Madras Agric. J. 89 (1-3) : 99-102 January-March 2002
https://doi.org/10.29321/MAJ.10.A00195

Enrichment and isolation of *Beggiatoa* spp. from rice ecosystem

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Abstract: *Beggiatoa*, an organism capable of oxidizing hydrogen sulfide has been isolated from rhizosphere of rice grown under submerged ecosystem. The isolation procedure involves prior enrichment with extracted hay and soil extract under various treatments *viz.*, addition of acetate or sodium sulfide or acetate + sodium sulfide, diluted and undiluted soil extract. The results indicated that diluted (1:1) soil extract with sodium sulfide enrichment had registered higher number of 22.5 trichomes ml^{-1}, followed by acetate + sodium sulfide treatment, apart from reducing the bacterial and protozoan contaminants to a minimal level. Formation of puff balls and mat in the sub-surface of the enrichment medium suggest that they are microaerophilic and grown in the interface between oxygen and hydrogen sulfide environments. Growth of *Beggiatoa* was also observed in *Beggiatoa* medium 2 as circuitance type of colony. Morphological characterizations of this isolate showed its close resemblances with that of *B. alba* *(Key words: Beggiatoa, Enrichment, Isolation, Rice rhizosphere)*

Beggiatoa, a gliding filamentous bacterium is capable of oxidizing sulfide to elemental sulfur, which it deposits in its cells. When the sulfide is depleted, the bacterium further oxidizes the deposited sulfur to sulfate, which is then released to the environment (Pringsheim, 1967). The presence of *Beggiatoa* in the rice rhizosphere lead to a mutualistic association in which the bacterium oxidizes H_2S in the root zone, thus protecting the plant from the toxic effects of H_2S, and the plant roots excrete catalase, which decomposes the toxic peroxides produced by the bacterium during its metabolism (Pitts *et al.* 1972; Joshi and Hollis, 1977). The H_2S production by reduction of sulfate in submerged soils are often due to the presence of anaerobic bacteria such as *Desulfovibrio* spp. and increase in the concentration of such gas in rice paddies have been shown to inhibit significantly the activities of cytochrome oxidase and other oxidase enzymes in rice seedling (Pitts *et al.* 1972). To exploit the mutualistic association between the rice plant and *Beggiatoa for* enhancing growth and yield of rice, an attempt was made to isolate *Beggiatoa* from rice ecosystem.

Materials and Methods

Beggiatoa can be isolated from rice soil by enrichment technique (Joshi and Hollis, 1976), which enables the isolation within 10-15 d. One hundred gram of dried bermuda grass *(Cyanodon dactylon)* was boiled in tap water for 10 min. The water was decanted and additional water added. Boiling and decanting was repeated 5 times. The hay was left in water overnight and the extraction procedure was again repeated thrice. The extracted hay was then spread out and dried at room temperature for two days.

Soil extract was prepared by the following procedure: 500 g of mud collected from blackish sediments where there is more of H_2S formation, was added to 1000 ml of tap water, mixed well and allowed to settle. The extract was filtered through coarse filter paper, then through Whatman No. 1 filter paper and the pH adjusted to 7.2. Approximately 0.5 g hay with 50 ml of soil extract diluted to 1:2(DSE) or undiluted soil extract (UDSE) was added to 125 ml saline glucose bottle. Each bottle was then inoculated with 1 g rhizosphere soil collected from rice field, closed with cotton plug and incubated at room temperature.

The following treatments are incorporated to find out suitable enrichment media for *Beggiatoa* isolation.

T_1 Diluted soil extract + hay
T_2 Diluted soil extract + hay + sodium sulfide
T_3 Diluted soil extract + hay + acetate
T_4 Diluted soil extract + hay + sodium sulfide + acetate
T_5 Undiluted soil extract + hay
T_6 Undiluted soil extract + hay + sodium sulfide

Table 1. Growth of *Beggiatoa* spp. from enrichment media.

Treat-ment	Number of trichomes (x 10^4 ml^{-1})	Type of colony	Remarks
T_1	3.0	Circuitans	Filaments dispersedin the medium
T_2	22.5	Circuitans	Puff-balls in sub-surface, white mats on glass surface, hay
T_3	6.0	Circuitans	Filaments dispersed in the medium
T_4	7.8	Circuitans	White mats on glass surface and hay. Filaments dispersed in the medium
T_5	2.0	Circuitans	Filaments dispersed in the medium
T_6	12.0	Circuitans	White mats on glass surface and hay. Filaments dispersed in the medium
T_7	1.0	Circuitans	Filaments dispersedin the medium
T_8	7.5	Circuitans	Filaments dispersedin the medium

Values represent mean of 3 replications.

Table 2. Salient characteristics of *Beggiatoa* isolated from rhizosphere of rice

Sl.No.	Characteristics	Results
A	*Trichome features*	
1	Filament diameter	2 to 4:m
2	Filament length	50 to 120:m
3	Nature of filaments	Flexible
4	Ends of filaments	Rounded
5	Grams reaction	Negative
6	Sulfur inclusions	+
7	PHB and Polyphosphate granules	+
8	Motility	+
B	*Growth on enrichment medium*	
1	Microaerophilic growth	+
2	Presence of tuft-balls	+
c	*Growth on agar medium*	
1	Slime production	+
2	Type of colony	Circuitance

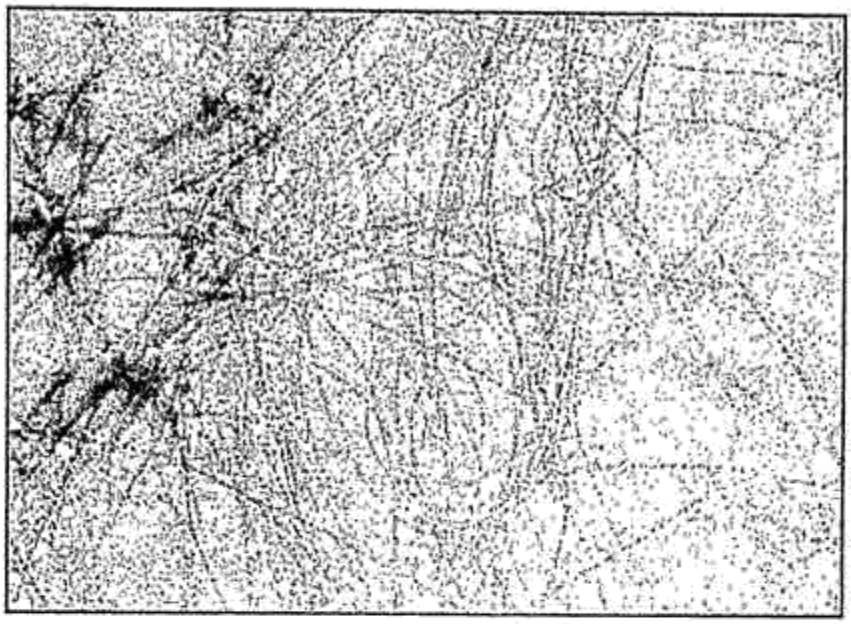

Fig.1. Typical appearance of *Beggiatoa* filaments (100 x magnification)

T_7 Undiluted soil extract + hay + acetate
T_8 Undiluted soil extract + hay + sodium sulfide + acetate

Sodium sulfide @ 0.03%
Acetate @ 0.10%
All treatments received cyclohexamide @ 20 mg ml^{-1}.

Beggiatoa growth was seen as whitish mat on surface of the medium and tuft balls below the surface of the medium after 7-10 d and 25-30 d of enrichment respectively. Tuft balls were transferred to MP medium (0.0001% sodium acetate, 0.03% Na_2S and 1.2% agar prepared in basal salt solution; Basel salt solution consisted of 5 ml of trace element solution, 20 ml of saturated $CaSO_4$ solution, 0.0001 g of K_2HPO_4 and 0.00019 g of $MgSO_4.7H_2O$). Trichomes of *Beggiatoa* were also seen on the glass sides of the bottle and on the hay. While transferring *Beggiatoa* from enrichment medium to the MP medium, puff-balls were washed in basal salt solution and placed on the agar surface of MP plate. The plates were incubated in a jar containing partially reduced environment (a pinch of pyrogallol dissolved in alkali was placed inside the jar for providing partially reduced aeration and to detoxify hydrogen peroxide produced by the organism). Tuft of filaments from enrichment medium was also transferred to *Beggiatoa* medium 2 (Anon, 2001) after washing in basal salts solution and upon incubation, the growth pattern was observed.

Results and Discussion

Microscopic observations of the culture from enrichment medium revealed Gram-negative colorless organisms with gliding motility. The trichome size ranged between 50-120 µm in length and 2-4 µm in width (Fig 1). Each filament is filled with a few cells to 50 or more cells. Trichomes contain inclusions of sulfur and their tips are rounded. Sulfur inclusions within the filaments were observed upon maturity or dehydration of the filament. In addition to sulfur inclusions, the presence of numerous retractile bodies in the matured trichomes also indicated the presence of PHB and polyphosphate inclusions, which is also in agreement with the earlier findings of Strohl and Larkin, (1978). Number of trichomes formed from each treatment was counted and presented in Table.1. The circuitans type of colony was also observed from enrichment medium.

On comparing various treatments for enrichment of *Beggiatoa*, addition of sodium sulfide @ 0.03 per cent alone in enrichment medium enhanced the trichome formation to the maximum of 22.5 x 10^4 ml^{-1} of medium with diluted soil extract, whereas the same enrichment medium containing undiluted soil extract has registered 12 x 10^4 trichomes ml^{-1}. Highest population recorded with diluted soil extract implies that these organisms preferred to grow under low nutrient status and is also in agreement with the earlier findings of Strohl and Larkin, (1978). More over, dilution of soil extract might have also reduced the level of other bacterial and protozoan contamination to a minimum level and allowed the *Beggiatoa* multiplication. Numerous puff- balls were also observed in the sub-surface area of the medium and this observation clearly indicates their micro-aerophilic nature of the organism. It was clearly demonstrated by earlier workers that the preference of this organism to grow in the interface between oxygen and hydrogen sulfide under submerged condition (Strohl and Larkin, 1978). The present findings with the enrichment study also demonstrated their growth at the interface (transition) between anoxic sulfide-emanating lower sediment and the oxic interstitial waters.

Irrespective of dilution of soil extract, the treatment enriched with sodium sulfide and sodium acetate recorded 7.8 x 10^4 trichomes ml^{-1} of enrichment medium. Addition of acetate in the enrichment medium lowered the population of *Beggiatoa* and this might be due to the fact that the increased carbon nutrient coupled with higher peroxide production, lead to the autolysis of cells. The direct correlation between higher levels of nutrients and peroxide accumulation with respect to autolysis of *Beggiatoa* has been well documented (Strohl and Larkin, 1978). On transferring the filaments into *Beggiatoa* medium No.2, the gliding movement of the filaments on agar surfaces was also observed within 24 h of incubation. The filaments also formed various patterns of waves and concentric rings. Extra cellular polysaccharide production around the colony on agar medium was also observed. The salient features of *Beggiatoa* isolated from rice rhizosphere are presented in Table 2.

Earlier works on the interaction of *Beggiatoa* and rice plant in wet land ecosystem (Pitts *et al.* 1972; Joshi and Hollis, 1977) suggest that *Beggiatoa* apparently plays an important role in rice, where the toxic peroxides produced by *Beggiatoa* may be decomposed by catalases from rice root and sulfides toxic to the rice root may be oxidized by the *Beggiatoa* and this kind of mutualistic association also promotes the growth and development of the plant at critical stages of rice. Nitrogen fixation demonstrated in several strains of *Beggiatoa (Nelson et al.* 1982) also suggested that the link between nitrogen and sulfur cycle must be studied. The present study paves the way for furtherance of research on exploitation of this mystery organism for nutrition, crop growth and yield of rice.

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(Received : October 2001; Revised: March 2002)