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Application of Microcalorimetry to the Screening of Halotolerant Yeast Strains

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Abstract

A calorimetric procedure was applied to study the inhibitory action of NaCl on the growth of three yeast strains: Saccharomyces cerevisiae HEBE - V 1, S. cerevisiae DP 127 - V 39 and S. cerevisiae DPG - V 40.

Using a conduction-type isothermal batch calorimeter with 24 units, the heat envolved during the growth of cultures in media with different amounts of NaCl (1-10%) was recorded as growth thermograms. The changes in the growth thermograms were observed when the salt concentrations increased and they were correlated to the determined values of growth rate and the time necessary for the culture to reach a selected level of calorimetric signal. These parameters made possible the calculation of 50% inhibitory concentration (K) and the 100% inhibitory concentration (MIC) for the growth of the yeast strain in the presence of salt.

The method can be used as a rapid technique for screening halotolerant yeast strains and for the study of the inhibitory action of salt on yeast growth Significance of the results, advantages and limits of the procedure are also discussed.

Keywords: screening of salt tolerant yeasts, quantitative determination of growth inhibition parameters, microcalorimetry technique.

Introduction

The molecular model for salt tolerance in plants is difficult to study, but the model can be established in microorganisms. The prokaryotic model – *Escherichia coli* – has provided very interesting examples regarding signal transduction mechanisms during salt stress and has suggested that the synthesis of organic solutes may be a crucial step in salt tolerance. Moreover, the eukaryotic model – *Saccharomyces cerevisiae* – is very useful because the fungi and plant cells share the same mechanisms of membrane energization and the stress signal transduction pathways may be conserved between yeasts and plants [7,8].

These models could not only illuminate the critical steps for salt tolerance, but also provide the tools for improving salt tolerance in plants. The utilization of the *Saccharomyces cerevisiae* yeast as a model system in the field of osmotic and salt stress provides a novel perspective for the genetic engineering of transgenic plants to which this model may be extended [7,9].

A major problem in the salt stress studies is how to generate a random collection of genetically modified organisms that need to be screened for salt tolerance. In addition, the

gene responsible for the halotolerance must be recovered from the selected salt-resistant transformants. These prerequisites are better suited to microorganisms with well-developed molecular genetic models, such as *Escherichia coli* and *Saccharomyces cerevisiae*. Working with plants would be much more complicated, even with the simplest plant *Arabidopsis thaliana.* In plants cloning by complementation of the desired phenotype has not yet been achieved. Therefore, even if a mutant with enhanced salt tolerance were isolated, the difficulty in isolating the responsible gene would further delay progress [7,8,9].

Starting from this premise our approach was to establish a simple and rapid method useful in research as well as for technological purposes, for the identification, selection and characterization of NaCl tolerance yeast strains. This procedure can complement other techniques used to study the action mechanisms of inhibitors at cellular level. The calorimetric method presented here is, in our opinion, suitable for the investigation of the salt effect on the growth of the yeast strains and precise quantitative information can be obtained by means of experiments similar with those employed here.

Materials and Methods

The yeasts used in this study were provided by the Culture Collection of the Microbiology Laboratory from the Faculty of Biotechnology – Bucharest (USAMV) and from Iwate Biotechnology Research Centre, Japan (IBRC).

For the qualitative analysis 41 yeast strains were tested for their ability to grow in saline conditions. Three halotolerant phenotypes were selected: *Saccharomyces cerevisiae* V 1,V 39 and V 40.

Adaptation to salt medium

The strains were cultivated on selective media (YEPD–NaCl) containing 1 – 10% NaCl. The colonies grown on plates were examined after 1–5 days and the cultural characteristics were recorded.

The biomass acumulation in liquid selective media YEPD-NaCl 5 - 10% was measured in adsorbance units at 650 nm on UV-VIS spectrometer.

Application of the microcalorimetry technique to the study of salt tolerance in yeasts

The growth activity of the microorganisms in culture media can be investigated by monitoring the heat produced by the microorganisms while growing under various conditions.

The calorimeter used to monitor the microbial growth belongs to the class of heat conduction calorimeters. The heat evolved during microbial growth is detected by the thermopile plates placed in each calorimetric unit, and the small temperature differences between sample units and a reference unit (which contains water) are transformed into voltage signals (μV). The apparatus detects the heat evolved during microbial growth and records it as a function of time, providing the so-called "growth thermograms" or "thermal profiles". The main advantage of the apparatus over other designs is the large number of calorimetric units; 24 microbial cultures can be monitored simultaneously.

Other detailed data regarding the apparatus and the calorimetric method have been previously published elsewhere [1-6].

Changes are visible among the growth thermograms recorded when an inhibitor is added to the cultures in various concentrations. These changes may be quantitative expressed using the value of the growth rate constant μ (that may be calculated from each thermogram) and the time necessary for the culture to reach a selected level of calorimetric signal. These parameters made possible the calculation of 50% inhibitory concentration (K) and the 100% inhibitory concentration (MIC) for the growth of the yeast strain in the presence of salt [1-6].

Inoculum: The strains *Saccharomyces cerevisiae* HEBE (V 1), *S. cerevisiae* DP 127 (V 39), and *S. cerevisiae* DPG (V 40) were grown on YEPD medium for 18–20 hours. The

suspension of yeast cells was prepared by diluting the resulting inoculum with distilled water until the cell concentration was between 1 and 9×10^6 cells/ml. Counting with a Thoma chamber was done to check the number of cells.

Strain	***Inoculum (cells/ml)***
Saccharomyces cerevisiae HEBE (V 1)	8.8×10^6
Saccharomyces cerevisiae DP 127 (V 39)	3.487×10^6
Saccharomyces cerevisiae DPG (V 40)	2.865×10^6

The 24 calorimetric units contain YEPD medium for control cultures and YEPD-NaCl media (2, 4, 5, 6, 8, 10% NaCl) for sample cultures.

All experiments were carried out in triplicate, but only the means of the data in each set of results are shown.

Results and Discussions

A calorimetric method for the study of the microbial inhibition by various chemical compounds was applied for the quantitative analysis of the inhibitory action of salt on the growth of three yeast strains. The method may prove useful for the identification, selection and characterization of NaCl tolerance yeast strains.

In the first stage we tested 41 yeast strains for their ability to grow in saline conditions and selected 3 halotolerant yeast strains: *Saccharomyces cerevisiae* V 1, V 34 are wine-making strains and strain V 39 is a baker's yeast strain. For these strains the halotolerant phenotypes isolated after natural selection showed a slower growth than the wild strains on a medium with 5% NaCl. These strains grew very well on a medium with 5% NaCl, but only V 1 and V 39 grew in medium with 10% NaCl. We observed in the qualitative analysis that the strains *Saccharomyces cerevisiae* V 1 and V 39 showed a very good growth on media with 10% NaCl (liquid and solid), while the strains *S. cerevisiae* V 40 did not grow on medium with 8% salt. We selected these strains for microcalorimetry studies.

For all the strains, the addition of NaCl in the culture at the beginning of the incubation affected the growth thermograms. Increased concentrations of salt led to a progressive decrease in the initial slope of the *g(t)* curves, usually lowered the peak height and delayed the peak time.

The actual heat evolution curve, called *f(t)* can be computed from the *g(t)* curve using the equation: $f(t) = g(t) + K\int g(t)\mathrm{d}t$

where *K* is the heat conduction constant of the calorimeter. The *f(t)* curve represents the heat amount developed in the calorimetric unit, as if it were recorded under adiabatic conditions.

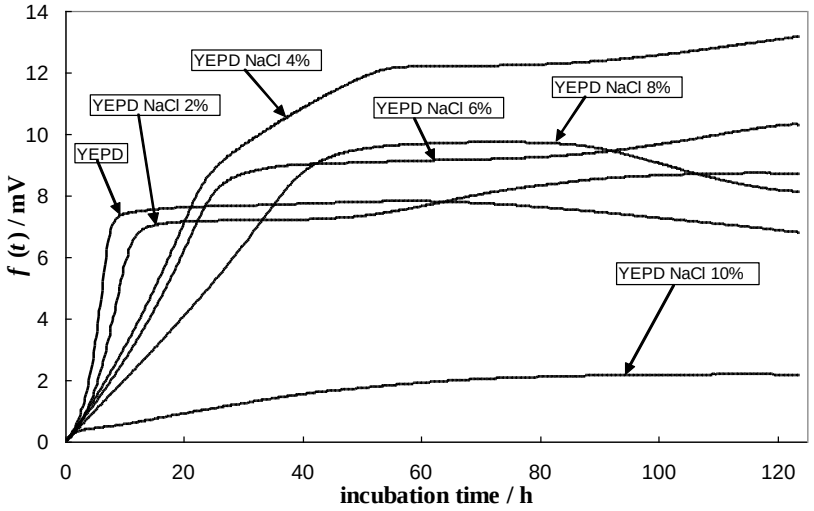

Figure 1. Actual heat evolution curves f(t) for the strain S. cerevisiae HEBE - V 1

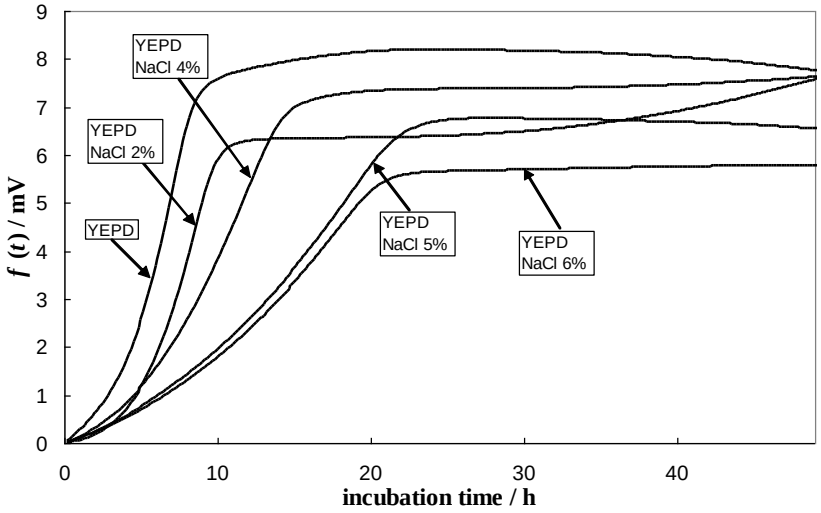

Figure 2. Actual heat evolution curves f(t) for the strain S. cerevisiae DPG - V 40

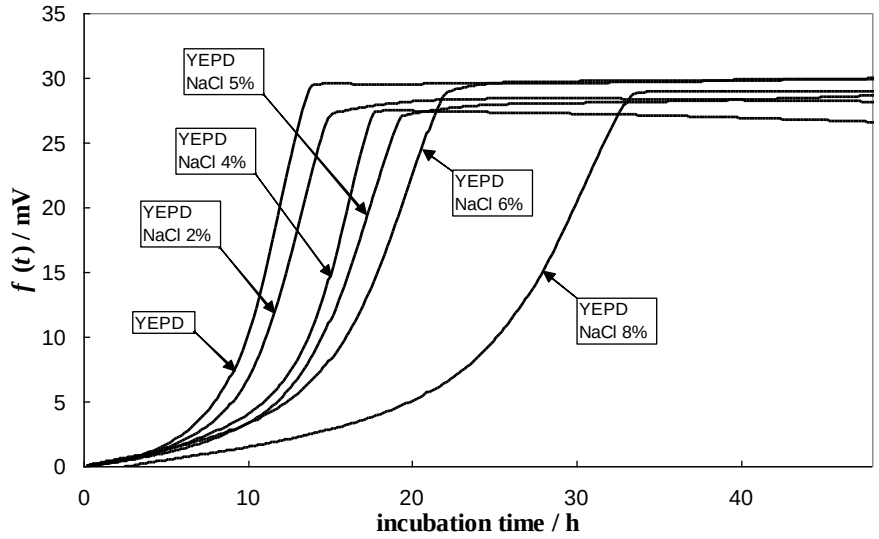

Figure 3. Actual heat evolution curves f(t) for the strain S. cerevisiae DP 127- V 39

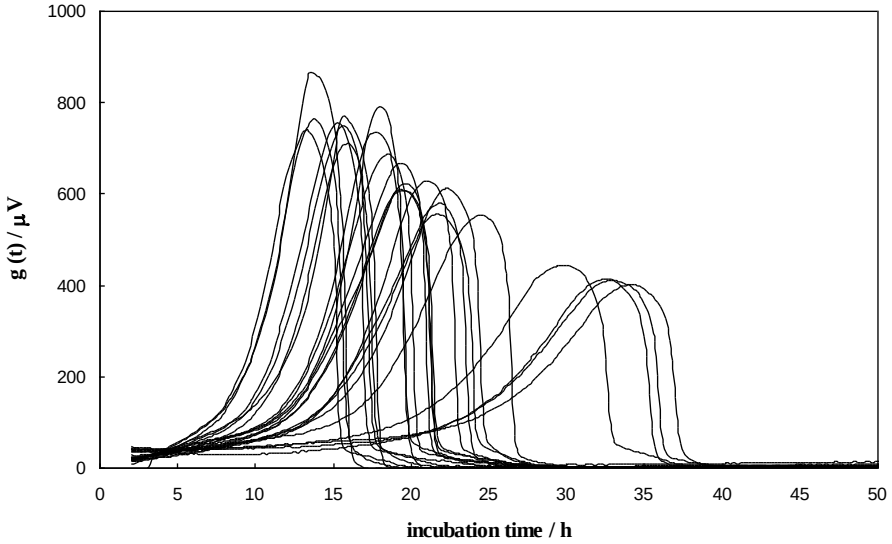

Figure 4. Growth thermograms g(t) for the strain S. cerevisiae V 39

For all the strains the changes observed in the growth thermograms, observed on the *g(t)* and *f(t)* curves, were gradual and evolved progressively; when the concentration of inhibitor increased the growth rate constant μ decreased and the time needed for growth increased.

The strains *Saccharomyces cerevisiae* V 1 and V 39 showed good growth on a medium with more than 10% NaCl, but the other strain *S. cerevisiae* V 40 did not grow on a medium with 8% NaCl. The same results were obtained in the qualitative analysis and on the growth thermograms (**Figures 1, 2, 3 and 4**).

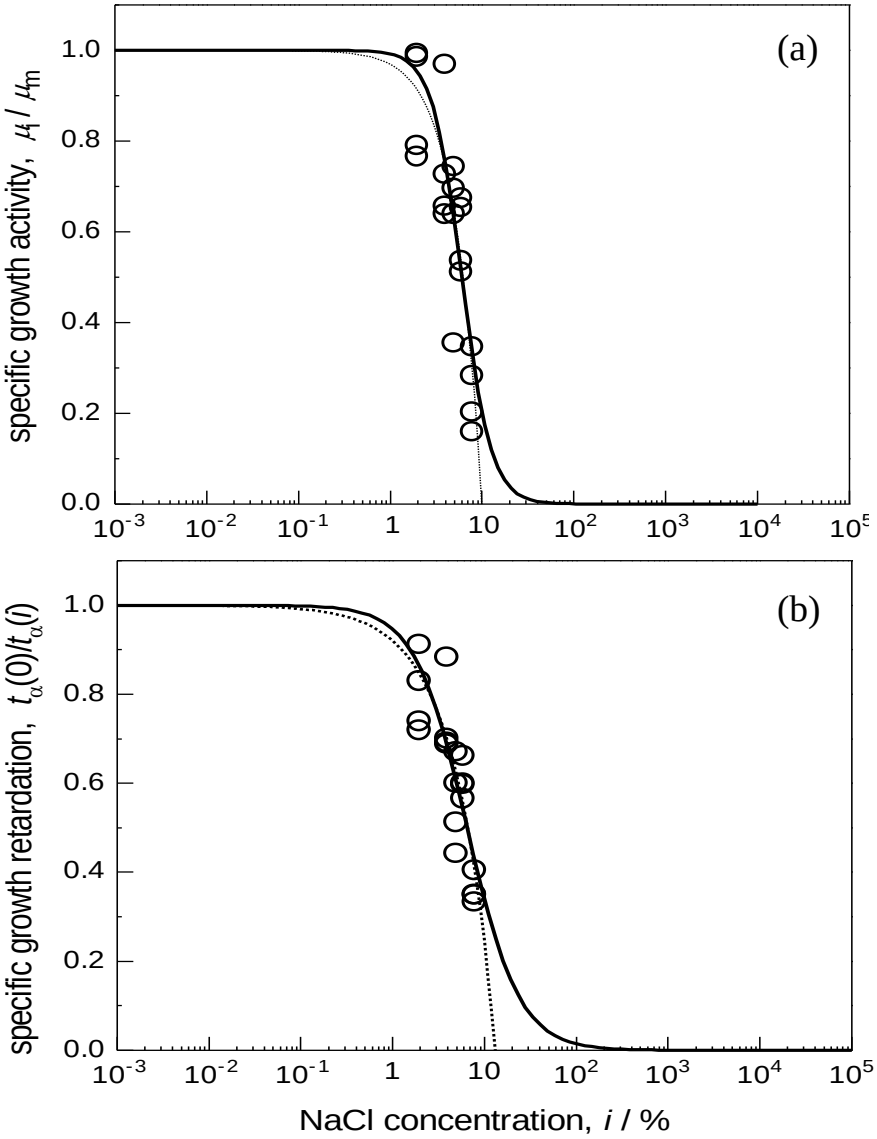

Figure 5. Determination of K and MIC to Saccharomyces cerevisiae V 39.
(a) based on the specific growth activity μ_i/μ_m ;
(b) based on the specific growth retardation $t_\alpha(0)/t_\alpha(i)$.

Figure 5 shows the method used for the determination of *K* and MIC, with an actual example obtained for the strain *Saccharomyces cerevisiae* DP 127 – V 39 (**Table 1**). These inhibitory parameters can be useful for assessing the tolerance of the various yeast strains to the inhibitors studied and for understanding the action mechanisms of inhibitors at the cellular level [1-3].

Table 1. The results obtained for strain *Saccharomyces cerevisiae* V 39

Results from μ_i/μ_m	Results from $t_\alpha(0)/t_\alpha(i)$
$K_\mu = 6.02 \pm 0.34$ (%)	$K_\theta = 6.48 \pm 0.48$ (%)
$MIC_\mu = 9.84 \pm 0.77$ (%)	$MIC_\theta = 13.21 \pm 1.74$ (%)

The results for *Saccharomyces cerevisiae* V 1 were:
$K_\mu = 22.95 \pm 0.95$ (g/l);
$MIC_\mu = 58.68 \pm 6.53$ (g/l).

Using microcalorimetry research (with these salt tolerant strains and with other strains with ethanol tolerance) we found that experiments with salt inhibitor concentrations up to the level of *K* lead to a satisfactory precision in the value of the determined MIC, while keeping the incubation time reasonably short. A very precise determination of MIC requires inhibitor concentrations as high as possible, which are not favorable for the calorimetric experiments because they require long incubation times.

Another important problem regarding values of inhibitory parameters is their variability with the experimental conditions (like culture media, pH value, temperature and inoculum size) and especially the effect of growth and fermentation products released in the medium by the yeast cells. Regarding this aspect, it can be argued that only the initial portion of the thermograms, between 3% and 30% of their total height, is actually used for the determination of the growth rate μ; therefore, the influence of such changes brought about by growth and fermentation is significantly reduced. Also, we found that variation of the inoculum size between 1 and 9 million cells per vial had no significant effect on the *g(t)* curves recorded.

Conclusions

In conclusion, the calorimetric method presented here is, in our opinion, suitable for the investigation of the salt effect on the growth of the yeast strains and precise quantitative information can be obtained through experiments similar with those employed here. It is obvious that the schematic mechanism proposed does not fully cover the complexity of the interaction between microorganisms and salt, but it allows the determination of useful inhibition parameters.

Some advantages of the method are evident: simplicity, improved accuracy of the results, applicability to almost any microbial growth process and growth medium and easy modification of growth conditions.

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