OnLine Journal of Biological Sciences 2 (11): 740-743, 2002
ISSN 1608-4127

Heterotrophic Marine Bacteria Attached to Leaves of *Avicennia marina* L. Along the Qatari Coast (Arabia Gulf)

Adel M. Mahasneh
Department of Biological Sciences, University of Jordan, Amman, Jordan

Abstract: Total viable heterotrophic aerobic marine bacteria attached to the leaves of *Avicennia marina* L. from two sites of a mangrove forest along the Qatari Coast did not show significant differences between sites. Maximum counts were recorded in both sites during September and October 1998 and lowest during May and April for site 1 and 2 respectively. Of the 5 bacterial groups studied, proteolytic bacterial counts in both sites were slightly higher then cellulolytic, amylolyic and lipolytic bacteria. Cellulolytic counts were rather prominent in site 2 during July 1998 to February 1999. However, spore formers were very low in both sites. The attached bacterial abundance and densities are correlated with counts in sea water of the mangrove sites. These bacterial variations may be related to atmospheric and leaf temperatures and probable inhibiting substances released by the plant leaves. The concept that only small part of the microbial community is active at any given time appears to be inviting in such cases.

Key words: *Avicennia marina*, marine environments, bacteria, sea water

Introduction

Invisible activity in the mangrove ecosystem could be estimated by just scooping few grams of mangrove soil or surrounding waters and examining it microscopically (Mahasneh, 2001a; O'Grady *et al*., 1996; Rivera-Monroy *et al*., 1995). One would be amazed to find greater than 10^9 bacterial cell per gram of soil, which keeps the system in equilibrium if not disturbed (Morita, 1988; Lugo and Snedaker, 1976). The indigenous marine bacteria help breaking down all leaf litter and other sources of nutrients (Twilley, 1985). In marine environments, most of the water in mangroves comes from the sea. Little fresh water may come from rainfall if there are no rivers. The presence of any individual mangrove species within the forest is reliant on several environmental factors including salinity, nutrient availability, atmospheric and water temperatures, oxygen and probably wave energy (Day *et al*., 1996; O'Grady *et al*., 1996). Unfortunately for a long time, the ecological importance of mangrove was not recognized (Miller, 1972). These plants live within diverse communities in the intertidal zone of tropical to subtropical coast, rivers, estuaries, bays and carbonate sediments around reef associated islands (McGuinness, 1977). The mangrove growth is essential to sustain life of other organisms in the system including macro and microorganisms (Mahasneh, 2001b). In mangrove ecosystem nothing is wasted, nutrient rich leaves are biodegraded by bacteria and fungi or eaten by organisms living on the forest floors (Wafar *et al*., 1997). Viable marine plants secrete varying photosynthates that are used by heterotrophic marine bacteria attached to different parts of the plant (Sieburth, 1979; Klug, 1980). Mangrove leaves are not an exception and they provide a large surface area for the attachment of diverse flora and fauna which may be consumed by other marine life (Ogden, 1980; Wahbeh and Mahasneh, 1984; Mahasneh and Al-Sayed, 1997). The extent of bacterial colonization and attachment to macrophyte surfaces has been studied partly (Harborne, 1977; Mahasneh, 2000). Review of literature indicates scarcity of published information on the abundance and seasonal distribution of certain marine bacteria associated with *Avicennia marina* leaves all over and specially in the Arabian Gulf region. Hence, the distribution and abundance of some bacteria attached to *A. marina* leaves as affected by some environmental factors are presented in this study. The bacterial group composition and seasonal variations are also studied.

Materials and Methods

Study sites and sample collection: *Avicennia marina* L. leaves samples were collected from two different sites, Al-Khor site 1 and Dakhira site 2.

Site 1 is located at a mangrove (*A. marina*) forest in the inter-tidal zone near Ras-Al-Matbach (Fig. 1). The neighbouring terrestrial area is covered with halophytic plants and Sabkhas. The site floor is rather sandy sediment with abundant fragments of shells with no obvious anaerobiosis of soil. Site 2 (Dakhira) located at a mangrove forest at about 7 km north of site 1 (Fig. 1). The site floor is sloppy gray sediment characterized by anaerobiosis activity exemplified by soil blackening. The site exhibits lower density of *A. marina* trees compared to site 1, but it is in the vicinity of Dakhira town. The neighbouring terrestrial area is heavily covered with halophytes.

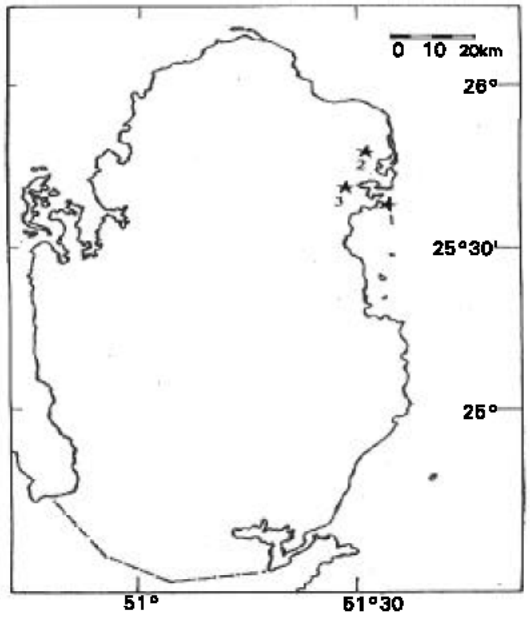

Fig. 1: Map of Qatar showing the study site 1 Al-Khor and site 2 Dakhira

Composite samples of *A. marina* leaves were collected monthly in sterile sampling bags and were placed in an ice box and were transported to the laboratory within 2-3 h of collection for analysis.

Bacteriological analysis and culture conditions: For bacterial analysis *A. marina* leaves (1 g) were washed with sea water to remove free bacteria. The rinsed leaves sample was cut into small pieces using sterile scissors and were then aseptically transferred

into 9 ml of sterile aged sea water, shaken vigorously using a vortex mixer and appropriate serial dilutions were prepared in order to be plated into suitable marine agar plates to enumerate the attached marine bacteria.
For bacterial spores determination, leaf samples were heated at 80°C for 20 min and samples were then plated into suitable plates of marine agar plates (Difco, USA). Bacterial counts were then carried out using the media, temperatures and incubation times listed below:

a) Marine agar (Difco, USA) plates for total viable marine bacteria and spore formers
b) Marine agar plates supplemented with 2% (w/v) cellulose to enumerate cellulase producers
c) Marine agar plates supplemented with 2% skimmed milk to detect bacterial colonies producing the protease enzyme
d) Marine agar plates supplemented with 2% (w/v) starch to detect amylase producers
e) Marine agar plates with added 2% (v/v) tween 80 to detect lipase producers.

All samples were plated in duplicates at least, incubated at 30 ± 1°C for 2-4 days and numbers were then recorded. Results of enumeration are reported as cfu. g^{-1} of leaves on weight bases. All culture media were prepared in accordance with the directions of the manufacturers.

Environmental parameters: Water and atmospheric temperature were recorded manually using a portable thermometer. pH was determined in the laboratory using a digital pH-meter, salinity was measured using a temperature compensated salinometer (Cambridge Instruments Inc., Buffalo, USA).
Analysis of variance was applied to the overall bacterial groups mean values recorded in different sites using the SPSS.

Results and Discussion

Bacteria attached to surfaces of *A. marina* leaves: At both sites Al-Khor 1 and Dakhira 2, annual mean atmospheric, water temperatures varied seasonally, however salinity and pH values did not vary significantly between sites to the extend of affecting bacterial numbers attached to *A. marina* leaves (Table 1). Total bacterial counts per gram wet leaf weight varied between 10^3 in May 1999 to $3.4x10^6$ in October 1998. Numbers for the other months ranged between $1.2x10^5$ to $7.4x10^5$. No significant changes were observed, however, maximum counts were recorded during November and January i.e. towards late fall to winter season (Tables 1, 2). Wahbeh and Mahasneh (1984) studying bacteria attached to leaves of sea-grass reported highest densities of total heterotrophic aerobic bacteria during summer which coincided with highest productivity of the grass and numbers were higher averaging between $11x10^8$ to 10^7.
For protease, amylase, cellulase, lipase producing bacteria, the maximum number per gram weight of *A. marina* leaves were recorded in October 1998 and ranged between $2.1x10^6$ for protease producers to $3.4x10^6$ for total counts and they followed closely related pattern of distribution, showing no defined seasonal pattern except the increase which appeared during October 1998 (Tables 2, 3). These bacterial counts fall within lower ranges reported for other species of macrophytes including some sea-grasses (Mazure and Field, 1980; Newell, 1981; Shiba and Taga, 1980). Wallberg *et al.* (1999) reported higher bacterial counts and primary production during rainy season in a tropical coastal ecosystem. Spore forming bacteria attached to *A. marina* leaves in this study were low 10^2 to 10^3 per gram of wet weight with no regular or seasonal pattern (Table 2). These numbers compare well with spore-forming bacterial numbers in the open water of the same site (Mahasneh, 2000, 2001a,b) and Dakhira site 2 of the same study (Table 4). The level of microbial colonization of

Table 1: Study sites and some environmental parameters

Site and location	Water depth (m)	Water temp. (°C)	Atmospheric temp. (°C)	Salinity (g L^{-1})	Water pH
Al-Khor (1)	1.5	26.4 ± 2.68	31.35 ± 2.95	48 ± 3.45	7.8 ± 0.18
25° 40 00N		(20-35)	(18-45)	(42-60)	(7.8-8.2)
51° 36 85E					
Dakhira (2)	1.5	27.25 ± 2.65	30.20 ± 1.75	41.5 ± 1.62	7.81 ± 0.31
25° 54 32N		(21-34)	(20-43)	(41-50)	(7.8-8.25)
51° 35 00E					

Table 2: Distribution of different bacteria (cfu. g^{-1}) of *A.marian* leaves at Al-Khor site 1 and Dakhira site 2. Annual log means ± SD for the period June 1998 to May 1999

	Bacteria					
Site	Tota	Amy	Celu	Prot	Lipa	Spor
Ak-Khor 1	5.44 ± 0.77	5.0 ± 0.86	5.1 ± 0.83	4.76 ± 0.95	4.92 ± 0.87	2.93 ± 0.73
	(3.3-6.56)	(3.08-6.41)	(3.08-6.38)	(3-6.62)	(3.4-6.49)	(2-4.34)
Dakhira 2	5.55 ± 0.76	5.0 ± 0.73	5.26 ± 0.75	5.0 ± 0.81	4.51 ± 0.85	3.19 ± 0.53
	(3.9-6.72)	(4.20-6.59)	(4.15-5.26)	(4-6.34)	(2.68-6.38)	(2.3-4.34)

Numbers in brackets indicate minimum and maximum numbers recorded during the study

Table 3: Bacterial counts (X10^5 g^{-1} wet weight) attached to *A. marina* laves from Al-Khor site 1 at different sampling dates

Type of Bacteria	Jun.	Jul.	Sept.	Oct.	Nov.	Dec.
1998						
Tota	6.7 ± 0.12	7.4 ± 0.10	0.17 ± 0.04	134.0 ± 0.21	2.2 ± 0.01	7.1 ± 0.34
Amy	0.205 ± 0.01	5.2 ± 0.35	1.7 ± 0.12	25.0 ± 0.42	0.28 ± 0.15	0.35 ± 0.05
Celu	0.18 ± 0.06	0.65 ± 0.10	4.5 ± 0.16	25.0 ± 0.42	1.55 ± 0.08	3.0 ± 0.18
Prot	7.65 ± 0.35	7.4 ± 0.48	0.12 ± 0.05	21.0 ± 0.95	0.16 ± 0.06	0.12
Lipa	2.0 ± 0.11	8.1 ± 0.62	2.3 ± 0.18	30.0 ± 0.85	0.46 ± 0.28	0.03 ± 0.01
Spor	0.21 ± 0.02	0.07 ± 0.003	0.01 ± 0.31	0.001 ± 0.001	0.005 ± 0.0001	0.001 ± 0.001
1999	Jan.	Feb.	Mar.	Apr.	May	
Tota	12.0 ± 0.51	1.35 ± 0.12	2.95 ± 0.1	1.6 ± 0.3	0.03 ± 0.01	
Amy	3.0 ± 0.21	2.5 ± 0.18	0.012 ± 0.01	3.5 ± 0.03	1.20 ± 0.1	
Celu	5.0 ± 0.50	0.021 ± 0.05	1.7 ± 0.22	1.55 ± 0.02	1.35 ± 0.08	
Prot	5.5 ± 0.25	0.69 ± 0.17	3.6 ± 0.18	1.25 ± 0.1	0.02 ± 0.01	
Lipa	0.37 ± 0.02	0.33 ± 0.12	3.8 ± 0.17	1.3 ± 0.10	0.05 ± 0.01	
Spor	0.005 ± 0.001	0.05 ± 0.001	0.4 ± 0.001	0.03 ± 0.002	0.06 ± 0.004	

Total bacterial counts (Tota), producers of amylase (Amy), cellulase (Cellu), protease (Prot), lipase (Lipa) and spore formers (Spor)

Table 4: Bacterial counts (10^5 g^{-1} wet weight) attached to *A. marina* leaves from Dakhira site 2 at different sampling dates (Means ± S.D)

Type of Bacteria	Jun.	Jul.	Sept.	Oct.	Nov.	Dec.
1998						
Tota	0.82 ± 0.12	35.00 ± 1.3	48.00 ± 1.5	3.50 ± 0.25	4.40 ± 0.42	6.15 ± 0.65
Amy	0.21 ± 0.02	37.50 ± 1.8	0.34 ± 0.13	0.49 ± 0.08	1.05 ± 0.03	0.24 ± 0.02
Celu	24.00 ± 3.8	46.00 ± 6.85	0.32 ± 0.05	4.60 ± 0.33	3.80 ± 0.28	0.25 ± 0.04
Prot	0.22 ± 0.03	21.00 ± 0.42	0.16 ± 0.02	2.90 ± 0.14	0.95 ± 0.08	0.11 ± 0.02
Lipa	5.3 ± 0.52	13.00 ± 1.24	0.24 ± 0.04	0.93 ± 0.18	1.50 ± 0.25	0.19 ± 0.1
Spor	0.21 ± 0.01	0.05 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.001 ± 0.001	0.004 ± 0.002
1999	Jan.	Feb.	Mar.	Apr.	May	
Tota	8.70 ± 0.71	6.80 ± 5.5	1.30 ± 0.10	0.89 ± 0.11	0.12 ± 0.02	
Amy	2.85 ± 0.12	9.10 ± 2.36	0.89 ± 0.09	1.50 ± 0.17	0.16 ± 0.03	
Celu	7.90 ± 0.78	5.90 ± 0.58	1.20 ± 0.17	0.64 ± 0.06	0.14 ± 0.01	
Prot	4.90 ± 0.72	9.50 ± 0.82	0.19 ± 0.03	2.00 ± 0.90	0.15 ± 0.02	
Lipa	0.34 ± 0.001	0.05 ± 0.001	0.32 ± 0.02	2.60 ± 1.10	0.22 ± 0.03	
Spo	0.01 ± 0.003	0.01 ± 0.0	0.03 ± 01	0.00 ± 0.0	0.01 ± 0.002	

Table 5: Percentage group composition of bacteria attached to leaves of *A. marina* in Al-Khor (site 1) and Dakhira (site 2) based on annual log means of cfu. g^{-1}

	Percentage composition	
Bacterial groups	site 1	site 2
Amylolytic	21.6	21.7
Cellulolytic	23.2	23.4
Proteolytic	21.2	21.7
Lipolytic	21.8	19.6
Spore formers	12.8	13.6

surfaces of marine plants appears to be at least partially regulated by antifouling substances as polyphenols, tannins and other inhibitory substances which have been reported in certain marine plants (Conover and Sieburth, 1964; McMillan *et al.*, 1980). Harborne (1977) reported that flavanoids, phenolic compounds and tannins are water soluble and could be antagonistic to microbial attachment and invasion of marine plants and *A. marina* would not be an exception and this require further investigation. Bacterial overall abundance and seasonal distribution of Dakhira site 2 leaf surfaces of *A. marina* did not differ significantly from that in Al-Khor site 1. Maximum total bacterial counts were recorded in September 1998 and reached 4.8×10^6 while minimum numbers were recorded during June 1998 and April 1999 (Tables 3, 4). Cellulase, amylase and protease producing bacteria appeared to be slightly higher than other bacterial counts during the study period (Table 4). This trend had been observed earlier in sea-water of the study sites (Mahasneh, 2000). Unique to *A. marina* leaves of Dakhira site 2 is the rather increased counts of cellulase producing bacteria during the period July 1998 to February 1999 (Table 4); no immediate explanation could be provided from these results. However, highly significant correlations ($P < 0.05$) have been observed between amylolytic group ($r = 0.82$), cellulolytic ($r = 0.89$), proteolytic ($r = 0.75$) and total counts in site 1 and the same trend more or less was observed for Dakhira site 2. No correlations were observed for spore formers in both sites. The concept that only small part of the microbial community is active at any given time appears to be inviting in such cases (Parkes *et al.*, 1984).

Table 5 presents the group composition of the bacteria studied based on the annual mean counts. Cellulase producing bacteria were rather dominant through out the study period in both sites 1 and 2. However, slightly significant differences ($P < 0.05$) were observed between these groups and protease producers in one hand and spore formers in the other hand both within the site and between the two sites. Generally, Dakhira site 2 appears to exhibit rather higher percentages of cellulase, protease producers as well as higher spore forming bacteria. Harborne (1977) and Tomlinson (1980) indicated that the extent of bacterial attachment to the surfaces of marine plants is thought to be linked to the antifouling substances such as poly phenols, flavons and tannins which have been reported in several marine plants (McMillan *et al.*, 1980). Wahbeh and Mahasneh (1984) and Bowen (1980) did not rule out the effect of factors related to microbial competition and microbial antagonistic interactions. This could be understood if amylolytic and proteolytic ability of bacteria is linked in most instances to their ability to produce certain antibiotics (Mahasneh, 1977).

Acknowledgment

This work was supported by a grant number 1-4/1998 from the Scientific and Applied Research Center (SARC) of the University of Qatar. Thanks are also due to the University of Jordan. Dr. A. Zugol is thanked for statistical analysis. Mrs. Smita Mehta is thanked for her technical assistance and for typing the manuscript.

References

Bowen, G.D., 1980. Misconceptions, Concepts and Approaches in Rhizosphere Biology. In: D.C. Ellwood, J.N. Hedger, M.J. Latham, J.M. Lynch and J.H. Slater (Editors). Contemporary Microbial Ecology, Academic Press London, pp: 283-304.

Conover, J.T. and J.M. Sieburth, 1964. Effect of *Sargassum* distribution on its, epibiota and antibacterial activity. Bot. Mar., 6: 147-157.

Day, J.W.Jr., C. Coronado-Molina, F.R. Verra-Herrera, V.H. Rivera-Monory, H. Alvarez-Guiellen, R. Day and W. Conner, 1996. A 7 year record of above-ground net primary production in southeastern Mexican mangrove forest. Aquat. Bot., 55: 39-60.

Harborne, J.B., 1977. Flavonoid sulphates: a new class of natural products of ecological significance in plants. Prog. Phytochem., 4: 189-208.

Klug, M.J., 1980. Detritus-decomposition relationships. In: R.C. Phillips and C.P. McRoy (Ed.), Hand Book of Seagrass Biology: An Ecosystem Perspective. Garland, New York, pp: 373-385.

Lugo, A.E. and S.C. Snedeker, 1976. Mangrove ecosystem analysis. In: B.C. Pattern (Ed.), System Analysis and Simulation in Ecology. Academic Press, New York, pp: 113-145.

Mahasneh, A.M., 1977. Genotypic manipulation of bacteria producing several glucanases. Ph.D. thesis, Heriot-Watt University, Edinburgh, Scotland, pp: 1-15.

Mahasneh, A.M. and H.A. Al-Sayed, 1997. Seasonal incidence of some heterotrophic aerobic bacteria in Bahrain Pelagic and near shore water and Oysters. Intern. J. Environ. Studies, 51: 301-312.

Mahasneh, A.M., 2000. Distribution, abundance and seasonal variation of aerobic heterotrophic marine bacteria attached to *A. marina* leaves and surrounding water at the Qatari Coast. A Report Prepared for SARC, University of Qatar, Doha, Qatar, pp: 22-52.

Mahasneh, A.M., 2001a. Bacterial decomposition of *Avicennia marina* leaf litter from Al-Khor (Qatar-Arabian Gulf). OnLine J. Biol. Sci., 1: 717-719.

Mahasneh, A.M., 2001b. Seasonal incidence of some heterotrophic aerobic marine bacteria in an *Avicennia marina* habitat along a Qatari Coast. OnLine J. Biol. Sci., 1:666-670.

Mazure, H.G.F. and J.G. Field, 1980. Density and ecological importance of bacteria on klep fronds in an upwelling region. J. Exp. Mar. Biol. Ecol., 48: 173-182.

McGuinness, K.A., 1977. Dispersal, establishment and survival of *Cariops australis* propagules in a north Australian mangrove forest. Ecology, 109: 80-87.

McMillan, C., O. Zapata and L. Escober, 1980. Sulphated phenolic compounds in seagrass. Aquat. Bot., 8: 267-278.

Miller, P.C., 1972. Bioclimate, leaf temperature and primary production in red mangrove canopies in South Florida. Ecol., 531: 22-45.

Morita, R.Y., 1988. Bio-availability of energy and its relationship to growth and starvation-survival in nature. Can. J. Microbiol., 34: 436-441.

Newell, Y., 1981. Fungi and bacteria in or on leaves of seagrass (*Zastra marina* L.) from Chesapeake Bay. Appl. Environ. Microbiol., 41: 1219-1224.

Ogden, J.C., 1980. Faunal Relationships in Caribbean Seagrass Beds. In: R.C. Phillips and C.P. McRoy (Editors). Hand Book of Seagrass Biology: An Ecosystem Perspective. Garland, New York, pp: 173-198.

O'Grady, A.P., P.K. McGuinness and D. Eamms, 1996. The abundance and growth of *A. marina* and *Rhiziphora stylosa* in the low shore zone of Darwin Harbour, Northern Territory. Aust. J. Ecol., 21: 272-279.

Parkes, R.J., J. Taylor and D. Jork-Ramberg, 1984. Demonstration using *Desulfovibrio* sp. of two pools of acetate with different biological availability in marine pore wales. Mar. Biol., 83: 271-276.

Rivera-Monroy, V.H., J.W. Day, R.R. Twilley, F. Vesa-Herrera and C. Coronade-Molina, 1995. Flow of nitrogen and sediment in a fringe of mangrove forest in Terminos Lagoon, Mexico. Est. Coast, Shelf Sci., 40: 139-160.

Shiba, T. and N. Taga, 1980. Heterotrophic bacteria attached to seawweeds. J. Exp. Mar. Biol. Ecol., 47: 251-258.

Sieburth, J.M., 1979. Sea Microbes. Oxford University Press, New York, pp: 360-491.

Tomlinson, P.B., 1980. Leaf morphology and anatomy in sea grasses. In: R.C. Phillips and C.P. McRoy (Ed.). Hand Book of Seagrass Biology: An Ecosystem Perspective. Garland, New York, pp: 7-28.

Twilley, R.R., 1985. The exchange of organic carbon in mangrove forest in a south west Florida estuary. Est. Coast. Shelf Sci., 20: 543-557.

Wafar, S., A.G. Untawale and M. Wafar, 1997. Litter-fall and energy flux in a mangrove ecosystem. Est. Coast. Shelf. Sci., 44: 111-124.

Wahbeh, M.I. and A.M. Mahasneh, 1984. Heterotrophic bacteria attached to leaves rhizomes and roots of three seagrass species from Aqaba (Jordan). Aqua. Bot., 20: 87-96.

Wallberg, P., P.R. Jonsson and R. Johnstone, 1999. Abundance biomass and growth rates of pelagic microorganisms in a tropical coastal ecosystem. Aqua. Microbiol. Ecol., 18: 175-185.