7. Govindachari, T. R., Sandhya, G. and Ganesh, R. S. P., *J. Nat. Prod.*, 1992, **55**, 596–601.
8. Rembold, H. and Puhlmann, I., in *The Neem Tree, Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes* (ed Schmuttere, H.), VCH Verlagsgesellschaft, Weinheim, Federal Republic Germany, 1995, pp. 224.
9. Schmutterer, H., in *The Neem Tree, Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes* (ed Schmuttere, H.), VCH Verlagsgesellschaft, Weinheim, Federal Republic Germany, 1995, pp. 28.
10. Govindachari, T. R., Sandhya, G. and Ganesh, R. S. P., *Chromatographia*, 1991, **31**, 303.
11. Yamasaki, R. B., Klocke, J. A., Lee, S. M., Stone, G. A. and Darlington, M. V., *J. Chromatogr.*, 1986, **356**, 220–226.

ACKNOWLEDGEMENTS. We thank Dr P. V. Sane, Director, for guidance. Financial assistance from the Council of Scientific and Industrial Research, New Delhi and the Ministry of Non-conventional Energy Sources, Govt of India is gratefully acknowledged.

Received 9 February 1996; revised accepted 3 May 1996

Effect of salinity on amino acid composition of the marine fungus *Cirrenalia pygmea*

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***Cirrenalia pygmea*, a mangrove fungus, was grown at various salinities and its amino acid composition determined. Higher salinity led to an increase in the amino acid pool size and the number of amino acids produced. Acidic amino acids were present in higher concentrations at 6.9‰ and 20.7‰ salinities. Gln, His, Thr, Arg and Val were present only when the fungus was exposed to high salinity conditions. The concentration of Gly increased with salinity. Arginine and ornithine were present in low salinity, but in high concentrations at higher salinities. Proline and Dragendorff-positive compounds were absent.**

CIRRENALIA PYGMEA Kohlmeyer, a marine fungus, occurs as a saprophyte on bark and wood of mangrove roots and on calcareous linings of empty shipworm tubes[1]. Although ecological studies on mangrove fungi have been carried out[2], there is little work on the physiology of this group of marine fungi[3]. Our earlier studies on the physiology of *C. pygmea* showed that the fatty acid profile[4] and the melanin content of the hyphae are altered by salinity[5]. Here we report the influence of salinity on the amino acid composition of this fungus.

A single conidial isolate of *C. pygmea* isolated from prop roots of *Rhizophora mucronata* Poiret was grown on cellophane disks[6] overlying malt agar medium (pH 8.0) made up with undiluted, filtered and aged sea water[1] of 34.5‰ salinity or sea water diluted with deionized water. The cultures were incubated at 30°C for 8 days. The free amino acid pool of the mycelium was analysed by HPLC after derivatization with *o*-pthaldialdehyde[7]. The HPLC had an Isco C18 Reverse Phase column and 0.1 M acetic acid buffer and methanol : tetrahydrofuran (97:3 v/v) as solvents in a gradient. The rate of flow was 1.5 ml/min. The detector was Isco V, 9 µl flow cell 7L-2 fluorescence. An excitation filter of 305–395 nm and an emission filter of 430–470 nm were used. The amino acids were identified by comparing their retention time and peak areas with those of standards obtained from Sigma, USA. Dragendorff-positive compounds was tested by thin layer chromatography[8]. The plates were developed in ethanol : ammoniacal solution : water (85:12:13)[9]. Betaine standard was used and visualized with Dragendorff reagent sprays[10].

The amino acid pool size and composition of *C. pygmea* were correlated by salinity (Table 1). The pool size increased from 5.4 to 7 times with the increase in salinity. The number of amino acids increased from 13 to 18 when the fungus was grown on high saline medium. These results were similar to those obtained for *Aphanothece halophytica*, a halophilic cyanobacterium[11]. Gln, His, Thr, Arg and Val were absent in the mycelium grown on 6.9‰ salinity. However, these amino acids were present in high concentrations in the

Table 1. Free amino acid composition (µg/g dry weight) of *C. pygmea* grown in different sea water concentrations

	Salinity (‰)		
Amino acids	6.9	20.7	34.5
Asp	30	650	1410
Glu	680	3940	430
Asn	550	1810	2400
Ser	10	440	440
Gln	nd	2220	70
His	nd	360	720
Gly	190	680	4010
Thr	nd	880	330
Arg	nd	100	1120
Ala	920	3460	300
Tyr	280	2150	3840
Met	980	2520	5370
Val	nd	120	140
Phe	20	60	1000
Ile	260	940	2120
Leu	trace	740	2430
Lys	trace	trace	420
Ornithine	trace	trace	300
Total	3920	21070	26850

nd = not detected; Analysis by HPLC; Figures are mean of 2 values.

mycelium exposed to high salinities. Similarly, Leu, Lys and ornithine, which were present only in traces in cultures grown on low salinity, were present in considerable amount when salinity increased. The major amino acids (more than 10% of the pool) of *C. pygmea* grown on 6.9‰ salinity were Glu, Asn, Met and Ala. Glu, Gln, Ala, Tyr and Met were the major amino acids in the mycelium reared on 20.7‰ salinity. In 34.5‰ salinity, Gly, Tyr and Met were the major amino acids (Table 1).

Marine organisms adopt different strategies to come to terms with their hypertonic environment. Halophilic vascular plants accumulate high concentrations of sodium and chloride ions to reduce cell water potential[3,12]. As sodium ions inhibit enzymes in the cytosol, they are sequestered in the large, metabolically inert vacuoles[13]. Some organisms synthesize and accumulate organic solutes in their cytoplasm. Such solutes, even in high concentrations, do not perturb cell metabolism[14] and are termed compatible solutes. *Dendryphiella salina*, the only marine fungus to have been studied in detail for its osmoregulation, synthesizes sugar alcohols as compatible solutes[13,14]. Amino acids function as compatible solutes in marine angiosperms[15], marine diatoms[16] and in halophilic bacteria[17]. In the marine fungus *Dendryphiella salina* and in some terrestrial fungi grown on high salt medium, amino acids do not control cell turgor since their amino acid composition is not altered by changes in salinity[6,18]. However, Luard[19] found that the amino acid proline is the principal compatible solute in some Oomycetes.

The most commonly occurring amino acid osmolytes in marine algae are glycine, glutamate and proline[16]. In *C. pygmea*, although proline was absent, the concentrations of glutamate and glycine increased 6 times and 3.5 times respectively at 20.7‰ salinity when compared with those at 6.9‰ salinity (Table 1); the concentrations of glycine increased 21 times on 34.5‰ salinity. However, the amount of glutamate fell at this salinity, a trend similar to that observed for the marine diatom *Nitzchia pungens f. multiseries*[16].

Acidic amino acids (Asp + Glu) were present in high concentrations at higher salinities (18.1% at 6.9‰ salinity; 21.8% at 20.7‰ salinity) than the basic amino acids (Lys + Arg + His–0 and 2.2% at 6.9‰ and 20.7‰ salinities respectively). This is similar to the results obtained for some obligate halophilic prokaryotes[11,20]. Being negatively charged at physiological pH, the acidic amino acids could be used to counter ion accumulation by the cell[21]. Luard[6] opined that, in addition to sugar alcohols, negatively charged amino acids contribute to osmoregulation in some terrestrial fungi.

Polyamines which are synthesized from arginine and Ornithine, are another group of nitrogenous-compatible solutes found in bacteria[22]. Since the concentrations of these amino acids in *C. pygmea* increased with salinity, it is likely that polyamines could also be involved in hyphal turgor regulation of this marine fungus. Betaines (Dragendorff-positive compounds) function as compatible solutes in halophilic eubacteria[22,23]. These compounds were absent from the mycelium of *C. pygmea* grown on high saline medium, suggesting that they may not play a role in turgor regulation of this fungus.

Our results suggest that amino acids could contribute to turgor regulation in marine fungi. The use of organic compounds such as amino acids as compatible solutes is a metabolically expensive strategy when compared to the alternative strategy of accumulating sodium ions[24]. *C. pygmea* appears to have evolved this mechanism, perhaps, due to the limited vacuole size that is obtained in a fungal hypha as has been suggested by Clipson and Jennings[3].

1. Kholmeyer, J. and Kohlmeyer, E., in *Marine Mycology: The Higher Fungi*, Academic Press, New York, 1979, pp. 690.
2. Jones, E. B. G. and Tan, T. K., *Trans. Br. Mycol. Soc.*, 1987, **89**, 390–392.
3. Clipson, N. J. W. and Jennings, D. H., *Can. J. Bot.*, 1992, **70**, 2097–2105.
4. Ravishankar, J. P., Muruganandam, V. and Suryanarayanan, T. S., *Bot. Mar.*, 1994, **37**, 479–481.
5. Ravishankar, J. P., Muruganandam, V. and Suryanarayanan, T. S., *Bot. Mar.*, 1995, **38**, 413–416.
6. Luard, E. J., *J. Gen. Microbiol.*, 1982, **128**, 2563–2574.
7. Rajendra, N., *J. Liquid. Chr.*, 1987, **10**, 941–954.
8. Wethered, J. M., Metcalf, E. C. and Jennings, D. H., *New Phytol.*, 1985, **101**, 631–649.
9. Blunden, G., Barouni, M. M. and Gordon, S. M., *Bot. Mar.*, 1981, **24**, 451–456.
10. Stahl, E., *Thin Layer Chromatography: A Laboratory Handbook*, 1969, Allen and Unwin, London, 1969.
11. Tindall, D. R., Yopp, J. H., Schmid, W. E. and Miller, D. M., *J. Phycol.*, 1977, **13**, 127–133.
12. Flowers, T. J., Troke, P. F. and Yeo, A. R., *Annu. Rev. Plant Physiol.*, 1977, **28**, 89–121.
13. Money, N. P., in *The Mycota* (eds Esser, K. and Lemke, P. A.), Springer, Berlin, 1994, vol. 1, pp. 67–88.
14. Jennings, D. H. and Bruke, R. M., *New Phytol.*, 1990, **116**, 277–28.
15. Flowers, T. J., Hajibagheri, M. A. and Clipson, N. J. W., *Q. Rev. Biol.*, 1986, **61**, 313–337.
16. Jackson, A. E., Ayer, S. W. and Laycock, M. V., *Can. J. Bot.*, 1992, **70**, 2198–2201.
17. Vreeland, R. H., Mierau, B. D., Litchfield, C. D. and Martin, E., *Can. J. Microbiol.*, 1983, **29**, 407–414.
18. Beever, R. E. and Laracy, E. P., *J. Bacteriol.*, 1986, **168**, 1358–1365.
19. Luard, E. J., *J. Gen. Microbiol.*, 1982, **128**, 2583–2590.
20. Lanyi, J. K., *Bacteriol. Rev.*, 1974, **38**, 272–290.
21. Hochachka, P. W. and Somero, G. M., in *Biochemical Adaptation*, Princeton University Press, NJ, 1984, p. 537.
22. Javor, B., *Hypersaline Environments*, Springer, New York, 1989, p. 328.
23. Imhoff, J. F. and Rodriguez-Valera, F., *J. Bacteriol.*, 1984, **160**, 478–479.
24. Yeo, A. R., *J. Exp. Bot.*, 1981, **32**, 487–497.

ACKNOWLEDGEMENTS. We thank Dr G. Subramanian, National Facility for Marine Cyanobacteria, Bharathidasan University, Trichirapalli, for HPLC facility. One of us (JPR) thanks the UGC, New Delhi for a fellowship.

Received 8 January 1996; accepted 3 May 1996