

Influence of salinity on the activity of polyol metabolism enzymes and peroxidase in the marine fungus *Cirrenalia pygmea* (Hyphomycetes)

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The activities of polyol metabolism enzymes such as polyol dehydrogenase (NADP⁺) and D-mannitol dehydrogenase as well as peroxidase of the marine fungus *Cirrenalia pygmea* were found to be influenced by salinity. Increasing salinity increased the activity of all the enzymes. Presumptive evidence for turgor regulation by polyols is presented.

Studies regarding the effects of salinity on enzyme activities in marine fungi are meager¹. To understand more about the physiology of marine fungi, we studied the influence of salinity on the activities of polyol dehydrogenase, mannitol dehydrogenase and peroxidase enzymes of the marine fungus *Cirrenalia pygmea* Kohlmeyer. This fungus is an obligate marine hyphomycete found growing on the stilt roots of mangrove trees. Being a manglicolous fungus, *C. pygmea* is exposed to alternating high and low tides and hence a continually changing salinity.

A single-spore isolate of the fungus, isolated from prop roots of *Rhizophora* sp., and maintained on malt seawater (malt 20g, Difco yeast extract 1g, agar 20g, filtered seawater diluted with deionized water to 60%) agar medium (pH 8.0) was used.

A culture of *C. pygmea* was grown on a disc of cellophane² overlying malt agar medium (pH 8.0) made up with different dilutions of seawater. Seawater was diluted with deionized water to give 20 and 60% dilutions; these dilutions were equal to 6.9 and 20.7‰ salinity. The salinity was measured using a salinometer. After 8 days of incubation at 28°C, the margin of the colony (about 0.5 cm wide) which was devoid of spores and represented young and actively growing mycelium³ was collected for analysis. The mycelium was homogenized with acid-washed sand using Tris-HCl buffer (pH 7.5, 0.2 M for polyol

dehydrogenase and 0.05 M for mannitol dehydrogenase) and centrifuged at 10,000 rpm for 30 min. The supernatant was used as enzyme preparation. All operations were done in a cold room at 4°C. Three replicates were maintained for enzyme extraction and assay.

Polyol dehydrogenase (NADP specific, E.C.1.1.1.21) was assayed following the method of Horitsu *et al.*⁴. The incubation mixture contained 0.2 M tris-HCl, pH 7.5, 0.067 M D-xylose or L-arabinose and 0.1 ml enzyme preparation. The reaction was initiated by addition of 0.001 M NADPH₂, and the change in absorbance at 340 nm for 3 min was recorded in a Shimadzu 160A double beam recording spectrophotometer. The specific activity was expressed as $\Delta 0.1$ OD min⁻¹mg⁻¹ protein. A substrate-free preparation served as control.

D-Mannitol dehydrogenase (E.C.1.1.1.138) was assayed by the method of Horikoshi *et al.*⁵. The assay mixture consisted of 0.1 M fructose solution, tris-HCl buffer (0.05 M, pH 7.5), 0.1 ml enzyme and 0.001 M NADP. The decrease in OD at 340 nm was measured. The specific activity was expressed as $\Delta 0.1$ OD min⁻¹mg⁻¹ protein. A substrate-free preparation served as control.

A spectrophotometric assay procedure⁶ for peroxidase (E.C.1.11.1.7) was used. The enzyme preparation and H₂O₂ (3%) were added to the pyrogallol substrate (0.5M) and the increase in OD

Table 1—Effect of salinity on activities of some enzymes of *Cirrenalia pygmea*

		Enzyme activity ($\times 10^2$) $\Delta 0.1$ $\text{min}^{-1} \text{mg}^{-1} \text{protein}$			
		Salinity (‰)			
		0	6.9	20.7	34.5
Polyol dehydrogenase (Arabinose substrate)	8	79	141	20	
Polyol dehydrogenase (Xylose substrate)	4	10	140	42	
Mannitol dehydrogenase	28	40	98	67	
Peroxidase	510	608	815	570	

at 420 nm was recorded at 30 sec intervals. The activity of the enzyme is express as $\Delta 0.1 \text{ OD min}^{-1} \text{mg}^{-1} \text{protein}$. A substrate-free preparation served as control.

External salinity influenced the activities of all the three enzymes of *C. pygmea* (Table 1). As salinity increased, polyol dehydrogenase activity increased and reached a maximum at 20.7‰ salinity. This was true for both the substrates used. The activity of mannitol dehydrogenase showed a similar trend. The activity of both the enzymes decreased at salinity above 20.7‰.

Marine fungi have to necessarily develop and maintain a more negative intracellular water potential than that of the seawater to survive in a marine environment. In *D. salina*, the only marine fungus to have been studied in detail, hyphal turgor regulation is through polyol synthesis⁷. The polyol content of this fungus increases with external salinity^{1,7}. The present results suggest that in *C. pygmea* also, polyols function as turgor regulator since activities of polyol metabolism enzymes increased with external salinity (Table 1).

In addition, *C. pygmea* synthesized more polyols with increasing external salinity⁸.

Peroxidase activity increased with increasing salinity and reached a maximum at 20.7‰ salinity. Peroxidase activities in the cyanobacterium *Phormidium ambiguum*⁹ and marine green alga *Ulva lactuca*¹⁰ are known to increase with salinity and fall at very high salinity. Though the role of peroxidase in salinity tolerance in marine organisms is unknown, it is pertinent to mention that this enzyme is implicated in stress tolerance in plants¹¹. Our earlier studies showed that fatty acids¹² and amino acids¹³ contribute to turgor regulation in *C. pygmea*. The present study suggests a role to polyols in this phenomenon.

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