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Total Culturable Bacteria and Bacterial Diversity in Northern intertidal areas of Persian Gulf

Ali Abolhasani Soorki, M. Sc.
Instructor, Faculty of Energy and Innovative Engineering, Shahid Beheshti University
Mohamad Roayaei Ardakani, Ph. D.
Assistant professor, Faculty of Science, Shahid Chamran University
Hossein Kermanian, Ph. D.
Assistant professor, Faculty of Energy and Innovative Engineering, Shahid Beheshti University

Abstract

Diversity of dominant marine bacteria was studied in nine different shoreline sites from Qeshm Island to Genaveh, in Northern Persian Gulf. Samples were collected from surface water and pelagic sediments. The total number of culturable bacteria in these sites ranged between 3.7×10^5 and 4.3×10^7, as determined by culture dilution method. These results showed relatively high concentration of marine bacteria. Serial dilution cultures were prepared from each sample on oligothrophic medium agar plates, made with filter sterilized natural seawater, and two isolates that were dominant in the number of colonies were chosen for identification. Using morphological and biochemical tests and determination of guanine plus cytosine content of DNA, members of the genera *Pseudomonas*, *Vibrio*, *Alteromonas*, *Flavobacterium*, *Bacillus*, *Mycobacterium* and *Alcaligenes* were identified and seemed to be abundant in investigation area.

Keywords: Microbial Biodiversity, Persian Gulf, Culturable Bacteria.

تنوع زیستی باکتریایی و تعداد کل باکتری‌های قابل کشت در منطقه جزری مدّی سواحل شمالی خلیج فارس

علی ابوالحسنی سورکی
کارشناس ارشد میکروبیولوژی، مربی دانشکده مهندسی فن‌آوری‌های نوین، دانشگاه شهید بهشتی
محمد رعایایی اردکانی
دکترای میکروبیولوژی، استادیار دانشکده علوم،
دانشگاه شهید چمران
حسین کرمانیان
دکترای صنایع خمیر کاغذ، استادیار دانشکده مهندسی فن‌آوری‌های نوین، دانشگاه شهید بهشتی

چکیده

تنوع زیستی باکتری‌های غالب دریایی در 9 ایستگاه در خط ساحلی شمالی خلیج فارس از جزیره قشم تا بندر گناوه مورد بررسی قرار گرفت. نمونه‌گیری از آب و رسوبات ساحلی انجام شد. شمارش باکتری‌ها با تکنیک most probable number نشان داد که تعداد کل باکتری‌های قابل کشت در این ایستگاه‌ها بین $3/7\times10^5$ تا $4/3\times10^7$ متغیر می‌باشد. این نتایج نشان دهنده تعداد نسبتاً بالای جمعیت‌های باکتریایی در آب و رسوبات خلیج فارس است. رقت‌های متوالی از نمونه‌ها بر روی پلیت‌های حاوی محیط کشت، تهیه شده از آب استریل دریا، تلقیح شد. پس از انکوباسیون و رشد مناسب کلنی‌ها، از هر نمونه دو کلنی با بیشترین تعداد، جهت شناسایی انتخاب گردید. با بررسی‌های مورفولوژیکی و بیوشیمیایی و همچنین تعیین درصد گوانین و سیتوزین DNA، اعضای جنس‌های سودوموناس، ویبریو، آلتروموناس، فلاووباکتریوم، باسیلوس، مایکوباکتریوم و آلکالیجنس شناسایی گردیدند و این‌گونه به‌نظر می‌رسد که در ایستگاه‌های مورد مطالعه جمعیت غالب را تشکیل می‌دهند.

کلیدواژه‌ها: تنوع زیستی میکروبی، خلیج فارس، باکتری‌های قابل کشت.

Introduction

Microorganisms play major roles in energy transformations and biochemical processes in exceedingly divers habitats especially in marine environments. Knowledge of both microbial diversity and microbial activity in costal area could be important for business and industry as well as in maintaining the health of near-shore ecosystems (Madigan *et al.* 2000). Studies in the past two decades show that heterotrophic bacteria not only function as decomposers, but also channel dissolved organic substances and inorganic nutrients into higher trophic levels through the microbial food web (Azam, 1998).

Several methods, in addition to plate counts, have been used to detect the number of active bacteria in natural samples. These have included microautoradiography (Hoppe, 1976) with various radioactive tracers, measurements of respiratory activity by the INT-formazan technique (Zimmermann *et al.,* 1978), and most probable numbers of marine bacteria in liquid media (Button *et al.*, 1993). The outcomes of these methods have one common feature: the numbers of active bacteria detected are always lower than the total counts determined by fluorescent staining. This observation has been taken as evidence for the presence of a large fraction of dormant or nonactive marine bacteria (Kjelleberg *et al*., 1993). On the other hand, data demonstrating the dominant occurrence of culturable bacteria in the sea have been reported. Rehnstam *et al.,* (1993), using 16 S rRNA probes, showed that single species of culturable bacteria can dominate the community DNA. Also Furham *et al.,* (1994) have reported that two culturable marine isolates each accounted for up to 20% of the bacterial community.

Assessment of the diversity of marine bacteria using identification with phenotypic tests and nutritional taxonomic approaches has shown a relatively small number of genera being found most frequently (Fry, 1987). Also it has been demonstrated that the major phylogenetic groups of bacteria most commonly abundant in marine habitates belonged to the gamma-proteobacteria, alpha-proteobacteria and Cytophaga-Flexibacter-Bacteroides (Stabili *et al.*, 2004). These groups contain many aerobic or facultative heterotrophs that are relatively easy to culture.

Unfortunately knowledge about culturable heterotrophic marine bacteria biodiversity of Persian Gulf is not available. Thus we focused our study on the quantitative composition of pelagic culturable heterotrophic bacterial communities in nine different sites of northern Persian Gulf.

Materials and Methods

Study site and sampling

Persian Gulf, arm of the Arabian Sea, southwestern Asia, is positioned between the Arabian Peninsula on the southwest and Iran on the northeast. The gulf extends northwest 970 km (600 mi) from the Strait of Hormuz to the Shatt al Arab, a river formed by the confluence of the Tigris and Euphrates Rivers (figure. 1, Microsoft Encarta Software). From nine sampling sites in northeastern shoreline, samples containing 25 ml surface water and around 5 g sediments were taken in sterilized sealed cap glass bottles and placed on ice. Study time was June 2004.

Media

Culture media used for counting of total culturable heterotrophic bacteria and isolation of dominant strains contained 0.75 g Bacto pepton (Difco) and 1.25 yeast extract (Merck) per liter filter sterilized seawater. For preparing solid media 12 grams agar agar (Merck) was added per liter. All chemicals used in this study were of analytical grade.

Total culturable bacteria

Counting of total culturable aerobic or facultative heterotrophic bacteria was carried out immediately after samples were taken. Each test included three series of culture dilutions (10^{-1}-10^{-9}) in microtest tissue culture plates, 96 well (Falcon). The plates were carried on ice to laboratory and were incubated at 20 °C over 7 days.

Isolation and maintenance

For isolation of numerically abundant bacteria, 100 microliter of serial dilutions of each sample were poured on marine yeast extract peptone agar plates and incubated at 22 °C for 7 days. All colonies were isolates and subcultured for further experiments. From each sample, two numerically dominant isolates were chosen based on colony and cellular morphology. For maintenance of isolates, dense pure cultures were prepared in marine yeast extract peptone broth containing 8% (v/v) dimethyl sulfoxide and frozen at -20 °C in tightly sealed 2 ml vials.

Identification

Chosen isolates were subcultured and identified using morphological, physiological and biochemical tests (Holt *et al.*, 1994). Total bacterial DNA was extracted and purified (Sambrook *et al.*, 1989) and Guanine plus cytosine content of DNA was determined by the HPLC method as described by Tamaoka & Komagata (1984). The Salmon sperm DNA and DNA obtained from *Bacillus subtilis* and *Pseudomonas aeroginusa* was used as control.

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2003.

Results and Discussion

Total Culturable heterotrophic bacteria counted in this study were higher than those reported by Stabili *et al.* (2004), possibly because of relatively higher temperature of seawater in Gulf than in other studied area. It also can be due to higher concentration of hydrocarbons and mineral nutrients discharged to the costal waters. Table 1 shows the confidence interval values (≥ 95%) of bacterial counts. The mean value of total bacteria counted was minimum 2.41×10^6 and maximum 2.54×10^7.

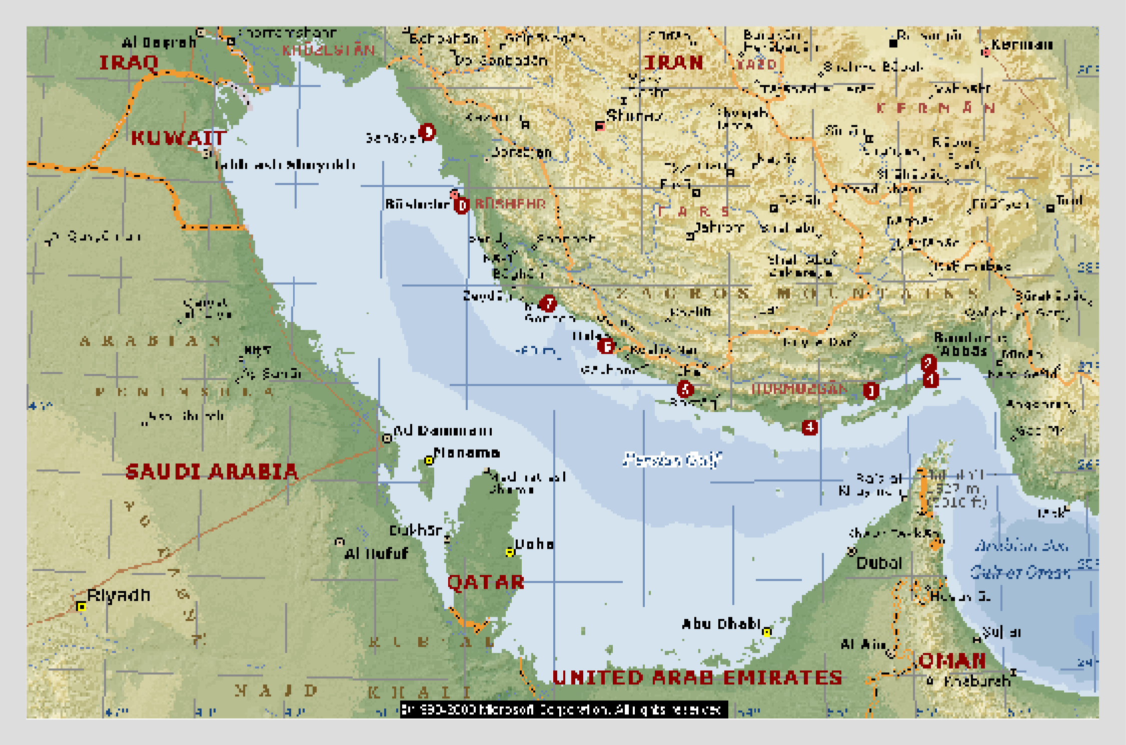

Figure 1- Map of Persian Gulf Showing the locations of sampling sites circled numbers 1-9.

Table 1- Total culturable heterotrophic bacteria in investigation sites as determined by culture dilution method.

Sample site	Confidence Interval ≥ 95%	
	Min.	*Max.*
1	$4.3*10^6$	$1.8*10^7$
2	$2.3*10^6$	$9.4*10^7$
3	$8.7*10^6$	$1.1*10^7$
4	$1.5*10^6$	$4.2*10^6$
5	$2.3*10^5$	$9.4*10^6$
6	$9.3*10^5$	$4.2*10^6$
7	$3.7*10^6$	$4.2*10^7$
8	$1.5*10^6$	$4.2*10^6$
9	$4.5*10^6$	$4.2*10^7$

Bacterial biodiversity

It was possible to identify about 77% of the isolates up to the genus level (table 2). Two strains from sampling sites 5 and 8 were lost during experiments, and also we were not able to put two of the remaining isolates in a known distinct genus, on the base of results from performed identification tests. Figure 2 shows the results in percentage of occurrences. As it is demonstrated, the most common genera isolated in this study belonged to the gamma subgroup of Proteobacteria, and included *Pseudomonas* (37%), *Vibrio* (14%) and *Alteromonas* (14%). Two genera of Gram positive bacteria, *Bacillus* (14%) and *Mycobacterium* (7%), were also found. *Flavobacterium*, a member of Bacteroides-Flavobacteria occupied 7% of the isolates. The remaining isolate, *Alcaligenes*, belongs to hydrogen-oxidizing bacteria of Kingdom Proteobacteria.

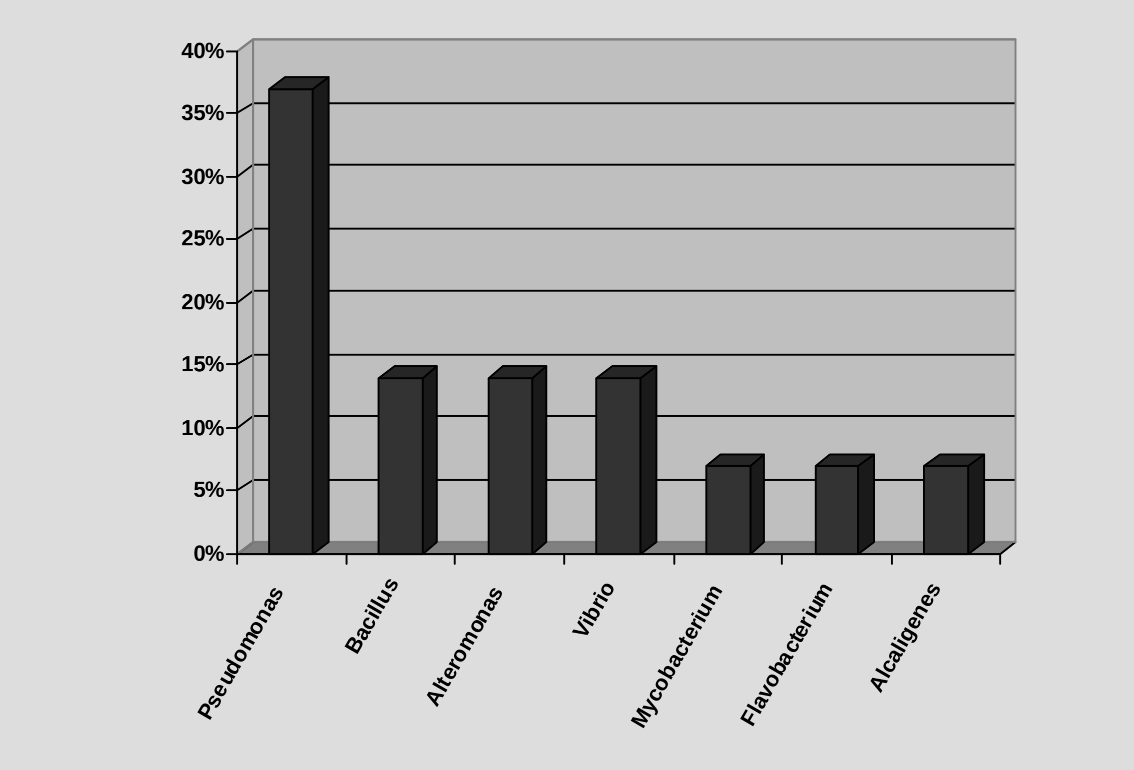

Figure 2- Bacterial genera isolated from northern shorelines of Persian Gulf and percentages of occurrence of each genus.

Table 2- Identification of two most abundant bacterial isolates from each sampling site. Two strains from investigation sites 5 and 8 were lost, and it was not possible to determine two other strains. M: marine (require high concentrations of magnesium and calcium ions for growth), ND: not detected.

Test		Site 1		Site 2		Site 3		Site 4		Site 5	Site 6		Site 7		Site 8	Site 9	
		Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 1	Strain 2
Colony	Shape	Circular	Punctiform	Irregular	Circular	Punctiform	Circular	Circular	Circular	ellipsoidal	Circular	Circular	Circular	Circular	Irregular	Circular	Circular
	Pigment	Orange	Reddish orange	Cream	-	Yellow	Reddish orange	-	White	Cream	Cream	-	Orange	-	-	-	-
	Diameter (mm)	1-2	1-2	4-7	3-5	1-2	1-2	5-9	4-5	3-4	7-8	3-4	1-2	2-3	ND	3-5	2-3
Cell Shape		Short rod	Short rod (curved)	Rod	Short rod	Short rod (curved)	Short rod	Short rod (curved)	Short rod	Rod	Rod	Rod	Rod (curved)	Short rod	Short rod	Short rod	Short rod
Size (m)		1.2	2.5	2.2	3.3	2.2	1.8	1.7	2	2.5	4.5	2.7	2.5	1.3	1.2	2	2.1
Gram Reaction		-	-	+	-	-	+ (weak)	-	-	-	+	-	-	-	-	-	-
Motility		-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+
Growth at 41 °C		-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Growth at salinity (%NaCl)		0-4	2-8 M	0-8	0-5	3-8	2-8	3-8 M	3-10 M	0-6	0-6	0-5	2-9 M	0-6	0-8	0-5	0-6
Endospore		-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
Capsule		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase		+	-	-	+	-	-	+	+	+	-	+	-	+	-	+	+

Continue Table 2-

Test		Site 1		Site 2		Site 3		Site 4		Site 5	Site 6		Site 7		Site 8	Site 9	
		Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 1	Strain 2
Gelatin hydrolysis		+	+	-	+	+	-	+	+	+	-	-	+	+	-	+	+
Starch hydrolysis		-	+	-	+	+	-	+	+	+	-	-	-	-	-	+	+
OF test	Oxidative	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+
	Fermentative	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Anaerobic growth		-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Urease		-	ND	-	+	ND	ND	+	-	+	-	-	-	-	-	-	-
Flagella		-	+	ND	+	+	ND	+	+	+	+	+	+	+	-	+	+
Acid Fast staining		-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Nittrate reduction		-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-
Denitrification		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mol% G + C		38.7	43.2	47.9	40.1	46.1	44.4								52.1		
Proposed genus		***Flavobacterium***	***Alteromonas***	***Bacillus***	***Pseudomonas***	**Not identified**	***Mycobacterium***	***Vibrio***	***Vibrio***	***Pseudomonas***	***Bacillus***	***Alcaligenes***	***Alteromonas***	***Pseudomonas***	**Not identified**	***Pseudomonas***	***Pseudomonas***

References

Azam, F. (1998). Microbial control of oceanic carbon flux: the plot thicken. *Science,* 280: 694-696.

Button, D. K., F. Schut, P. Quang, R. Martin, and B. R. Robertson (1993). Viability and isolation of marine bacteria by dilution culture: theory, procedures, and initial results. *Appl. Environ. Microbiol.,* 59:881–891.

Fry, J.C. (1987). Functional role of major groups of bacteria associated with detritus. In: D.J.W. Moriarty and R.S.V. Pullin, (eds.). *Detritus and Microbial Ecology in Agriculture.* PP. 83-122. International Center for Living Aquatic Resources Management, Manila, Philippines.

Furham J.A., S.H. Lee, Y. Masuchi, A.A. Davis and R.M. Wilcox (1994). Characterization of marine prokaryotic communities via DNA and RNA. *MIicrob. Ecol.,* 28: 133-145.

Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T.Staley and S.T. Williams (1994). *Bergey's Manual of Determinative Bacteriology.* Baltimore, Williams & Wilkins.

Hoppe, H.G. (1976). Determination and properties of actively metabolizing heterotrophic bacteria in the sea, investigated by means of microautoradiography. *Mar. Biol.,* 36:291–302.

Kjelleberg, S., B. G. Flardh, T. Nystrom, and D. J. W. Moriarty. (1993). Growth limitation and starvation of bacteria, p. 289–320. In T. E. Ford (ed.), *Aquatic microbiology.* Blackwell Scientific Publications, Boston.

Madigan M., J. Martinko and J. Parker (2000). *Brock biology of microorganisms.* 9th ed.

Sambrook, J., E.F. Fritsch & T. Maniatis (1989). Molecular Cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Stabili L. and R.A. Cavallo (2004). Biodiversity of culturable heterotrophic bacteria in the southern Andeiatic sea Italian coastal waters. *Scienta Marina.,* 68: 31-41.

Tamaoka, J., and K. & Komagata (1984). Determination of DNA base composition by reversed-phase high performance liquid chromatography. *FEMS Microbiol Lett.,* 25, 125-128.

Zimmermann, R., R. Iturriaga, and J. Becker-Birck (1978). Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. *Appl. Environ. Microbiol.,* 36:926–935.