Indian J Microbiol (Oct–Dec 2010) 50(4):474–477
DOI 10.1007/s12088-011-0144-z

SCIENTIFIC CORRESPONDENCE

Candidate OP Phyla: Importance, Ecology and Cultivation Prospects

M. Rohini Kumar · V. S. Saravanan

Received: 21 September 2010 / Accepted: 22 December 2010 / Published online: 25 January 2011

Absract OP phyla were created in the domain bacteria, based on the group of 16S rRNA gene sequences recovered from the Obsidian Pool. However, due to the lack of cultured representative it is referred to as candidate phyla. Wider ecological occurrence was predicted for the OP phyla, especially OP3, OP10 and OP11. Recently, members of phylum OP5 and OP10 were cultured, providing clues to their cultivation prospects. At last the bioprospecting potentials of the OP members are discussed herein.

Keywords Candidate OP group phyla · Uncultivated · Obsidian pool

Introduction

Nearly a decade ago, some novel 16S rRNA gene sequences were recovered from the Obsidian pool, a hot spring (75–95°C) located in Yellow Stone National Park (Fig. 1), rich in iron, sulfide, carbon dioxide and hydrogen, supporting a unique ecosystem dominated by lithotrophic communities [1, 2]. Those sequences could not be assigned to any other phylum known at that time, thus, novel OP group phyla were created [1]. Based on the variability of the sequences recovered, 12 OP phyla were initially created [1]. However, currently only seven phyla are validated (OP 1, 3, 5, 8, 9, 10 and 11). In this letter, the importance of Candidate phyla, ecological occurrence of OP phyla, recent cultivation efforts to underpin the OP10 and OP5 phylotypes and possible biotechnological applications are emphasized.

M. Rohini Kumar
Hex-226 Lab, School of Bio Sciences and Technology,
Vellore Institute of Technology, University,
Vellore, Tamilnadu 632 014, India

V. S. Saravanan (✉)
Department of Microbiology, Indira Gandhi College
of Arts and Science, Puducherry 605 007, India
e-mail: saraphd2003@yahoo.com

Radiance of Bacterial Phyla

The enormous diversity of bacteria in nature was revealed through the community sampling of ribosomal RNA genes (16s rRNA). It is believed that at least 100 phyla of bacteria are probably in existence. Among these, bacteria belonging to at least 40 phyla are well identified [3], among these validly identified phyla, only about 25 have cultured representatives (Fig. 2). The remaining phyla are referred to as Candidate, denoting phyla without cultured representatives, assigned by directly obtained gene sequences (16S rRNA sequences), at present they number 39 [4]. Thus candidate status indicates their unconfirmed position as a valid phylum due to the absence of cultured representatives. However, their members are abundant and widely distributed in soil ecosystems and ecologically they may play key roles in soil processes [5]. Candidatus nature is observed even among bacterial groups occurring in defined phyla including the dominant soil dwelling Acidobacteria [6] and Planctomycetes accommodating the anaerobic ammonium oxidizers (ANAMMOX) an important player in the nitrogen cycle [7]. The promise of being able to study the genome without cultivation led to the emergence of metagenomics as a popular, cultivation independent approach to bacterial community studies. Paradoxically, completed genomes posed a concern due to genomic micro heterogeneity and high species diversity within samples. Ultimately, a situation has arisen where 40% or more of the proteins in any

Fig. 1 Obsidian Pool, The Yellow Stone National Park (Courtesy: Bob Lindstrom, NPS)

genome cannot be assigned a function [8]. Even though cultivation independent efforts are empirical to ascertain the presence or detection of bacterial phyla, culturing is essential in understanding the basic features of a bacterium like its physiology, gene expression and function (Transcriptomics and Proteomics) under different ecological conditions and to conduct various biotechnological studies thereafter.

Ecological Occurrence of OP Phyla and Their Cultivation

After the initial discovery from the Obisidian Pool of Yellowstone National Park, they were subsequently detected in other niches. Members of these phyla were found distributed in different extremophilic environments like geothermal and mineral rich soils, [9] deep subsurface [10] aquifers [11, 12] and hydrothermal vents [13]. Recently, they were also observed in certain environmental niches, including mesophilic lake water [14] and flooded paddy soils [4]. Yet, several members of the OP phyla remain uncultured in nature till date, more than 700 valid sequences have been deposited in the rRNA public database (SILVA rRNA database project) [15].

Based on the 16S rRNA sequences recovered, a diverse habitat was identified for the members of OP3 phylum. They are found in many anoxic environments including marine sediments, hypersaline deep sea, fresh water lakes, aquifiers, cave seepage waters, flooded paddy environments and methanogenic reactors.

In a significant study, the 16S rRNA sequences of OP10 phylum maintained in the data base (NCBI) were critically analyzed and it was found that more than 357 different sequences clustering in the OP10 phylum were wrongly deposited in other phylum [16]. The study also pointed out that OP10 is an important bacterial phylum and it could constitute an average of 5% among the total bacterial sequences recovered in environments like arable soils, hyper saline soils, geothermal springs, lake and river environments, bioreactors and endolithic environments. Among OP phyla, a wider geographical distribution was noticed in OP11, as it is found in anaerobic niches [12, 13]. Analysis of rarefaction curves constructed from the OP11 sequences revealed that species richness in OP11 is not significantly different from that of β-Proteobacteria [17], however, till date they were not represented in culture.

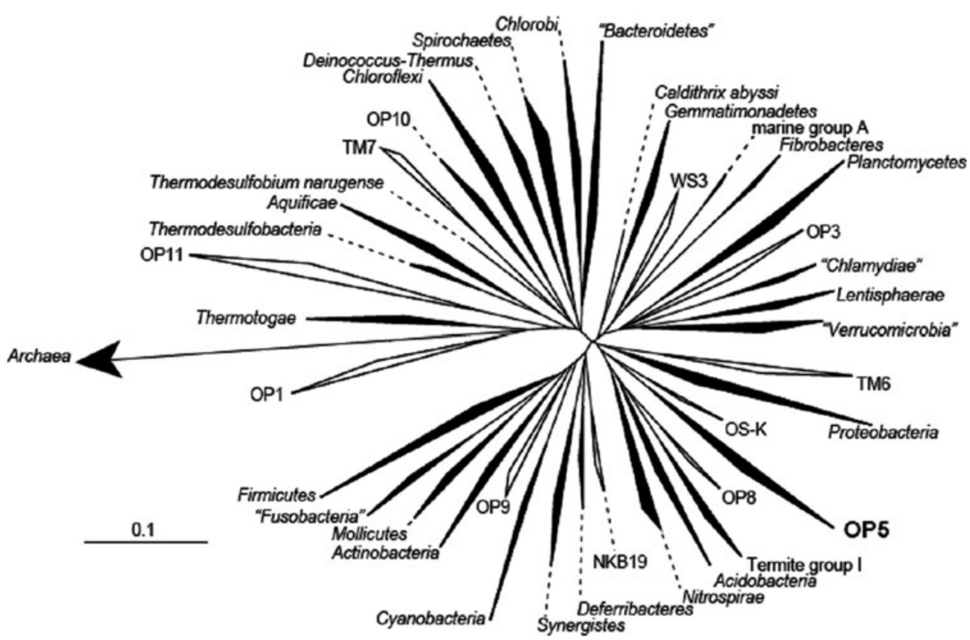

Fig. 2 Phylogenetic relationship based on 16S rRNA gene sequences showing major recognized bacterial phyla [19]. *Solid wedges* indicate phyla with cultivated representatives and *open wedges* indicate phyla (candidate phyla) currently lack cultured representatives and referred only by environmental clone sequences. *Bar*, 0.1 substitutions per compared nucleotide site

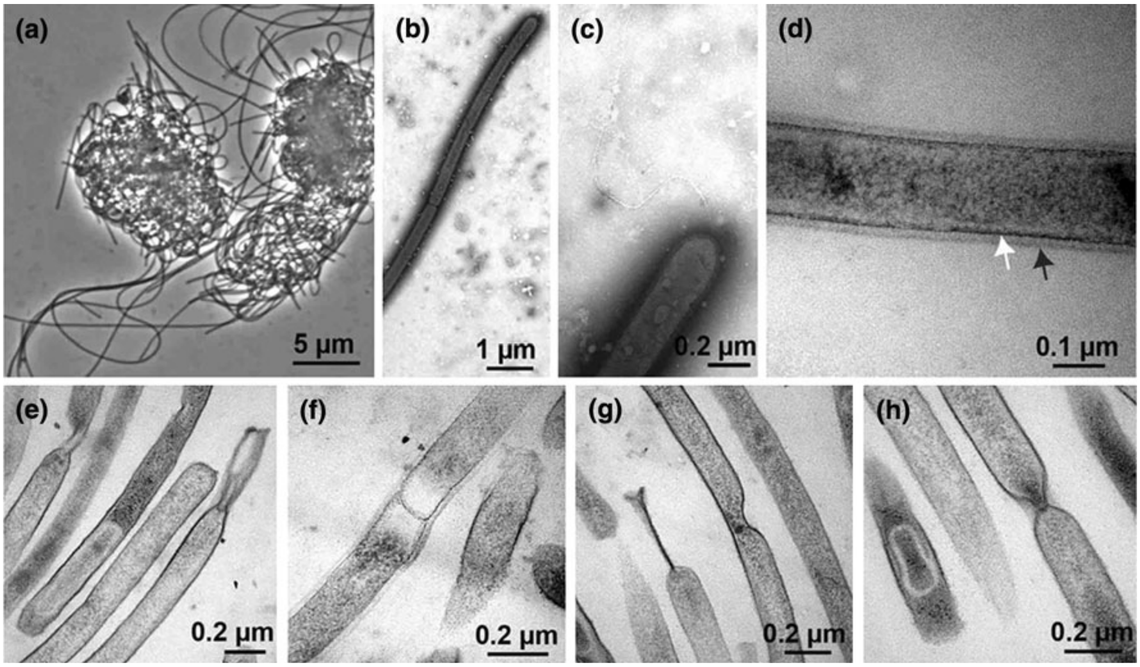

Fig. 3 Phase-contrast (**a**) and transmission electron (**b**–**h**) images of *C. exile*. Negatively stained cells are shown in **b** and **c**. Ultra-thin sections of the cells are shown in **d**–**h**. The cellular membrane and outer envelope are indicated by *open and solid arrows*, respectively (**d**) [19]

Recently, a few strains belonging to the members of OP5 and OP10 have been cultured [9, 14, 18]. Interestingly, their culturing conditions give us clues to the successful isolation of other members belonging to OP series. Members of OP phyla are ovoid rod (OP10) to filamentous (OP5) in shape, litho or chemo organotrophs and represent either aerobic or anaerobic form of metabolism.

A member of OP5 phylum has been isolated from hot springs of Japan. It is a Gram negative, filamentous (Fig. 3) chemoheterotrophic and anaerobic bacteria. It has been named as *Caldisericum exile* and its taxonomic position was validly published [9, 19].

In another study, members belonging to OP10 phylum were successfully isolated from the geothermally heated soils of Taupo volcanic zone, New Zealand. These isolates (P488 and T49) are thermophilic in nature, and grow only in the mineral medium. They are aerobes and able to utilize complex polysaccharides like gellan, xanthan and carboxymethylcellulose [18]. The success behind their cultivation was mainly attributed to maintenance of low pH medium and the use of gellan as gelling agent as an alternate to agar. Recently, another member of this phylum was cultured from the rhizoplanes of the aquatic plant, *Phagmites australis* (reed), commonly grown in the mesophilic fresh water lakes of Japan using low nutrient medium [14]. Both New Zealand and Japanese OP10 strains are chemoheterotrophic and aerobic however, the mesophilic strain isolated from Japan can be easily differentiated from those recovered from geothermal soils of New Zealand based on the temperature, pH range of growth, complex polysaccharide utilization and differences in certain biochemical features. Polyphasic taxonomic analysis placed the Japanese strain into a novel genera and it was validly published as *Armatimonas rosea*. Besides, the candidate OP10 phylum was renamed as phylum *Armatimonadetes* [14].

Bioprospecting Potentials

Based on the habitat diversity of OP phylum series, it can be hypothesized that it is a highly widespread and ecologically diverse phyla. Further, cultivation studies could pave the way to understanding the genetic, metabolic and species diversity in these phyla. The isolates obtained till date may throw more light on the cultivation aspects of OP phyla in the future, further phylum level identity could be better resolved for various OP phyla. The available evidence envisages their putative role in biogeochemical cycling, which is unexplored at present [12]. Interestingly, sequences of OP phyla have been recovered from high or low temperature environments including hydrocarbon and chlorinated solvent contaminated aquifers [11]. Therefore, bioprospecting potentials in the areas of extremophilic enzymes development and intrinsic bioremediation approaches could be explored on cultivation of those OP members or identifying the functional genes associated with their survival in those environments.

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