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Microbial Decomposition of the Floating Weed *Salvinia molesta* Aublet in Cochin Backwaters

E V RADHAKRISHNAN*, P S GORE, O RAVEENDRAN & R V UNNITHAN
National Institute of Oceanography, Regional Centre, Cochin 682018

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From the live and decomposing *S. molesta* 13 strains of bacteria were isolated and the growth promoting effect of the weed extract in various concentrations on 5 selected strains of bacteria and a mixed culture was studied. Turbidity measurements gave maximum percentage transmission in 5% extract. Dry weight loss of dried weed in the laboratory was 20 and 25% after 10 and 25 days of incubation respectively. Yield efficiency of 3 bacterial strains calculated for 48 hr was 20%. The results suggest the potential value of this primary source of organic material in the food web of Cochin backwaters.

Nutritional significance of detritus along with associated micro-organisms in estuarine ecosystem has been studied[1-6]. The Cochin backwater system receives allochthonus organic matter, primarily from the floating weed *Salvinia molesta* during monsoon.

There is no information on the microbial association and detritus formation from the weed in Cochin backwaters though some information is available on the animal association[7] and seasonal fluctuation and chemical characteristics[8] of detritus in the backwaters. Present study attempts to understand (i) utilisation of soluble weed components by aerobic, heterotrophic bacteria, isolated from fresh as well as decomposing weed in the backwaters, (ii) microbial decomposition of dried weed in the laboratory and (iii) yield efficiency (conversion of low protein weed biomass into bacterial protoplasm) of selected strains of bacteria.

Materials and Methods

Fresh and decomposing *S. molesta* from backwaters was collected (September and October 1975) in sterile glass containers and carried to the laboratory in iced condition. The weed (5-10 g) was aseptically chopped into fine pieces and enumeration of viable counts carried out on the suitably diluted samples using sea water agar (SWA) and freshwater agar (FWA) media by the pour plate methods. Morphological and biochemical tests were performed[9]. Large samples of the fresh weed were also collected from the same area to prepare weed extracts of desired concentrations[10] (0.5, 1, 5 and 10%) to study the growth promoting effect on selected strains of bacteria. Millipore filtered extract was dispensed (20 ml/100 ml conical flask) and sterilised at 15 lb pressure for 15 min. The generic classification of 13 strains of aerobic, heterotrophic bacteria was done as per the modified scheme of Simidu and Aiso[11]. Morphological and biochemical characteristics of the isolates are given in Tables 1 and 2.

Each of the 13 isolates maintained on sea water agar slant were tested for their ability to grow in 5% weed extract broth. Growth of 5 isolates (E_1 *Serratium*, E_2 *Flavobacterium*, E_3 an unidentified gram negative yellow cocci, 8 *Pseudomonas* and 13 *Bacillus*) and a mixed culture of bacteria (MC), in different concentrations of sterilised weed extract (0.5, 1, 5 and 10%) was studied at $28 \pm 2°C$ (Fig. 1). The extracts inoculated with the bacterial isolates were incubated for 48 hr in a waterbath shaker. Growth response after 48 hr incubation was measured at 620 nm in a 'Specometer' with corrections to exclude absorption by the pale brown weed extract (Fig. 1). To study variations in growth between the strains and between concentrations of the extract the results were subjected to analysis of variance.

Loss in dry weight of dried weed in the laboratory due to microbial activity was studied[10]. For estimating conversion of weed into microbial biomass, 3 strains of bacteria (E_1 *Serratium*, 8 *Pseudomonas* and 13 *Bacillus*) were allowed to grow in 3 different concentrations (0.5, 1 and 1.5%) of weed extract prepared in sea water. Weed extract dispensed (20 ml) into conical flasks was sterilised and inoculated with 2 ml of actively growing nutrient broth culture. Turbidity was measured using a Specometer (Elico model: C4-21). After 48 hr incubation dry weight of bacteria was estimated by the filtration method[10]. The values for dry weight of 3 cultures determined at each level of weed extract are presented in Fig. 2. In this

*Present address: Madras Research Centre of CMFRI, 29 Commander-in-Chief Road, Egmore, Madras 600008.

Table 1—Morphological and Growth Characteristics

Strain No.	Description of colony on SWA	Growth		
		Nutrient broth	SWA slant	FWA
1	Round, surface, convex, rose coloured colony	Uniform turbidity, thin pellicle at top	Filiform, dry growth	No visible growth
2	Round, surface, white colony with complete margin	Medium turbid, no pellicle	Filiform	do
3	Round, surface, cream coloured colony with complete margin	Medium turbid, membraneous pellicle at top	do	Poor growth
4	Horse-shoe shaped, surface white colony with lobated margin	Uniform turbidity, no pellicle	do	—
5	Round, surface, pale yellow colony	Medium turbid, no pellicle	do	Luxuriant growth
7	Round, sub-surface, white colony with smooth surface	Uniform turbidity, thin pellicle at top	do	No growth
8	Oval shaped, sub-surface white colony with frilled margin	Uniform turbidity thin pellicle	do	Poor growth
9	Round, surface, white colony	Uniform turbidity, no pellicle	do	Fair growth
10	Round, sub-surface small white colony with metallic shining	Uniform turbidity	do	No growth
11	Round, surface, small, white colony	Uniform turbidity thin pellicle at top	do	Poor growth
13	Round, white, sub-surface, feather margined colony	Uniform turbidity, no pellicle	Filiform luxuriant growth	Thick luxuriant growth
14	Oval, cream coloured, surface colony with frilled margin	Uniform turbidity, no pellicle	Filiform	Poor growth
15	Oval, pale yellow, surface colony	Uniform turbidity	do	do
E-1	Oval, red surface colony	do	do	Good growth

Other morphological characteristics:

Motility	:	All strains motile except 10 and 11 which were passive
Growth at *p*H 6	:	All showed good growth except strain 1 which showed satisfactory growth with turbidity
Pigmentation	:	Strain 1, rose-pink; strain 5, yellow; and strain E-1, red
Irridescence	:	Strains 2 and 3, greenish and strains 8, 11 and 14, bluish

experiment the number of bacteria per ml determined by dilution and plating after 48 hr in the medium containing 500 mg of extract per 100 ml was isolate E_1, 1.03×10^8; isolate 8, 1.64×10^8 and isolate 13, 2.06×10^8.

Results and Discussion

Growth response in 5% weed extract broth indicated that 8 bacterial isolates (strain Nos. 1, 3, 4, 5, 8, 10, 13 and 15) grew well in the extract while the rest exhibited slight growth. When all the isolates were tested for their ability to grow in standard nutrient broth and sea water broth, only 3 (strains 5, 9 and 13) showed appreciable growth in standard nutrient broth, 4 showed no growth and 5 showed poor growth (Table 1). All isolates showed appreciable growth in sea water broth. Of the isolates 50% grew better in weed extract medium than in sea water nutrient broth indicating the bacterial growth promoting factor of the weed. Many of the isolates appeared to be marine bacteria with respect to their requirements of salinity for their growth[12].

The growth promoting activity of the soluble components of the weed is clearly evident from the experiment. Data were analysed using analysis of variance. For framing the analysis of variance, the percentage transmission data were converted to angles using angular transformation, in order to stabilise the variance. There was significant ($P < 0.01$) variation between percentage transmission values. The variation between strains was not significant at 5% level. The least significant difference (LSD) at 5% level was formed and the mean of percentage transmission compared. Percentage transmission in 5% was highest compared to percentage transmission in 0.5 and 1%. The percentage transmission in 10% was less than that of 0.5%.

The percentage loss of organic matter during the initial 10 days of incubation was 20% whereas only 5% decomposition was noticed in the next 15 days. The initial loss of 20% may be attributed to the ready decomposition of protein and other soluble extractives, which were estimated[13] to be 42%. These components are easily susceptible to microbial attack.

Table 2—Biochemical Characteristics

Strain No.	Hugh & Leifson's Test	Fermentation of sugars (sucrose, glucose, maltose, mannitol and lactose)	Gram staining	Bacteria
1	NF	Nil	(−)ve elongated rods	*Pseudomonas* sp.
2	FNG	All except lactose	(−)ve short rods	*Vibrio* sp.*
3	NF	Nil	(−)ve elongated rods	*Pseudomonas* sp.
4	do	Nil	do	*Pseudomonas* sp.
5	FNG	All except lactose	(+)ve rods with central spores	*Bacillus* sp.*
7	NF	Nil	(−)ve elongated rods	*Pseudomonas* sp.
8	do	Nil	(−)ve short rods	*Pseudomonas* sp.
9	do	Nil	(−)ve elongated rods	*Achromobacter* sp.*
10	do	Nil	do	*Pseudomonas* sp.*
11	do	Nil	(−)ve short rods	*Pseudomonas* sp.
13	FNG	All except lactose	(+)ve spore-forming rods with central spores	*Bacillus* sp.
14	NF	Nil	(−)ve short rods	*Achromobacter* sp.
15	do	Nil	(−)ve elongated rods	*Achromobacter* sp.
E-1	do	All except maltose & lactose	(−)ve short rods	*Serratium* sp.

NF = non-fermentative and FNG = fermentative, no gas
*Denitrifier

Other biochemical characteristics:

Indole production	:	Strain No. 2 positive; rest all negative
VP reaction	:	Strain Nos. 5, 13, and E-1 positive, rest all negative
Gelatin-liquefaction	:	Eight strains (1, 2, 5, 9, 13, 14, 15 and E-1) positive; rest negative
Nitrate reduction	:	All strains positive
Starch digestion	:	Six strains (1, 2, 9, 13, 14 and E-1) positive; rest negative
Sensitivity towards penicillin	:	Five strains (5, 9, 13, 14 and E-1) only sensitive

The remaining 5% loss may be due to the slow decomposition of more complex fractions such as cellulose and hemicellulose (27% of the dry weight)[14], which are less susceptible to microbial attack.

The yield efficiency for the isolates E_1, 8 and 13 for 48 hr is 20%. This is comparable to the yield obtained for salt marsh grass[10] for 48 hr incubation. According to Gosselink and Kirby[15] yield efficiency calculated for 48 hr may not reflect the maximum bacterial biomass that has been achieved during the period. They have further shown that yield efficiency can be reached even up to 46% for a longer time interval as decomposition of more refractory fractions were expected within that period.

Burkholder and Bornsby[10] assumed that 11.2% of the annual crop of marsh grass may be available for rapid production of bacterial biomass if 20% of the soluble extractives and protein is readily converted. Using the same relationship it may also be assumed that 8.4% of the weed will be available for bacterial biomass production in Cochin backwater system also ($0.42 \times 0.2 = 0.084$, i.e. 8.4%).

Role of allochthonous organic matter as a major energy source in salt marsh ecosystems and streams have been well emphasised[3,6]. According to Tait[16] in detritus based ecosystem, some of the energy is directly available to the benthic fauna by digestion and assimilation of detritus, but many detritic materials

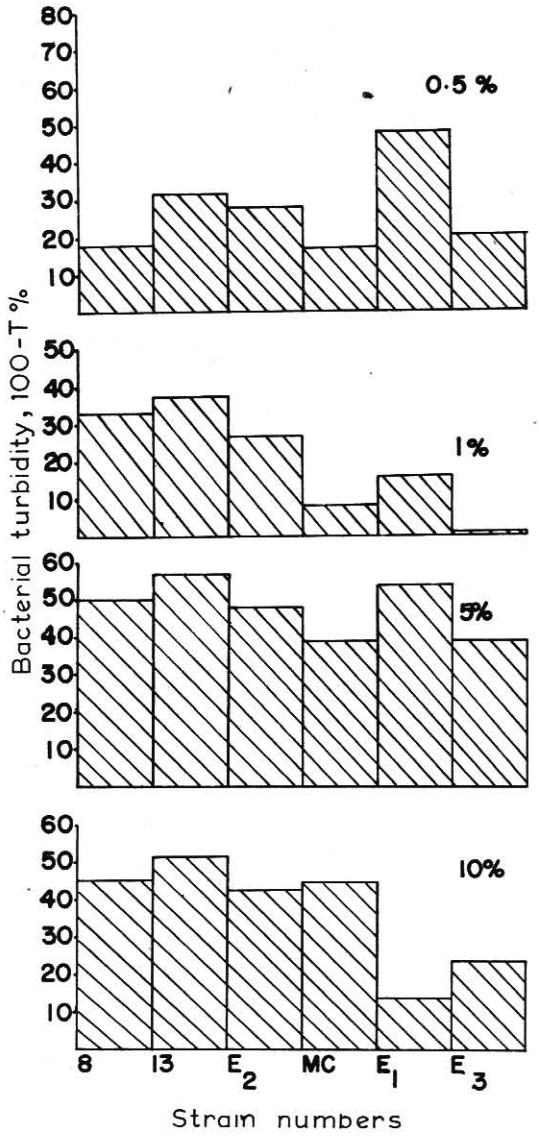

Fig. 1—Growth of 6 bacterial isolates in different concentrations of weed extract in sea water

reaching the bottom cannot be digested by animals. From the experimental evidence available he further assumed that 25% of the energy intake of the benthic herbivores come directly from detritus and 75% from the consumption of associated bacteria. From the present study it is seen that *Salvinia* detritus of Cochin estuary is acted upon by bacteria which may contribute to a significant proportion of the food of benthic animals in the estuary.

Detailed studies of energy flow in natural ecosystems are available[3,17,18]. But studies of the energetics of a tropical estuarine ecosystem like Cochin backwater system are limited. From the studies of organic production and the food chain of Cochin backwaters[8,19] it is known that a major portion of energy in the form of detritus is available. The detritus

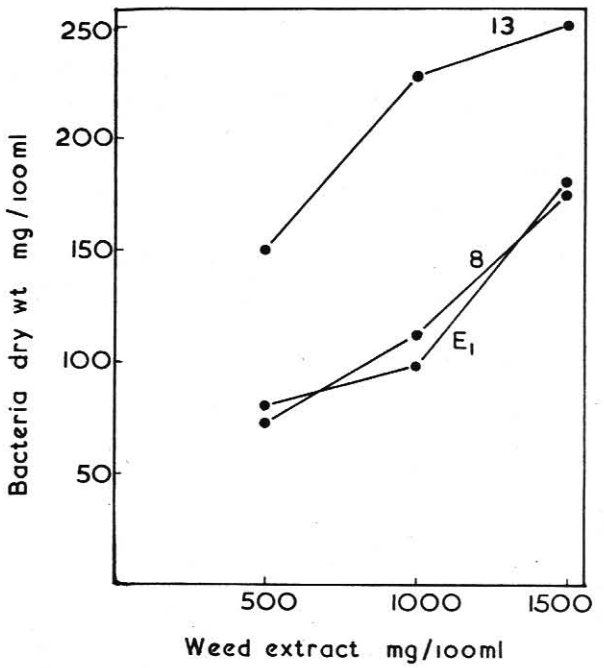

Fig. 2—Dry weight of bacterial culture grown in different weed extract concentrations

constitutes an additional pathway between organic production and animal nutrition increasing the efficiency of energy transfer from one trophic level to other. The production of *Salvinia* detritus is seasonal in that the fresh weeds come to the estuarine system in monsoon months, and undergo decomposition leaving the detritus, which accumulates in the bottom. If a large proportion of detritus exists in the sediments then even a slow rate of decomposition would be sufficient, to support the consumer populations. Fenchal[6] has stated that the existence of a large quantity of slowly decomposing plant body may enable the ecosystem to continue functioning even when the primary producers are temporarily removed. Similarly the existence of decomposing *Salvinia* to some extent may prove beneficial, especially in the absence of other primary producers.

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