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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Hemolytic activity of *Streptomyces* VITSDK1 spp. isolated from marine sediments in Southern India

Activité hémolytique de la souche Streptomyces *VITSDK1 sp. isolée de sédiments marins en Inde du Sud*

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Summary A moderately halophilic actinomycete strain was isolated from marine sediments collected at the Marakkanam coast of Tamil Nadu, India. The taxonomic position was evaluated by polyphasic approach and the strain was identified as a member of the genus *Streptomyces*. The strain is congruent with the description of the genus *Streptomyces* due to the characteristics of white aerial cell mass, spiral spore chains and a rough spore surface, menaquinones of MK-9 (H_4, H_2 and H_6) types and belongs to cell-wall type I. The G + C content of the isolated DNA is 71.3 mol% and the 16S rRNA sequence of the strain shows a maximum of 93% similarity with *Streptomyces HBUM 49447*. The comparison of phenotypic data of closest strains with the isolate clearly indicates that our strain belongs to the genus *Streptomyces*. Based on the phenotypic and phylogenetic analysis, the strain was classified as a new species of the genus *Streptomyces* and designated as VITSDK1. The media and cultural conditions for maximal growth have been optimized under shake-flask conditions by measuring the dry weight of the mycelium. The maximal growth was attained with the use of optimized production medium, pH of 7.2 and incubation temperature of 27 °C. The growth of the strain was salt concentration dependent and maximal growth was attained at 15% salt concentration which indicates the halophilic nature of the isolate. *Streptomyces* VITSDK1 spp. exhibits significant hemolytic activity against rat erythrocytes with the EC_{50} value of 127 μg/ml and for human erythrocytes 168 μg/ml. Further, it shows moderate antibiosis against fungi and bacterial pathogens.

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MOTS CLÉS
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Halophilie ;
Activité antifongique et antibactérienne

Résumé Une souche d'actinomycète modérément halophile a été isolée de sédiments marins récoltés sur la côte de Marakkanam du Tamil Nadu à l'est de l'Inde. La souche a été identifiée comme appartenant au genre *Streptomyces*. Elle est conforme à la description du genre *Streptomyces* par ses caractéristiques de culture, ses chaînes spiralées de spores à paroi rugueuse, ses ménaquinones MK-9 (H_2, H_4 et H_6) et son appartenance à la paroi de type I. Le G + C de l'ADN isolé est de 73 % et la séquence du 16S ARN montre un maximum de similarité de 93 % avec la souche de *Streptomyces HBUM 49447*. La comparaison des travaux sur le phénotype de souches proches avec la souche isolée montre clairement qu'elle appartient au genre *Streptomyces*. Compte tenu de l'analyse phénotypique et génotypique, elle a été identifiée comme une nouvelle souche du genre *Streptomyces* et dénommée VITSDK1. Les milieux et les conditions de culture pour une croissance maximale ont été optimisés en culture en ballons agités par la mesure du poids sec du mycélium. Le maximum de culture est atteint à l'aide d'un milieu optimisé, à pH 7,2 et à température de 27 °C. La croissance de la souche est concentration dépendante en sel et la croissance maximale est obtenue pour 15 % de concentration saline qui montre l'halophilie de cette souche. *Streptomyces* VITSDK1 sp. montre une activité hémolytique significative contre les érythrocytes de souris et les érythrocytes humains avec une EC_{50} respectivement de 127 et 168 μg/ml. Par ailleurs, elle montre une activité antimicrobienne modérée contre des champignons et des bactéries pathogènes.

Introduction

The order Actinomycetales, commonly called as actinomycetes, represent one of the most studied and exploited classes of bacteria for their ability to make a wide range of biologically active metabolites [9]. The actinobacteria play an important role among the marine bacterial communities, because of its diversity and ability to produce novel chemical compounds of high commercial value [1,8,10]. The genus *Streptomyces*, within the family Streptomycetaceae was first proposed by Waksman and Henrici [36]. It was then well defined by chemotaxonomic and molecular identification methods [31]. A number of terrestrial *Streptomyces* are being extensively used for commercial production of different medically important compounds. *Streptomyces* has been reported to contribute nearly 70% of metabolites described under actinomycetes [32]. The enormous potential of marine actinomycetes was confirmed by the analysis of genome sequences of two *Streptomyces* species, *S. coelicolor* [4] and *S. avermitilis* [9] revealed that the presence of more than 20 gene clusters encoding for the synthesis polyketides (PKs) or non-ribosomal peptides (NRPs), the two important biosynthetic classes of microbial secondary metabolites.

In Indian peninsula 41 species of marine actinomycetes were reported in which the genus *Streptomyces* was more frequently recorded. Of nine maritime states in India, only four states (Maharastra, Kerala, Tamil Nadu and Andhra Pradesh) have been extensively studied for the diversity of actinobacteria [30]. Much of the studies reported were focused on antibacterial and antifungical properties of the strains. This paper deals with the moderately halophilic actinomycetes isolated from the marine sediments of Marakkanam, the Bay of Bengal coast of Tamil Nadu, India.

Materials and methods

Sampling and isolation of actinomycetes

The strain was isolated from the marine sediment samples collected at the depth of 400 cm in the Marakkanam, (Latitude [N] 12°20′; Longitude [E] 79°95′) the southeast costal region of the Bay of Bengal, India. The sediment samples were dried in laminar air flow for 8—12 h and then kept at 42 °C for 10—30 days in a sterile Petri dish and these preheated samples were used for the isolation of actinomycetes. The International *Streptomyces* Project (ISP) No. I media, Starch casein agar and Bennett's agar with 25% sea water, 25% soil extract was used for the isolation of actinomycetes and the growth media was supplemented with antibiotics, cycloheximide (25 μg/ml) and nalidixic acid (25 μg/ml) (Himedia, Mumbai, India). The soil extract was prepared by mixing 400 g of air-dried marine soil (used for the isolation of actinomycetes) with 1000 ml distilled water and sterilized for one hour at 121 °C. The mixture was allowed to settle for few hours at room temperature. Then the supernatant was decanted and filtered through Whatman No. 1 filter paper. The clear supernatant solution obtained was adjusted to pH 6.8—7.0, sterilized and kept as stock. Plates were incubated at 28 ± 2 °C for 7—18 days. All the medias were prepared with varying salt concentrations (3, 5, 7, 9, 12, 15, 18 and 21% [w/v]) to isolate the halophilic actinomycetes. The isolate was subcultured and maintained in slant culture at 4 °C as well as at 20% (v/v) glycerol stock at −80 °C.

Optimization of nutritional and cultural conditions

To determine the optimal nutritional and cultural conditions and to identify the suitable media for growth, the strain was inoculated in different culture medias (SCA, ISP 2, ISP 3, ISP 4, ISP 5, ISP 6, ISP 7, Potato agar, Czapek's agar, modified Bennett's agar, sucrose/nitrate agar, glucose/nitrate agar, water agar (20 g agar/1 L distilled water), glucose/peptone agar, glycerol/calcium malate agar (Waksman medium No. 7) and nutrient agar) and the growth was investigated. The effect of cultural conditions like different incubation temperatures (15, 27, 37 and 50 °C), different pH (5.0, 6.0, 7.4 and 9.0) and NaCl concentrations (6, 12, 15, 18, 22, 24 and 26%) on the growth of the isolate was also studied. The carbon and nitrogen sources required were also studied by inoculating the isolates into the starch agar (starch: 10 g; K_2HP0_4: 1 g; $MgSO_4$: 1 g; NaC1: 1 g; $(NH_4)_2SO_4$: 2 g; $CaCO_3$: