

Growth of marine yeast on different strength of stress solutes

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ABSTRACT

Sixteen isolates of marine yeasts belonging to genera *Candida*, *Debaryomyces*, *Rhodotorula* and *Saccharomyces*, isolated from the (EEZ) of India were screened for their growth on different concentrations of sodium chloride (NaCl). Most of them showed excellent growth at 4% and good growth at 6% NaCl. Species of red yeast *Rhodotorula* lost its pigment production at 6%, except *R. rubra* which grew up to 16% NaCl with faint pigmentation. Six species of genera *Debaryomyces*, *Saccharomyces* and *Rhodotorula* were tested against the stress solutes NaCl (0 -16%), potassium chloride (KCl) and sodium sulphate (Na_2SO_4) at 0.4%, 4% and 8% concentrations. *D.hansenii* and *D. marama* were the most versatile isolates exhibiting excellent growth in all the concentrations of the three stress solutes, while *S.cerevisae* and *R.glutinis* exhibited good tolerance in NaCl, but failed to do so in KCl and Na_2SO_4 .

INTRODUCTION

Organisms are found living from fresh water to saturated brine (6M NaCl) conditions and are thus confronted with the potential problem of water gain or loss. Particularly among euryhalime osmoconformers, intracellular osmotic pressure may fluctuate greatly (Yancey *et al.* 1982). Cellular growth is generally inhibited by extremes of environmental salinity. However, several species of prokaryotic and eukaryotic microorganisms have acquired the ability to tolerate wide range of salt stress (Brown 1976; Gould 1977). The obligate halophilic bacterium *Halobacterium salinarium*, whose enzymes require 25% NaCl for optimal activity was studied in detail (Holmes *et al.* 1965). Extremely halotolerant *Halomonas* sp. was isolated from tar balls and its cellular fatty acid composition has been studied by Naganuma & Horikoshi (1993). In yeasts the widely distributed species *Debaryomyces hansenii* and *Saccharomyces cerevisae* were studied in detail (Alder & Gustafsson 1980;

Alder *et al.* 1985; Lars *et al.* 1988). Preliminary studies have been carried out on *Rhodotorula rubra* and *R. glutinis* (Ito & Takada, 1978; Lan, 1990).

This paper presents a general screening of 16 marine yeasts for their capacity to grow on different concentrations of NaCl. Out of the 6 isolates 2 belonging to *Saccharomyces*, 3 to *Debaryomyces* and one to *Rhodotorula* were tested on different concentrations of stress solutes NaCl, KCl and Na₂SO₄.

MATERIALS AND METHODS

Yeast cultures used in the study were isolated from water and mud samples of the EEZ of India during the cruises of *FORV Sagar Sampada* (Gupta & Prabhakaran, 1990). The cultures are maintained as stock culture in the Regional Centre of National Institute of Oceanography, Cochin, India. Screening of the yeast cultures were carried out by observing their growth on YEPD agar (yeast extract 1%, peptone 2%, dextrose 2% and agar 2% w/v) with different percentages of NaCl i.e. 0%, 4%, 6%, 12%, and 16%. Inoculum from actively growing slants of yeasts were streaked on the plates and the plates were incubated at 26±1°C. Observations were made after 6 days of incubation. Growth was estimated by count of viable colony forming units (cfu) on the plates (Lars *et al.* 1988).

Cells were cultured in a defined liquid mineral salt medium (Alder & Gustafsson, 1980) with supplements as stated further. Starter culture was prepared by inoculating a loopful of actively growing yeast culture in 50 ml of the above medium supplemented with 1% w/v glucose in 250 ml Erlenmeyer flasks. The flasks were incubated at 26±1°C on rotary shaker (at 110 rpm) for 24 to 48 hours when the cell density reached 2×10^4 cells/ml. These cultures (0.1 ml) were used as inoculum for 100 ml of the above medium in 250 ml Erlenmeyer flasks supplemented with different concentrations of stress solutes. Concentrations tested were NaCl : 0%, 4%, 6%, 12% and 16%; KCl and Na₂SO₄ : 0.4%, 4%, and 8%. Tests were run in triplicate. The flasks were run on rotary shaker at 110 rpm and 26±1°C. After 24 hours incubation 0.05 ml culture was added to 50 ml of molten medium (having same composition with 1% w/v glucose and the same percentage of the stress solute plus 2% bactoagar) and poured into replicate plates. The cfu were counted after 6 days of incubation. For all the media pH was adjusted to 6, incubation temperature was 26±1°C and the plates were incubated enclosed in polythene bags to avoid desiccation.

RESULTS AND DISCUSSION

Details of the results of screening 16 yeast isolates for their growth at different percentage of NaCl concentration is given in Table 1. Two species of *Debaryomyces*, *D. hansenii* and *D. marama* showed excellent growth from 0% to 16% NaCl concentrations, while *D. vanriji* did not grow at 16% although it grew well from 0% to 12% NaCl concentrations. *Rhodotorula graminis* was the most sensitive of all the *Rhodotorula* isolates tested, exhibiting poor growth at 0% and 12% onwards, its optimal

Table 1 — Growth of yeast isolates in different concentrations of sodium chloride

Yeast species	Concentration of NaCl				
	0%	4%	6%	12%	16%
<i>Candida</i> sp.	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	⊕⊕
<i>Candida albicans</i>	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕	⊕	⊕
<i>Candida guilliermondii</i>	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	—
<i>Candida membranaefaciens</i>	⊕⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	—
<i>Candida parapsilosis</i>	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	—
<i>Candida parapsilosis</i>	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	⊕
<i>Debaryomyces hansenii</i>	⊕⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕
<i>Debaryomyces marama</i>	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕⊕
<i>Debaryomyces vanriji</i>	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕⊕	—
<i>Saccharomyces kluyveri</i>	⊕⊕⊕⊕	⊕⊕⊕⊕	⊕⊕	⊕	—
<i>Saccharomyces cerevisae</i>	⊕⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕	—
<i>Rhodotorula</i> sp.	⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	⊕
<i>Rhodotorula glutinis</i>	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	⊕
<i>Rhodotorula minuta</i>	⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	⊕
<i>Rhodotorula graminis</i>	⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕	—
<i>Rhodotorula rubra</i>	⊕⊕	⊕⊕⊕⊕	⊕⊕⊕	⊕⊕	⊕

⊕⊕⊕⊕ (250 - 150 cfu) Excellent growth, ⊕⊕⊕ (150 - 50 cfu) good growth, ⊕⊕ (50 - 10 cfu) moderate growth, + (10 - 1 cfu) poor growth, — no growth.

growth was at 4% NaCl concentration while *R.rubra* proved most tolerant with optimal growth at 4% NaCl concentration. Most of the species of red yeast *Rhodotorula* lost their pigmentation from NaCl concentration of 6% onwards but *R.rubra* maintained light pigmentation even at 16% NaCl concentration. All the species of *Candida* exhibited wide range of flexibility except for *C.albicans* which grew well up to 4% NaCl concentration. *Candida* sp. was most versatile exhibiting excellent growth at 4% and it grew moderately at 12% and 16% NaCl concentrations. Most of the other yeast isolates tested showed excellent growth up to 4% and good growth up to 6% but grew poorly above 12% concentration.

Details of the results of testing 6 yeast isolates for their growth on different concentrations of stress solutes are given in Table 2. *Debaryomyces hansenii* exhibited equally good growth from 0% to 16% NaCl concentrations with 206 cfu at 0%, 260 cfu at 4%, 220 cfu at 12%, and even 184 cfu at 16% NaCl concentration

Table 2 — Growth of six yeast isolates in different concentrations of stress solutes: colony forming units (cfu)/plate

Yeast species	NaCl					KCl			Na ₂ SO ₄		
	0%	4%	6%	12%	16%	0.4%	4%	8%	0.4%	4%	8%
<i>Debaryomyces hansenii</i>	206	260	248	220	184	210	210	204	220	208	220
<i>Debaryomyces marama</i>	188	260	240	180	174	188	84	25	132	174	-
<i>Debaryomyces vanriji</i>	160	240	180	174	-	180	32	30	178	180	184
<i>Rhodotorula glutinis</i>	170	220	160	20	5	6	4	-	10	20	10
<i>Saccharomyces cerevisiae</i>	200	208	180	5	-	2	5	-	8	28	22
<i>Saccharomyces kluyveri</i>	204	200	25	2	-	88	28	-	160	26	20

D. marama could also grow in wide range of NaCl concentrations, optimal viable cfu 260 at 4%, 188 cfu at 0%, and 174 cfu at 16%. *Debaryomyces vanriji* too had good growth from 0% to 12% but failed at 16% NaCl concentration. *Saccharomyces cerevisiae* had good growth with 180 cfu at 8% but poor at 12% NaCl concentration. *S. kluyveri* showed good growth with 200 cfu at 4%, but at 6% growth became poor. Both the *Saccharomyces* sp. could not grow at 16% NaCl concentration. *Rhodotorula glutinis* had good growth with 229 cfu till 8% but had only a few cfu (5) at 16% NaCl concentration.

In KCl and Na₂SO₄ test concentration (Table 2) *D. hansenii* exhibited steady growth from 0% to 8% concentrations, whereas *D. vanriji* and *D. marama* could grow up to 8% of KCl and not in Na₂SO₄. *Rhodotorula glutinis* had poor growth of 4 cfu at 8% KCl and Na₂SO₄, while nil at 8% KCl and 10 cfu at 8% Na₂SO₄ respectively.

All the three species of *Debaryomyces* exhibited very good growth in a wide range of salt concentrations (stress solutes) of NaCl, KCl, Na₂SO₄. *Debaryomyces hansenii* was the most versatile species.

This study proves that a number of species of *Candida* and *Rhodotorula* which regularly occur in the marine environment are also highly versatile in their salt tolerance and can stay viable and grow in high concentration of stress solutes as well as in very low concentrations. This establishes them to be of euryhaline nature. Further work is in progress.

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