Biological N_2-fixation on Mangrove Pneumatophores: Preliminary Observations and Perspectives

Mangroves are highly productive ecosystems in tropical marine environment (1, 2). It is generally accepted that mangrove swamps supply both organic and inorganic material to adjacent coastal waters and related biotopes (3–5). Despite their high production rates, mangrove trees grow and flourish in highly oligotrophic tropical areas. Cyanobacteria, common in mangrove swamps may play an important role as atmospheric nitrogen fixers in these habitats (6, 7). Other diazotrophic but heterotrophic eubacteria are also common in marine sediments and are suggested to contribute substantially to fixed nitrogen inputs (8, 9). In addition, some nitrogen-fixing bacteria inhabiting barks of mangrove trees has been isolated and characterized (10). A previous survey in mangrove areas around Zanzibar revealed a high diversity among free-living cyanobacteria ranging from unicellular to non-heterocystous filamentous and heterocystous species (11). A heterocystous cy anobacterium of the genus *Rivularia* was particularly common on mangrove pneumatophores. Other potential diazotrophic cyanobacteria described from mangrove areas include the genera *Aphanocapsa*, *Lyngbya, Nodularia*, and *Scytonema* (11). The current study was aimed to assess nitrogenase activity associated with cyanobacteria and other diazotrophs on mangrove pneumatophores and in adjacent sediments.

PRELIMINARY STUDY

Mazizini is located within Zanzibar municipality about 5 km south of central Zanzibar town. In the intertidal mudflats of this area there is a small mangrove stand that is primarily composed of *Sonneratia alba* and a few *Avicenia marina* trees. As a preliminary observation, Mazizini location was chosen due to its easy access from the laboratory. Biological N_2-fixation in the area was determined by using the acetylene reduction assay (12). Incubations were done *in situ* during low tide at around noon using 5 cm diameter plexiglass corers sealed by rubber corks. On one occasion night-time incubations were performed from 22.00–24.00 hrs. On each occasion, one corer was incubated without addition of acetylene to monitor endogenous production of ethylene in sediments and served as an experimental blank. Corers were fixed on pneumatophore and in sediments to a depth of ca. 5 cm and the rubber cork tightened. Then 10% of the air phase in each corer was replaced by acetylene (generated from calcium carbide) through a rubber sealed sampling port using a syringe. After 2 hrs incubation gas samples were taken and stored in 7 ml pre-evacuated FMAB vacutainers (Sweden) and transported to Sweden for analysis. Analyses were performed using a gas chromatograph (Shimadzu GC-8A) equipped with Porapak-N column and flame ionization detector at oven temperature of 120°C. In order to estimate the occurrence of *Rivularia* on the mangrove pneumatophore, 20 0.5 x 0.5 m quadrants were randomly 'thrown' on mangrove pneumatophores on each sampling occasion and the pneumatophores with and without *Rivularia* quantified.

DISCUSSION

The vegetative cells of the cyanobacterium *Rivularia* ranged between 3.1 to 8.3 μm with terminal heterocyst of about 9.5 μm in diameter as previously reported by Lugomela et al. (11). Acetylene reduction was significantly higher on mangrove pneumatophore with attached *Rivularia* crusts compared to pneumatophores without *Rivularia*. The mean activity ranged between 27 and 118.5 nmol C_2H_4 cm^{-2} hr^{-1} on pneumatophores with *Rivularia* and between 6 and 25.9 nmol C_2H_4 cm^{-2} hr^{-1} on pneumatophores without *Rivularia* (Fig. 1). Out of the 1810 pneumatophores examined, 16.9% harbored the cyanobacterium *Rivularia*. Higher acetylene reduction rates were observed in adjacent sediments with well-developed cyanobacterial dominated microbial mats than in seemingly bare sediment (Fig. 2). The mean nitrogenase activity ranged between 6 to 16.8 nmol C_2H_4 cm^{-2} hr^{-1} in sediment with a microbial mat and between 1.3 to 3.8 nmol C_2H_4 cm^{-2} hr^{-1} in bare sediment.

Comparison between rates of nitrogenase activity in mangrove ecosystems and intertidal mud flats recorded for different habitats around Zanzibar and in other geographic regions are given in Table 1. The rates obtained are generally similar to those reported for microbial mats in mangrove sediments from Gulf of Eilat (13) and intertidal mudflats in the North Sea (14), and North Carolina (15). The variations observed between these studies could be due to variability in composition or biomass of diazotrophic organisms and/or variations on environmental conditions such as temperature, light regime and availability of inorganic nutrients. Ethylene production on intact and excised pneumatophores of the black mangroves in Balandra lagoon, northwestern Mexico, was 27.6 and 32.7 nmol ethylene μg Chl a^{-1} pneumatophore^{-1} hr^{-1}, respectively

Figure 1. Rates of nitrogenase activity associated with mangrove pneumatophores encrusted by *Rivularia* (gray bars) and without *Rivularia* (black bars) in Mazizini mangrove stand, Zanzibar (error bars = SD, n = 4).

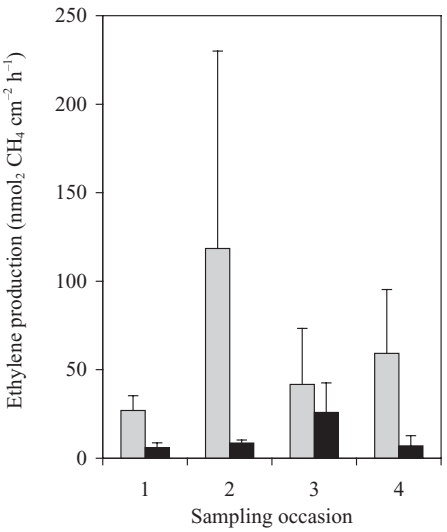

Figure 2. Rates of nitrogenase activity in sediments on a visible microbial mat (gray bars) and on seemingly bare sediment (black bars) in a Mazizini mangrove stand, Zanzibar (error bars = SD, n = 4).