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Full Length Research Paper

Trichosporon cutaneum resistance to hazardous substances as measured by dielectric

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Conditioning of cultures involves growing cells in the presence of increasing amounts of a toxic chemical. In order to investigate how conditioning affects a culture's ability to resist new challenges, the resistance of *Trichosporon cutaneum* to various toxic chemicals, before and after conditioning to growth on phenol, was investigated by measuring the capacitance of cell suspensions at 0.4 MHz following a toxic challenge. The results show that cells grown on phenol are more resistant to the influence of polar aromatic toxic chemicals such as phenol (log P_{ow} = 1.48) and benzylalcohol (log P_{ow} = 1.1), but less resistant against less polar non-aromatic compounds such as *n*-octanol (log P_{ow} = 2.9). In reverse, cells grown on glucose were found to be more resistant against *n*-octanol, but less so against phenol and benzylalcohol. The results indicate that cells, adapted to be more resistant to one type of substance, may become more susceptible to other compounds.

Key words: conditioning, adaptation, membrane, *Trichosporon cutaneum*, capacitance.

INTRODUCTION

The use of yeasts in the bioremediation of phenol-containing wastewaters has a number of advantages, including their high growth rate, their ability to degrade higher concentrations of phenol (1 g/L), and their ability to work at low pH values (4 - 5) as typically encountered in phenol containing wastewaters. *Trichosporon cutaneum* is a yeast which is able to use phenol as its sole carbon source as well as well as a wide range of other substrates (Aleksieva et al., 2002; Ivanova et al., 2002; Godjevargova et al., 2003). In addition, it has found use in a number of biotransformations (Stahl et al., 1999; Conceição et al., 2003).

Before it can be used to biodegrade phenol-containing wastewater, *Trichosporon* needs to be conditioned by repeated growth in phenol-containing media (Godjevargova et al., 2003). Once in wastewater, *Trichosporon* will simultaneously encounter a wide range of different chemicals with different properties and hence different toxicological effects.

Dielectric measurements in the radiofrequencies have previously been found to be an excellent method for the rapid study of the toxicity of solvents (Stoicheva et al., 1989; Davey et al., 1993).

The success of the method is based on the large differences in the electrical properties of the membrane (dielectric constant or permittivity and conductivity) compared to that of the cytoplasm and suspending medium. Charge separation in the electric field causes the cell to obtain a dipolar moment, whose magnitude in a specific part of the radiofrequency range (0.1 - 20 MHz) is highly dependent on the membrane capacitance (Harris et al., 1987; Markx and Davey, 1999).

Addition of solvents to a cell suspension leads to the insertion of solvent molecules into the membranes, which, when at a sufficiently high concentration, destabilizes the membrane and can lead to its rupture. These phenomena can often be observed as an increase in the suspension capacitance as the solvent molecules are taken up by the membrane, followed by a decrease as the membrane is ruptured (Stoicheva et al., 1989; Davey et al., 1993).

In this paper we describe the investigation of the resistance of phenol-conditioned and unconditioned *Trichosporon cutaneum* cells against phenol, benzylalcohol and *n*-octanol using dielectric spectroscopy.

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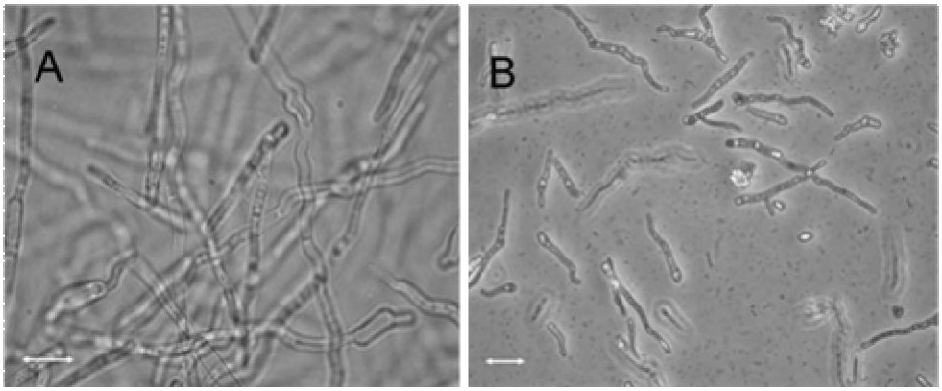

Figure 1. *T. cutaneum* cells grown on A) glucose and B) phenol. (Arrow = 50 m).

MATERIALS AND METHODS

Yeast strain and media

T. cutaneum, registered by Ivanova et al. and maintained in the Bulgarian National Bank of Industrial Microorganisms and Cell Cultures under N 2414, was used for the experiments. Trichosporon is an opportunistic fungal pathogen. All direct contact with the yeast was avoided, and all used material was autoclaved before disposal. A 1% Virkon$^{(TM)}$ solution was immediately applied to any spillages. The strain was maintained by periodic culturing on agar medium at 28°C every 48 h with the following composition: glucose 20 g/L, bacterial peptone 20 g/L, yeast extract 10 g/L, agar 20 g/L. First, a solution was made of all components except the agar in distilled water, and its pH was set to a value of 6 with a 10% NaOH solution. Following this, the agar was added, and the volume adjusted to its final value. The medium was then sterilized at 120°C for 20 min.

After incubation colonies were picked and suspended in a mineral salt medium with a glucose concentration of 20 g l^{-1} at 28°C for 24 h in 250 ml Erlenmeyer flasks with 100 ml liquid medium. The composition of the medium was as follows: glucose 20 g/L, $(NH_4)_2SO_4$ 4 g/L, $Na_2HPO_4.2H_2O$ 0.75 g/L, KH_2PO_4 1.7 g/L, $MgSO_4.7H_2O$ 0.02 g/L, $FeSO_4.2H_2O$ 0.001 g/L, $MnSO_4.H_2O$ 0.001 g/L, $CaCl_2$ 0.002 g/L and yeast extract 1 g/L . A solution was made of all components except yeast extract, after which the pH value was adjusted to 6.8 with a 10 % KOH solution. Yeast extract was then added, and the medium was sterilized at 120°C for 20 min.

Adaptation of the cells to phenol

10 ml of a suspension of cells that were grown on glucose medium on a shaker at 28°C for 24 h was resuspended in 100 ml of a medium of the same composition, but containing phenol (1g/L) instead of glucose, and incubated on a shaker at 28°C for 24 h. Following this, 10 ml of cell suspension was again resuspended in 100 ml phenol medium, and allowed to grow for 24 h before harvesting.

Dielectric measurements

Dielectric measurements were performed using an Aber Instruments Biomass Monitor model 220 (Aber Instruments, Aberystwyth, UK), with a probe with a nominal cell constant of 1.0 cm^{-1}. The probe is solvent resistant, and can be autoclaved.

Cell suspensions that were obtained by growth of *T. cutaneum* in Erlenmeyer shake flasks on either glucose medium, phenol medium (1 time) or phenol medium (2 times) were harvested, centrifuged and concentrated until 100 ml cell suspension was obtained with a capacitance value of 10 pF. The cells remained suspended in spent growth medium. The suspension was transferred to a magnetically stirred beaker. Phenol, benzylalcohol or *n*-octanol was added to the cell suspension at different concentrations, and the capacitance of the suspension was followed over time and recorded using a chart recorder.

RESULTS

T. cutaneum was grown on glucose and phenol medium (Figure 1). The yeast grew in long, clear filaments on glucose, whilst cells grown in batch on phenol medium for 2 times appeared darker. Cells grown on phenol medium only once were intermediate in appearance.

Suspensions of *Trichosporon* cells grown on glucose and phenol (for one or two consecutive batch cultures) were prepared, and phenol, benzylalcohol and *n*-octanol were added to the suspension at different concentrations, and the capacitance of the suspension was measured in time. The results, shown in Figure 2- 4, show a change in the suspension capacitance similar to the ones recorded previously for other organic compounds (Stoicheva et al., 1989; Davey et al., 1993), i.e. a rise in the capacitance as the solvent is taken up by the membrane, followed by a decline in the capacitance as the membrane is breached. It has previously been shown (Stoicheva et al., 1989; Davey et al., 1993) that the decline in the capacitance correlates well with the decline in cell viability. The capacitance does not decline to a value of zero, indicating that dead cells have a residual capacitance.

As shown in Figure 2 - 4, it was found that *Trichosporon* cells, when repeatedly grown on phenol, became increasingly more resistant against phenol and benzylalcohol. However, when confronted with *n*-octanol, the preconditioned cells proved to be less resistant against this compound, and died more quickly, as judged by the more rapid decline in the suspension capacitance when the phenol-grown cells were exposed to this chemical. In

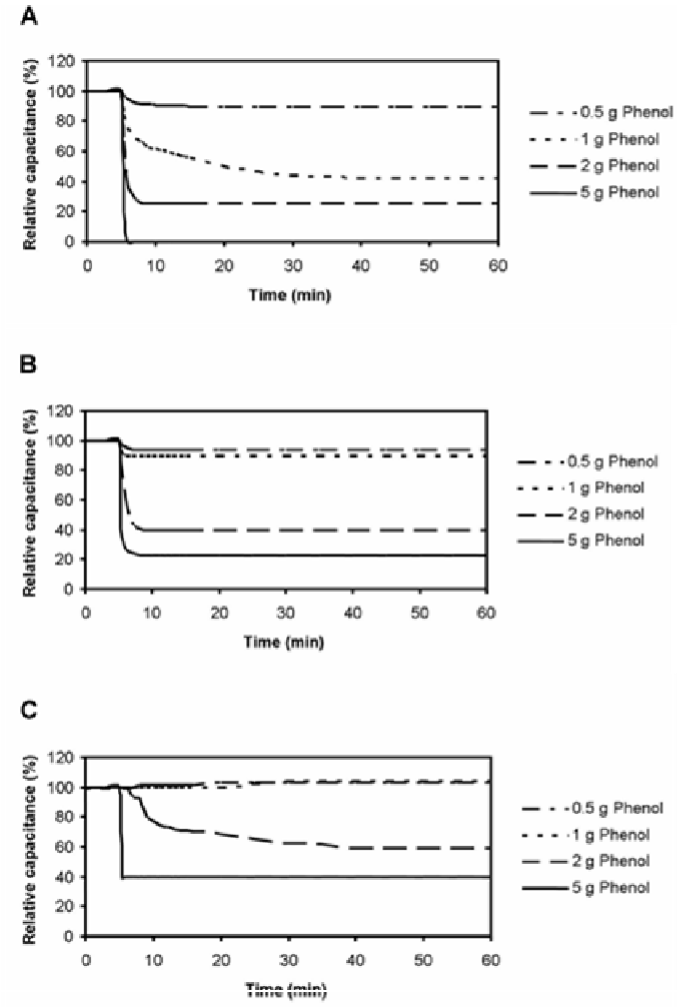

Figure 2. Changes in *T. cutaneum* suspension capacitance after the addition of different amounts of phenol to 100 ml cell suspension. A: Unconditioned cells grown on glucose (2%). B: After conditioning on phenol medium (1 g/L) for the first time. C: After conditioning on phenol medium (1 g/L) for the second time.

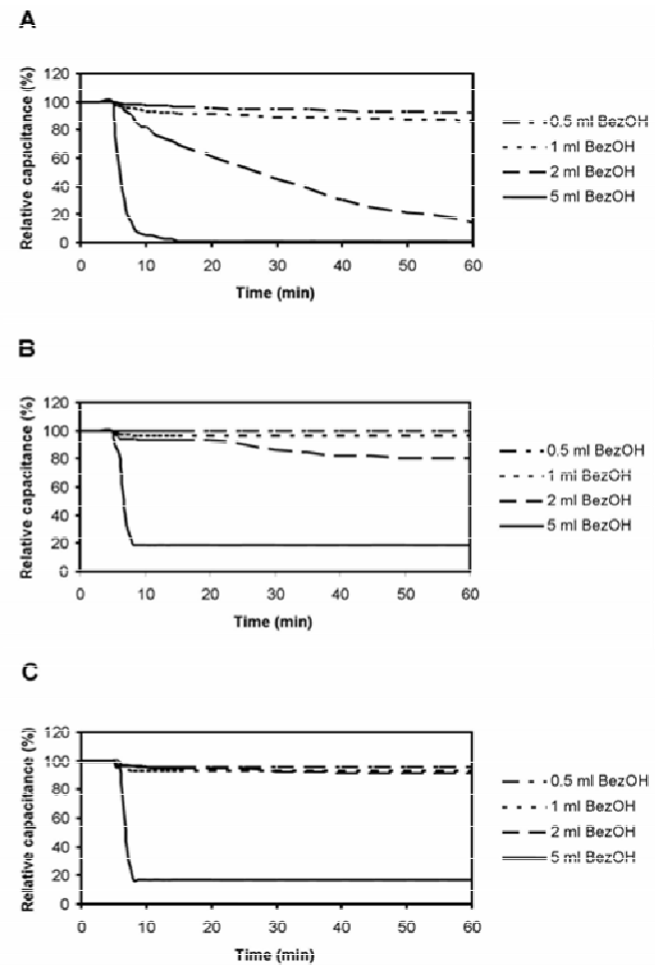

Figure 3. Changes in *Trichosporon cutaneum* suspension capacitance after the addition of different volumes of benzylalcohol to 100 ml cell suspension. A: Unconditioned cells grown on glucose (2%). B: After conditioning on phenol medium (1 g/L) for the first time. C: After conditioning on phenol medium (1 g/L) for the second time.

contrast, the *Trichosporon* cells grown on glucose were relatively more resistant to *n*-octanol, but less so to phenol and benzylalcohol.

Discussion and Conclusions

A wide variety of different mechanisms have been found to be employed by different micro- organisms to increase their resistance against solvents, including changes in membrane composition, active efflux of solvents, and the induction of detoxifying enzymes (Sikkema et al., 1995; Ramos et al., 1995; Weber and de Bont, 1996; Kieboom et al., 1998). Although the exact mechanisms of adaptation of *Trichosporon* yeast to growth on phenol are not fully known, they are likely to include the induction of enzymes of phenol-degrading pathways, as well as the adaptation of its membrane properties.

The resistance of the yeast to benzylalcohol was comparable to that to phenol, even though the yeast had not encountered this compound as a substrate previously. This indicates that the conditioning of *Trichosporon* to phenol leads at least partly due to an adaptation of its membrane properties.

It is well-known that microorganisms change the properties of their membrane in response to the presence of solvents (Sikkema et al., 1995; Weber and de Bont, 1996; Sardessai and Bholse, 2002; Ingram, 1977; Keweloh et al., 1990; Denich et al., 2003). In particular, relatively polar solvents and short-chain alcohols have been observed (Ingram, 1977) to increase the synthesis of lipids containing unsaturated fatty acids, whilst apolar solvents and long-chain alcohols have had the opposite effect, increasing the amount of fatty acids.

The polarity of a compound is expressed in terms of their P_{ow}, which is the partitioning coefficient of the

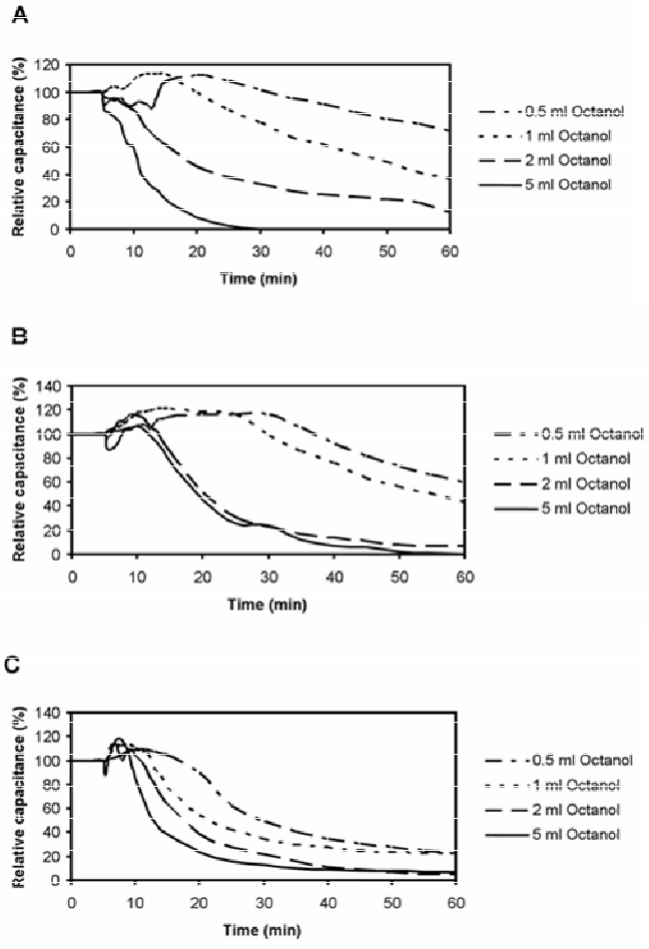

Figure 4. Changes in *Trichosporon cutaneum* suspension capacitance after the addition of different volumes of *n*-octanol to 100 ml cell suspension. A: Unconditioned cells grown on glucose (2%). B: After conditioning on phenol medium (1 g/L) for the first time. C: After conditioning on phenol medium (1 g/L) for the second time.

compound over an octanol/water two phase system. Phenol and benzylalcohol are relatively polar compounds, with log P_{ow} = 1.48 and log P_{ow} = 1.1, respectively. In contrast, *n*-octanol is relatively apolar (log P_{ow} = 2.9).

In our experiments, cells grown on phenol were found to be more resistant to phenol and benzylalcohol, but less so against *n*-octanol. In reverse, cells grown on glucose were found to be more resistant against *n*-octanol, but less so against phenol and benzylalcohol. The results indicate that cells adapted to polar compounds may become more susceptible to other, more apolar compounds.

The findings may be of general relevance to wastewater treatment, biotransformations of toxic compound and the development of biocidal treatments as it points to possible method for enhancing or reducing the cell death induced by toxic compounds. For example, the separation of polar substances from wastewater from apolar substances prior or during biodegradation could potentially be used to improve wastewater treatment.

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