Isolation of myxobacteria from the marine environment

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Abstract

In an attempt to isolate indigenous marine myxobacteria from coastal samples, we obtained two swarm forming bacteria. Both isolates formed cell aggregates which, at least in one isolate, developed to fruiting body-like structures consisting of a mass of myxospore-like cells. The optimum NaCl concentrations for their growth were between 2 and 3%, comparable to the NaCl concentration of seawater. This growth characteristic strongly suggests that the two isolates are specific marine bacteria. The 16S rDNA sequence studies indicated that the two isolates were related to the genus Nannocystis. Based on the phylogenetic distances between branches, we concluded that the isolates should be assigned to two new myxobacterial genera. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

The myxobacteria are Gram-negative, rod-shaped gliding bacteria. They are unique among prokaryotes by virtue of their elaborate intercellular communication and morphological development [1]. On starvation, the cells aggregate by gliding to form masses which later differentiate into fruiting bodies [1]. Another characteristic is their predatory activity on other microorganisms. Most of the myxobacteria are able to lyse a variety of bacteria and fungi, and obtain nutrients from the lysis products.

As for their habitat, the myxobacteria have long been regarded as terrestrial bacteria and have been mainly isolated from soils and plant debris, which are rich in microbial communities [1]. Only a few investigators have reported the isolation of myxobacteria from marine environments. Those reports, however, lack detailed descriptions of the physiological and phylogenetic characteristics of the isolates [2,3].

Recently, phylogenetic analyses of 16S rDNA fragments amplified from marine sediment DNA indicated the presence of bacterial phylotypes which are closely related to myxobacteria [4,5], but live cultures have not been isolated.

To clarify the existence of indigenous marine myxobacteria, we attempted to isolate myxobacteria from coastal samples. In this study, we report evidence for the presence of myxobacteria which are physiologically adapted to marine environments.
2. Materials and methods

2.1. Microorganisms

Strain SHI-1 was isolated from a wet sand sample collected from a rocky coast (Ogata-ura) of Hachijo-jima, Japan, and strain SMP-2 from a dry Laminariales seaweed sample collected from a sandy beach (Aburatsubo-wan) of the Miura Peninsula, Japan. The following strains of terrestrial myxobacteria were used in the physiological studies as reference strains: *Archangiun gephrya*\(^T\) (T = type strain) ATCC 25201, *Chondromyces apiculatus* \(^T\) DSM 436, *Cystobacter fuscus* \(^T\) ATCC 25194, *Myxococcus xanthus* \(^T\) ATCC 25232, *Myxococcus fulus* \(^T\) ATCC 25199, *Myxococcus virescens* \(^T\) ATCC 25203, *Nannocystis exedens* ATCC 35989, *Nannocystis exedens*\(^T\) DSM71.

2.2. Media

Enrichments were initiated on the ASW-WCX agar medium [1], in which 0.75× artificial seawater (ASW) was used. The composition of the medium was as follows (per liter): cyanocobalamin, 0.5 mg; cycloheximide, 25 mg; agar, 15 g. ASW was prepared by Jamarine-S kit (Jamarine Laboratory, Osaka, Japan), according to the manufacturer’s directions (pH 7.5). For morphological observations and strain preservation, we used VY/2-ASW agar [1], which contained (per liter): Baker’s yeast cake, 5 g; cyanocobalamin, 0.5 mg; agar, 15 g. To prepare the cell mass of isolates, either 0.2 or 1× CY-SWS medium was used. CY-SWS contained (per liter of Sea Water Salts solution; SWS): Bacto Casitone (Difco), 3 g; Bacto Yeast Extract (Difco), 1 g; NaCl, 20 g (pH 7.5, NaOH). SWS contained (per liter of distilled water): ferrous citrate, 0.1 g; MgSO\(_4\)-7H\(_2\)O, 8.0 g, CaCl\(_2\)-2H\(_2\)O, 1.0 g; KCl, 0.5 g; NaHCO\(_3\), 0.16 g; H\(_3\)BO\(_3\), 0.02 g; KBr, 0.08 g; SrCl\(_2\), 0.03 g; glycerophosphate-2Na, 0.01 g; trace element solution [1], 1 ml (pH 7.5, KOH).

2.3. Isolation

Fifty samples were collected from the coast of Hachijo-jima in February, 1997, and 40 samples were collected from the coast of the Miura Peninsula in July, 1997. Samples contained sands, wood pieces, small dead animals (fish, molluscs and crustaceans), and seaweeds. All samples were found in the intertidal area. Isolation procedures were performed on the wet samples within one day of sampling. The isolation method, based on predatory activity [6], was applied with slight modifications. A cross streak of live *E. coli* was smeared on an ASW-WCX agar plate. The central part of the streaks was inoculated with a pea-sized amount of sample, then incubated at 25°C and checked daily under a dissection microscope. The occurrence of myxobacteria was recognized by the emergence of a diffusive colony (swarm [1]) usually after 2–4 weeks of incubation. For purification, 0.5×0.5 mm agar pieces from little contaminated areas of the swarms were picked up with sharp needles and transferred to fresh *E. coli* streaks. The procedure was repeated until a pure culture was obtained.

2.4. Taxonomy

2.4.1. Quinone analysis

Respiratory quinones were extracted with acetone, fractionated by silica gel TLC and separated by HPLC [7].

2.4.2. GC content of DNA

The mol % G+C of the DNA was measured with the HPLC method [8]. Equimolar nucleotide mixture (Yamasa, Chiba, Japan) was used as a standard.

2.4.3. 16S rDNA sequencing

16S rDNA fragments were amplified by PCR directly from the crude lysate [9]. PCR products were sequenced with a SequiTherm Cycle Sequencing kit (Epicenter Technologies, Madison, WI, USA), followed by detection with a Pharmacia laser fluorescent DNA sequencer [10].

2.4.4. Phylogenetic analysis

Nearly complete 16S rRNA genes of the two new isolates were sequenced and compared with the 16S rDNA sequences of known terrestrial myxobacterial representatives. Distance matrix trees were constructed by the N-J method with the CLUSTAL W program, and the topologies of the phylogenetic trees were built by bootstrap analysis [10]. To con-
firm the robustness of the determined tree, other
treeing methods, maximum parsimony (Dnapars)
and maximum likelihood (Dnaml), were applied
[11]. They provide the same tree topology as that
obtained by the N-J method. The 16S rDNA se-
cquence data of both isolates will appear in the
DDBJ/EMBL/GenBank nucleotide sequence data-
bases with the accession nos. AB016469 and
AB016470 for SHI-1 and SMP-2, respectively.

2.5. The effect of NaCl on growth

The effect of NaCl on growth was investigated on
VY/2-SWS agar media with 0–10% (w/v) NaCl
added as indicated in the text (Fig. 3). Each strain
was inoculated on the center of the agar plate, and
incubated at 27°C in the dark for 14–30 days. The
relative growth rate was determined by measuring
the diameter of swarms.

3. Results

3.1. Isolation

Swarm formations were recognized in 2 of the 50
Hachijo samples, and in 4 of the 40 Miura samples.
We succeeded in the purification of two isolates
(SHI-1, SMP-2) from the above 6 samples. The other
4 swarm forming strains are still undergoing purifi-
cation, so taxonomic and phylogenetic analyses were
performed on the two isolates.

3.2. Taxonomic characteristics

3.2.1. Morphology

The swarms of strain SHI-1 appeared as shallow
sunken craters in the agar (Fig. 1A). Radial patterns
were recognized within the swarm area. Strain SMP-
2 formed thin film-like swarms (Fig. 1C). Wavelet-
like ridges were observed at the swarm periphery.
Vegetative cells of both strains were rod-shaped
with blunt ends (Fig. 1B,D), between 1.5–7.0 μm in
length and 0.5–0.8 μm in width. Strain SMP-2
formed fruiting body-like structures which were 50–
200 μm in diameter (Fig. 1E), colored chrome-yellow
to lemon-yellow. Within them, spherical cells which
were 0.5–1.0 μm in diameter could be observed (Fig.
1F). These cells were similar in shape to the spherical
myxospores of Nannocystis strains [6]. In strain SHI-
1, spherical to oval cell clumps, which were 50–150
μm in diameter and colored orange-white to light
orange appeared. However, we could not identify
distinctive myxospores within them.

3.2.2. Quinone analysis

The major respiratory quinone from both strains
was menaquinone 8.

Fig. 2. Dendrogram based on 16S rRNA gene sequences showing the position of strain SHI-1 and SMP-2 within the Proteobacteria delta-
subclass radiation. The topology shown was obtained by the N-J method. Bootstrap values, expressed as a percentage of 1000 replica-
tions, are given at the branching points. Bar indicates the evolutionary distance.
3.2.3. DNA base composition

The G+C contents of the DNA from strains SHI-1 and SMP-2 were 70.0 mol% and 66.7 mol%, respectively.

3.2.4. 16S rDNA sequence studies

The phylogenetic analyses demonstrated that the two marine isolates were members of the order Myxococcales and that they share a common line of descent with the genus *Nannocystis* (Fig. 2). The closest relative for strain SHI-1 was *N. exedens* (level of similarity 89.3%), and those for strain SMP-2 were *N. exedens* (level of similarity 83.2%) and *Chondromyces apiculatus* (level of similarity 82.9%). The average similarity value between the two isolates was 84.6%.

3.3. Effect of NaCl on growth

As evident in Fig. 3, the growth of all reference terrestrial strains was strongly inhibited by increased salinity. No terrestrial strains could grow at NaCl concentrations higher than 2% (w/v). In contrast, both isolates from coastal samples exhibited growth stimulation at salinities of up to 2% NaCl. Growth of strain SHI-1 occurred at NaCl concentrations ranging from 1 to 4%, with optimal growth occurring between 2 and 2.5%. Strain SMP-2 grew at NaCl concentrations ranging from 0.2 to 5%, with optimal growth occurring between 2 and 3%.

4. Discussion

The two marine isolates showed characteristic morphological and chemotaxonomic features of the myxobacteria. In particular, the unique appearances of the swarm colonies strongly suggests that both isolates belong to the suborder Soranginiae [1]. Phylogenetic analysis based on 16S rRNA similarity indicated that the two isolates are closely related to the genus *Nannocystis*, a member of the suborder Soranginiae. The levels of similarity among the marine isolates and the closest terrestrial relatives were rather low (between 82 and 90%), suggesting that the two marine isolates should be assigned into two new myxobacterial genera. To determine the taxonomic position of the two isolates more definitely, further studies are now in progress.

The genus *Nannocystis* is an ubiquitous myxobacteria and has been reported to inhabit a wide range of terrestrial environments, from tropical to Antarctic soil [1]. Its ubiquitous nature may be due to its rapid evolutionary pace in comparison to other myxobacterial genera [12]. Therefore, we speculate that an ancestral strain of the genus *Nannocystis* diverged into various types and that some adapted to marine environments. Since we know nothing about the ancestral strain, it is not clear whether it originally inhabited a marine or terrestrial environment.

Marine bacteria are generally defined as those living in marine environments and requiring sodium for their growth. Moreover, a higher concentration of magnesium is also required for their growth as compared with terrestrial strains [13]. To demonstrate if these requirements applied to the two marine isolates, we investigated the salinity dependence of growth. In contrast to the terrestrial strains, both marine isolates showed a specific requirement for sodium, and optimal NaCl concentrations for growth were roughly equivalent to that of seawater. Other components, such as magnesium, calcium, potassium and lithium did not compensate for the sodium requirement. In addition, both isolates required a higher concentration of magnesium than the ter-
restrial reference strains (data not shown). From the above results, we concluded that the two isolates were specifically adapted to marine environments.

No myxobacterium able to grow at the concentration of salt found in seawater had been previously known [1], therefore this is the first report to describe the phylogenetic and physiological features of myxobacteria from marine ecosystems. The accumulation of marine myxobacterial isolates would provide us with profound new knowledge of the taxonomy, physiology and ecology of myxobacteria.

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References