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Isolation and characterization of actinobacteria from Yalujiang coastal wetland, North China

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ABSTRACT

Objective: To evaluate various types of samples from the different marine environments as sources of actinomycetes from the Yalujiang coastal wetland, North China, and to screen their antimicrobial properties. Further, the identified actinomycetes were characterized based on morphological, biochemical, and physiological characteristics.

Methods: Eight different production media were used to isolate actinomycetes from different stations of marine soil sediments in Yalujiang coastal wetland and the genotypic positions were established by 16S rDNA.

Results: A total of 172 actinomycetal isolates were obtained from 13 samples using five media. The most effective culture media in the isolation of actinobacteria were Gause's Synthetic agar and Starch-casein agar. Among 172 isolates, 46 isolates (26.74%) showed antibacterial activity, 70.93% belonged to the genus *Streptomyces*, others were *Micromonospora* spp. and *Rhodococcus* spp. Out of the 46 isolates, two cultures were further supported by morphological characterization analysis.

Conclusions: This is the first report about actinomycetes isolated from Yalujiang coastal wetland and it seems that the promising isolates from the unusual/unexplored wetland may prove to be an important step in the development of microbial natural product research.

1. Introduction

The marine ecosystem has been widely recognized as a source that nurtures abundant compounds that contain novel composition and organic characteristics. Wetlands are considered to be the most biologically important and productive ecosystems on earth [1]. They provide habitat, food, and spawning grounds for a large number of plants and animals and therefore exhibit great biodiversity [2].

Actinomycetes are the dominant group of soil population together with bacteria and fungi. Some reports described China wetland as a major source of actinomycetes [3,4]. Actinomycetes are one of the major microbial dominant groups and are well known for their saprophytic behavior as well as for production of diverse bioactive secondary metabolites. They are also recognised for their capacity to survive in extreme habitats [5].

Recently, many scientists are searching new antibiotics from different untouched wetland to find out for their productions of antibiotics. Although hundreds of Chinese rivers discharge into the Northwest Pacific Ocean, little is known about the diversity of actinomycetes in marine sediments, which is an inexhaustible resource that has not been properly exploited for search and discovery of novel actinomycetes. The Yalujiang coastal wetland is one of the largest wetlands near Dandong City, located in North China and covers 108 057 ha. The temperature is minimum of −28.2 °C, maximum of 33.9 °C and the mean annual temperature is 9.9 °C.

In order to exploit more laudable actinomycetes, it is necessary to better understand the diversity of actinomycetes in a single ecosystem of the Yalujiang coastal wetland, which covers both fresh water as well as marine ecotones and possesses hostile environments enriched with immense biodiverse values. The present study was designed to evaluate various types of

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samples from the different marine environments as sources of actinomycetes from the Yalujiang coastal wetland, North China, and to screen their antimicrobial properties. Further, the identified actinomycetes were characterized based on morphological, biochemical, and physiological characteristics.

2. Materials and methods

2.1. Sample collection

Our research was located in the Yalujiang coastal wetland (120°12′9″ E to 123°30′50″ E, 39°40′50″ N to 40°30′ N), North China. Altogether 15 points were sampled, in October 2012 and October 2013, from different zones, areas and marine source of Yalujiang coastal wetland (Table 1). Random sediment samples were collected in triplicates by using sterile bottles and they were stored at 4 °C in the laboratory for further study.

2.2. Bacterial isolation

The samples were processed using the dilution and heat-shock method as the selective method [6]. After the dilution, 100 μL of the 10^{-3} and 10^{-4} diluted suspension was inoculated by spreading onto eight different production media [glycerol asparagine agar (ISP5), starch casein agar (SC), Gause's synthetic agar (GS), starch-yeast extract-peptone agar (M1, modified), minimal salts agar (M5), glycerol arginine agar (M2), humic acid vitamin (HV), and trehalose dehydrate proline (T-p)]. All the sample aliquots were analyzed in duplicate. The plates were incubated at 28 °C for 1–3 weeks. All media were prepared with 50% filtered natural seawater. After autoclaving, all of the isolation media were complemented with 50 μg/mL nystatin and 25 μg/mL nalidixic acid in order to minimize contamination with fungi and Gram-negative bacteria.

2.3. Morphological characterization

The isolated strains were initially characterized by morphological criteria according to Bensultana *et al.* [7].

2.4. Antimicrobial testing

The antimicrobial activity was tested by the plate diffusion method [8]. Three strains of pathogenic microbes [*Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and *Fusarium oxysporum* (*F. oxysporum*)] were chosen as the test organisms. Isolates were inoculated onto the modified ISP5 medium at 28 °C for 7 days and discs (6 mm in diameter) were cut and placed on Luria-Bertani agar medium (for *S. aureus* and *E. coli*) or potato dextrose agar medium (for *F. oxysporum*) which was seeded with appropriate test organism. Plates were incubated at 37 °C. Inhibition zones were determined after 48 h for *S. aureus* and *E. coli* and after 72 h for *F. oxysporum*.

2.5. Molecular identification

Selected isolates were subjected to 16S rDNA sequence analysis for establishment of their genotypic position. DNA was prepared according to the method described earlier [9]. The 16S rDNA was amplified as described by with eubacterial specific primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1522R (5′-TGCGGCTGGATCACCTCCTT-3′) [10]. The PCR cycling conditions included an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, followed by a final extension for a period of 10 min at 72 °C. The amplified PCR product was checked on 1% agarose in 1× TAE buffer, and purified with a mixture of 20% polyethylene glycol and 2.5 mol/L NaCl. The sequence obtained was compared with 16S rRNA gene sequences of cultured species available from EzBioCloud using BLAST (http://eztaxon-e.ezbiocloud.net/) [11]. Sequences were aligned using CLUSTAL W software [12]. Phylogenetic analyses were performed by using three treemaking algorithms and phylogenetic trees were constructed using the Neighbor-Joining [13], Maximum-Parsimony [14] and Maximum-Likelihood [15] tree-making algorithms by using the software packages MEGA version 5.0 [16]. Kimura's two parameter model was used to calculate evolutionary distance matrices of the Neighbor-Joining method [17]. The topologies of the resultant trees were evaluated by using the bootstrap resampling method with 1000 replicates [18].

3. Results

3.1. Isolation medium

A total of 15 samples were collected from 3 different locations in Yalujiang coastal wetland, China (Table 1). A total number of 172 different actinomycetes were isolated from different media, different places and different soil samples on the basis of color of aerial and substrate mycelium, pigmentation and microscopic examination.

Table 1
Characterization of the collection sites from Yalujiang coastal wetland, North China.

No.	Locations	Sample code	Site denomination	Sampling material
1	Yalu River estuary	Z1	Yalu River estuary (salinity, 0%)	Sediment
2		Z12	Yalu River estuary (water resource)	Sediment
3		Z2	Yalu River estuary (salinity, 1.21%)	Sediment
4		Z4	Yalu River estuary (salinity, 7.63%)	Sediment
5		Z6	Yalu River estuary (salinity, 15.04%)	Sediment
6		Z9	Yalu River estuary (salinity, 21.6%)	Sediment
7		Z17	Yalu River estuary (salinity, 30.4%)	Sediment
8	Trepang habitat	T4	500 m from trepang habitat	Sediment
9		T6	Outside the trepang habitat	Sediment
10		T21	Inside the trepang habitat	Sediment
11	Rhizosphere	T7	Straw rhizosphere	Sediment
12		T8	Seepweed rhizosphere	Sediment
13		T8jl	Between the seepweed and root rhizosphere	Sediment
14		T9	Outside of the root rhizosphere	Sediment
15		T20	Central of the root rhizosphere	Sediment