



Microbial Diversity of Marine Biofilm

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We isolated culturable bacteria that have inhabited on natural marine biofilms, and identified them by phylogenetic analysis using 16S rDNA sequences. In marine environment, biofilms cover most of subtidal and intertidal solid surfaces such as rocks, ships, loops, marine animal, algae, etc. The bacteria in most biofilms are embedded in extracellular polymeric substances that comprise mainly exopolysaccharides. The main component exopolysaccharides are excreted from multiple bacterial species, therefore, biofilms are good source for screening exopolysaccharide-producing bacteria. Total 31 strains were cultured, among them 14 strains were overlapped and 17 strains were finally identified. Phylogenetic analysis using 16S rDNA indicated that the 17 strains belonged to α -Proteobacteria (*Ochrobactrum anthropi*, *Paracoccus carotinifaciens*); γ -Proteobacteria (*Pseudoalteromonas piscicida*, *P. agarovorans*, *Pseudomonas aeruginosa*, *Shewanella baltica*, *Vibrio parahaemolyticus*, *V. pomeroyi*); CFB group bacteria (*Cytophaga latercula*, *Tenacibaculum mesophilum*); high GC, Gram positive bacteria (*Arthrobacter nicotianae*, *Brevibacterium casei*, *B. epidermidis*, *Tsukamurella inchonensis*); and low GC, Gram positive bacteria (*Bacillus macroides*, *Staphylococcus haemolyticus*, *S. warneri*).

Introduction

Bacteria can adhere to natural or artificial surfaces, and form sessile multicellular communities known as biofilms. The natural and artificial surfaces that are covered by biofilms, include animal and plant cells, soils, sediments, pore in glaciers, thermal vent, pipelines, heat exchangers, separation membranes, filters, etc. In marine environment, biofilms cover most of subtidal and intertidal solid surfaces such as rocks, ships, loops, marine animal, algae, etc. The bacteria in most biofilms are embedded in extracellular polymeric substances (Lawrence *et al.*, 1991). The extracellular polymeric substances offer microniche with stable arrangements and a certain degree of homeostasis (Wimpenny, 2000). The extracellular polymeric substances can sequester nutrients from the environment and is, thus, part of a general microbial strategy for survival under oligotrophic conditions (Decho, 2000). Extracellular polymeric substances also have the potential to physically prevent access of certain antimicrobial agents by acting as an ion exchanger, thereby restricting diffusion of compounds into the biofilm (Gilbert *et al.*, 1997). Extracellular polymeric substances have also been reported to provide protection from a variety of environmental stresses, such as UV radiation, pH shifts, osmotic shock, and desiccation (Mayer, 1999). But the composition and characteristics of the extracellular polymeric substances have been studied from few model organisms such as *Pseudomonas aeruginosa* (Wingender *et al.*, 2001).

Extracellular polymeric substances comprise mainly exopolysaccharides (40-95%), protein (1-60%), nucleic acids (1-10%), and lipids (1-40%), which form hydrogel matrices (Davey and O'Toole 2000). The main component exopolysaccharides are excreted from multiple bacterial species; therefore, biofilms are good source for screening exopolysaccharide-producing bacteria. The bacterial species forming biofilm community are largely unknown for most natural biofilms, and members of exopolysaccharide-producing



bacteria has scarcely been cultured and identified. Biofilm formation may require coordination with, interaction of, and communication between multiple bacterial species (Davey and O'Toole, 2000).

In this study, we isolated and cultured bacteria that have inhabited on natural marine biofilms, and identified them by phylogenetic analysis using 16S rDNA sequences. This is the first step to screen marine bacteria producing exopolysaccharides that have good prospect for industrial and medical applications.

Results and Discussion

Total 31 strains were cultured, among them, 14 strains were overlapped and 17 strains were finally identified (Table 1). Phylogenetic analysis using 16S rDNA indicated that the 17 strains belonged to α -Proteobacteria (*Ochrobactrum anthropi*, *Paracoccus carotinifaciens*); γ -Proteobacteria (*Pseudoalteromonas piscicida*, *P. agarovorans*, *Pseudomonas aeruginosa*, *Shewanella baltica*, *Vibrio parahaemolyticus*, *V. pomeroyi*); CFB group bacteria (*Cytophaga latercula*, *Tenacibaculum mesophilum*); high GC, Gram positive bacteria (*Arthrobacter nicotianae*, *Brevibacterium casei*, *B. epidermidis*, *Tsukamurella inchonensis*); and low GC, Gram positive bacteria (*Bacillus macroides*, *Staphylococcus haemolyticus*, *S. warneri*).

Several species such as *Arthrobacter nicotianae*, *Cytophaga laterculai*, *Pseudoalteromonas agarovorans*, *P. piscicida*, *Shewanella baltica*, *Tenacibaculum mesophilum*, and *Vibrio pomeroyi* have been reported that they were isolated from seawater or marine organisms (Nakagawa and Yamasato, 1993; Ziemke *et al.*, 1998; Venkateswaran *et al.*, 2000; Radwan *et al.*, 2001; Suzuki *et al.*, 2001; Romanenko *et al.*, 2003; Thompson *et al.*, 2003). But *Ochrobactrum anthropi*, *Staphylococcus haemolyticus*, *S. warneri*, and *Tsukamurella inchonensis* that are originated from human samples, they may be putative pathogen inhabited in marine environment like *Vibrio cholerae*.

Most of strains cultured in this study belong to α -Proteobacteria, γ -Proteobacteria, CFB, and gram-positive bacteria (Table 1). α -Proteobacteria, γ -Proteobacteria and CFB group bacteria are dominant groups in marine environment (Kelly and Chistoserdov, 2000) and also have been reported from various marine biofilms. α -Proteobacteria, γ -Proteobacteria and CFB group bacteria were dominant in dead coral surface (Frias-Lopez *et al.*, 2002); α -Proteobacteria, γ -Proteobacteria, CFB, and gram-positive bacteria were dominant in the shrimp-attached bacteria (Lau *et al.*, 2002); α -Proteobacteria, γ -Proteobacteria and CFB group bacteria were associated with tubes of a worm living in deep-sea hyperthermal vent (Lopez-Garcia *et al.*, 2002); α -Proteobacteria, γ -Proteobacteria, CFB, and gram-positive bacteria were isolated from hydrocarbon-degrading biofilms (Stach and Burns, 2002).

Bacteria forming raised colonies are expected to produce abundant exopolysaccharides, thus, further studies of exopolysaccharides are progressed for strains 13705, 13716, 13721, 13724 and 13731. Strains forming convex colonies are also putative exopolysaccharides-producing bacteria. This study provides an initial step in deciphering the bacterial diversity of marine biofilms, and in screening the exopolysaccharides-producing bacteria.

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Table 1. List of bacterial species isolated from marine natural biofilms

Strain No.	Colony				Closest match	Homology (%)
	Color	Form	Elevation	Margin		
13685	orange	circular	Convex	entire	<i>Tenacibaculum mesophilum</i>	100
13686	yellow/orange	irregular	Convex	erose	<i>Brevibacterium epidermidis</i>	98
13687	cream	circular	Flat	erose	<i>Pseudomonas agarovorans</i>	100
13690	orange	irregular	Flat	erose	<i>Tsukamurella inchonensis</i>	99
13691	white	circular	Convex	entire	<i>Staphylococcus warneri</i>	100
13703	yellow	circular	Convex	entire	<i>Pseudoalteromonas piscicida</i>	100
13705	ivory	irregular	Raised	erose	<i>Vibrio parahaemolyticus</i>	100
13707	yellow	circular	Convex	entire	<i>Arthrobacter nicotianae</i>	98
13716	yellow/brown	irregular	Raised	undulate	<i>Pseudomonas aeruginosa</i>	100
13717	white	circular	Convex	entire	<i>Staphylococcus haemolyticus</i>	99
13721	ivory/brown	circular	Raised	entire	<i>Vibrio pomeroyi</i>	100
13723	yellow/brown	circular	Convex	entire	<i>Shewanella baltica</i>	100
13724	yellow	filamentous	Raised	erose	<i>Bacillus macroides</i>	100
13729	white	circular	Convex	entire	<i>Brevibacterium casei</i>	99
13731	yellow	circular	Raised	entire	<i>Cytophaga latercula</i>	100
13733	orange	circular	Convex	entire	<i>Paracoccus carotinifaciens</i>	100
13737	ivory/yellow	circular	convex	entire	<i>Ochrobactrum anthropi</i>	100