks. Colors were determined according to Prauser (1964). Carbohydrate utilization was determined by growth on carbon utilization medium (ISP 9) (Pridham and Gottlieb, 1948) supplemented with 1% carbon sources at 28 °C. Temperature range for growth was determined on inorganic salts-starch agar medium (ISP 4) using a temperature gradient incubator. Hydrolysis of starch and milk were evaluated by using the media of Gordon et al. (1974). Reduction of nitrate and production of melanoid pigment were determined by the method of ISP (Shirling and Gottlieb, 1966). Liquefaction of gelatin was evaluated by the method of Waksman (1961). All cultural characteristics were recorded after 14 days.

Antimicrobial activity of the strain was determined by standard cup plate method using Gram (+) and (−) bacteria, fungi and yeast including the pathogenic methicillin resistant *Staphylococcus aureus* (MRSA) as test organisms. Assay plates were prepared by inoculating 20 ml of Mueller–Hinton agar medium with test organism. Agar-cups (6 mm diameter) were filled with 50 μl of mycelia-free culture filtrate in triplicate and the plates were incubated at 37 °C for 24 h. Inhibition zone diameter was measured.

Optimization of nutritional and cultural conditions

To determine the optimal nutritional and cultural conditions for growth and antibiotic production, Pridham and Gottlieb's inorganic salts medium (Pridham and Gottlieb, 1948) was used as the base. It was supplemented with different carbon and nitrogen sources to study their effect on growth and antibiotic production. The effect of different concentrations of K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ in the medium was also investigated. The medium (50 ml in 250 ml Erlenmeyer flask) was inoculated with 1.5 ml of homogenous spore suspension (0.2 O.D.) in 0.05% Tween 20 solution and incubated at 28 °C on a rotary shaker (220 rpm) for 4 days. The effect