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Bacterial Decomposition of *Avicennia marina* Leaf Litter from Al-khor (Qatar-Arabian Gulf)

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Abstract: Overall rate of bacterial decomposition of *Avicennia marina* L. leaves of 0.34% day^{-1} was recorded on dry weight basis after 256 days of in situ incubation. Initial loss of 68.45% was observed after first 108 days and additional 20.66% loss was achieved at the end of the experiment after 256 days. Decomposing leaf litter samples harbored variable mean counts of bacteria ranging from 146 x10^6 of amylolytic bacteria to the lowest of 0.029X10^6 of the spore formers per gram wet weight. The dominant bacterial groups during decomposition were non spore formers and were in the order amylolytic > proteolytic > cellulolytic > lipolytic > spore formers. Highest decomposition percentage was observed during warm months.

Key words: Marine bacteria, *A.marina*, decomposition, sea-water.

Introduction

Mangrove forests are part of a productive ecosystem that supports abundant life through food chains that start with the trees in that ecosystem (Wahbeh and Mahasneh, 1985; Mackey and Smail, 1996). In the mangrove system nutrient rich leaves are biodegraded by fungi (Tan and Pek, 1997), bacteria (Wafar *et al*., 1997; Shome *et al*., 1995), or eaten by marine animals and macro fauna that live on the forest floor (Kohlmeyer *et al*., 1995; Schrijvers *et al*., 1995; Lee, 1995). Decaying organic material breaks down into small particles and detritus covered with protein rich bacterial films (Mahasneh, 2000). The nutrients which are released into the water through bacterial degradation of fallen leaves would be available for fishes, prawns, mollusks and crustaceans and finally to planktons (O'Grady *et al*., 1996). The decomposition process is not confined to mangrove environments but the decomposition products are transported to the sea through tidal cycles (Findlay *et al*., 1990). Measurement of weight loss and marine bacterial counts associated to detritus are commonly used in studying detrital decomposition (Rublee and Roman 1982; Wahbeh and Mahasneh, 1985). This article investigates the decomposition rate of *Avicennia marina* L. leaves and the quantitative as well as ecological group composition of the heterotrophic aerobic marine bacteria involved in the process.

Materials and Methods

Decomposition experiment: *Avicennia marina* L. composite leaves samples were collected from *A.marina* forest at Al-Khor (Arabian Gulf Qatari Coast). The leaves were rinsed clean and air-dried for about 72 h. These leaves samples were further dried at 105^{0}C to constant weight. Subsamples (10 g) were placed in each of 14 one litre glass jars. 1mm mesh nylon netting was used to seal the jars in order to allow the exchange of bacteria and meofauna and protozoans less than 1mm in size. The jars were then placed on the bottom of 1.5 m deep seawater of *A.marina* forest. At varying time intervals starting on June 1998 to February 1999 two jars at a time were taken from the seawater and checked for changes in the dry weight of the decomposing *A.marina* leaves. The samples of each interval were dried at 105^{0}C to constant weight. Weight loss was the difference between the initial weight of the incubated leaves sample and that of the sample after each time interval.

Bacterial enumeration and culture conditions: The jar incubated sub-samples of *A.marina* decomposing leaves were aseptically transferred and gently rinsed in sterile seawater to remove bacteria of seawater origin. Bacteria was then extracted from the decomposing leaves by vortexing for 5 minutes 1 g wet weight material in sterile screw-cap test tube containing 9ml of sterile seawater. Appropriate serial dilutions were prepared and 0.1 ml aliquots were plated onto suitable marine agar plates.

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. For bacterial spore determination, samples were heat treated at 80^{0}C for 20 min. and then plated.
b. Marine agar plates supplemented with 2% (w/v) cellulose to enumerate cellulase producers.
c. Marine agar plates supplemented with 2% (w/v) 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 incubated at 30 $\pm$1^{0}C for 2-4 days and numbers were then recorded. Results of enumeration are reported as cfu. g^{-1} of sample on wet weight basis. Water, atmospheric temperature, pH and salinity was also measured.

Table 1: Some environmental characteristics of *A.marina* decaying leaves experiment. The site of incubation was 1.5 m deep at Al-Khor coastal waters (25^{0}40 00 N and 51^{0}36 25 E)

Date and days of decomposition	Atmospheric T (C^0)	Water T (C^0)	Water (pH)	Water salinity g L^{-1}
2.7.98 (16)	44	31	7.81	49
30.5 (108)	35	33	7.95	49
25.10 (133)	31	28	8.10	41
29.11 (168)	23	19	8.19	41
31.1.99 (231)	22	18	8.00	41
25.2 (256)	25	18	8.00	43
Mean $\pm$ S.D	31.35$\pm$ 2.95	26.4$\pm$ 2.68	7.8$\pm$ 0.18	48$\pm$ 3.45

Table 2: Bacterial counts (*10^6cfu.g^{-1}) associated with decomposing *A .marina* leaves at different incubation times. (Mean ± SD)

Date and decomposition	Days of	Bacteria					
		Tota	Prot	Amy	Celu	Lipo	Spor
2.7.98	16	37±1.20	120±22.5	145±2.40	115.5±4.30	16.6±3.0	0.07±0.024
30.9	108	80±2.10	0.16±0.12	14±2.30	1.7±0.15	0.5±0.04	0.0027±0.00036
25.10	133	1.9±0.85	2.3±1.10	7.7±0.75	0.51±0.22	1.3±0.08	0.00±0.00
29.11	168	1.4±0.06	0.75±0.04	0.52±0.32	0.71±0.025	0.59±0.01	0.0008±0.00011
31.199	231	0.88±0.05	0.51±0.31	0.18±0.08	1.1±0.21	0.15±0.07	0.046±0.0022
25.2	256	1.7±1.32	1.60±0.68	0.8±0.023	1.50±0.11	0.52±0.02	0.058±0.0032
overall mean		20.50±0.75	20±0.83	27±2.50	19.83±1.80	3.34±0.76	0.029±0.00024

Total bacteria (Tota), Proteolytic (Prot), Amylolytic (Amy), Cellulolytic (Celu), Lipolytic (Lipo) and Spore forming (Spor) bacteria.

Results

Table 1 presents some environmental parameters of Al-Khor study area. Water temperatures were strongly correlated with the atmospheric temperatures ($r = 0.9812$; $P < 0.001$). Mean water temperature during incubation was 26.4° C±2.68. Water pH and salinity were rather stable (annual means = 7.8±0.18 and 48±3.45 g L^{-1}) respectively. Maximum total bacterial counts reached 80X10^6 cfu.g^{-1} weight of decaying leaves after 108 days then numbers decreased to the level of 1 to $^{1.7 \times 106}$ cfu.g^{-1} and remained so until the experiment terminated after 256 days (Table 2). Proteolytic, amylolytic, cellulolytic and lipolytic bacterial counts maximum densities were recorded after 16 days of decomposition and were 120, 145, 115 and $^{16 \times 106}$ cfu.g^{-1} respectively (Table 2). These numbers stayed rather high from 16-133 days of decomposition and then declined and remained so to the end of the experiment after 256 days. Spore formers stayed almost at the same level all through the study period. Among the 5 groups studied the overall mean counts indicate the dominance of the amylolytic group of bacteria (30.32%) followed by proteolytic and cellulolytic which formed about 21% each (Table 3). The lowest were the lipolytic with 3.45% and spore-forming about 0.03%. All groups were present except the spore-formers group which was not detected in samples taken after 133 days of decomposition only. Spore-forming bacterial counts were very low and they were 6 to 7 orders of magnitude lower than other bacterial groups. However strong correlation was observed between amylolytic and each of proteolytic ($r = 0.9542$; $P < 0.001$) and cellulolytic ($r = 0.9654$; $P < 0.001$) at early stages of decomposition. No significant correlations were observed between spore formers and these groups. The decomposition of *A.marina* leaves expressed as weight loss of the incubated leaves was significantly correlated ($r = 0.9642$, $P < 0.001$) with decomposition time as well as with amylolytic ($r = 0.9624$; $P < 0.001$), proteolytic bacteria ($r = 0.9345$, $P < 0.001$) at the early stages of decomposition. The highest percentage rate of decomposition (68.45%) occurred after 108 days in summer time. However, decomposition was continued at slower rates reaching about 89.11% after 256 days with slow rate of decomposition during the colder months (Table 4).

Table 3: Distribution of bacterial groups (percentage of overall means) associated with decomposing *A.marina* leaves

Bacterial group	Percentage composition
Proteolytic	21.86
Amylolytic	30.32
Cellulolytic	20.70
Lipolytic	3.45
Spore formers	0.03

Table 4: weight loss (percentage) of *A.marina* decaying leaves in Al-Khor seawater

Sampling	days of incubation	weight loss
2.7.98	16	8.54
30.9.98	108	68.45
25.10.98	133	71.38
29.11.98	167	78.25
31.1.99	231	85.48
25.2.99	256	89.11

Discussion

Mean pH and salinity of seawater did not vary during the decomposition period (Table 1) to the extend of affecting decomposition rate as well as the different bacterial counts (Table 2) and bacterial group composition (Table 3). O'Grady *et al.* (1996) noted that low shore environment was heterogenous for parameters such as salinity and light which may affect the decomposition rate in space and time. Phillips *et al.* (1996) indicated that microbial distribution in *A.marina* habitat is affected by tidal phenomenon, salinity, light intensity and temperature. Highest bacterial counts (Table 2) were recorded for all groups after 16 and 108 days and at the warmer months of June and September. The counts per gram of decomposing leaves were as follows: Amylolytic> Proteolytic> Cellulolytic> Lipolytic> Spore formers. The first three groups were 1.5-2 orders of magnitude higher than the lipolytic bacteria and 2-5 orders higher than spore formers. Bacterial numbers then declined after 137 days and stayed almost within the same range (Table 2) after 256 days. This decline in the bacterial numbers may be linked to increase meiofauna in *A.marina* study site as well as decreasing water temperatures. Such meiofauna determined the bacterial community structure and nutrients regeneration (Wahbeh and Mahasneh (1985). Distribution of the different bacterial groups is linked to the nature of the decomposing *A.marina* leaves. The highest groups were amylolytic, proteolytic and cellulolytic and appeared earlier during decomposition indicating the correlation between the more labile starchy and proteinaceous components of the leaves which are easily metabolized, leaving the more resistant components. Lee (1980) and Wahbeh and Mahasneh (1985) reported similar observations with the decomposing *Halophila stipulacea* leaves and other marine detritus. De-Godoy *et al.*, (1997) indicated the high protein content in *A.marina* leaves and pointed out the absence of taninns in *A.marina* leaves. Concerning the low density of spore-formers, however, highest counts coincided with early decomposition (16 days) and declined greatly later on indicating probably ample nutrients as a result of

decomposition by other groups of bacteria. Wafar *et al.* (1997) claimed that mangrove decomposition is important mainly for the carbon budget which is consistent with our rationale.

The decomposing bacterial community (Table 3) was dominated by amylolytic group (30.32%) followed by proteolytic and cellulolytic groups (21.86%; 20.70%) respectively and negligible percentages of lipolytic and Spore forming bacterial groups. *A.marina* leaves lost 68.45% after 108 days of incubation (Table 4) and decomposition slowed down reaching 78.25% after 167 days and 89.11% after 256 days. Mackey and Smail (1996) found that decomposing *A.marina* leaf litter to be higher on the shore line and in summer time and 50% loss was achieved after 59 and 98 days in summer and winter respectively. There is an exponential relationship between leaf litter decomposition in one hand and water temperature and season on the other hand, indicating their importance in mangrove systems(Ashton *et al.*, 1999). Several reports have emphasized the role of the water temperature, leaf decomposition and particle size (Hargrave, 1972) and water depth and currents (Wahbeh and Mahasneh, 1985). The relatively high rate of decomposition may be attributed to the leaf composition (no tannins which are bacterial inhibitors) and being rich in proteins (De-Godoy *et al.*,1997) as well as to the shallow waters of the site of incubation. The mode of leaf decomposition showing very rapid rate of weight loss followed by rather slower rate (Table 4). This rapid initial loss is linked to leaching and microbial utilization of easily decomposed contents of the incubated leaves (Wahbeh and Mahasneh, 1985) leaving the more resistant contents to decompose slowly.

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