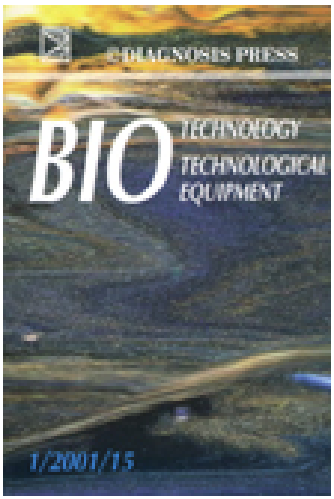

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EFFECT OF NIKEL ON GROWTH AND ULTRA-STRUCTURE OF SCHIZOSACCAROMYCES POMBE

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ABSTRACT

In this project, we investigated effects of nickel (Ni) on the growth and ultrastructure of Schizosaccharomyces pombe wild type strain. It was found that cells were tolerant against a concentration of 2 mM Ni^{+2} but the generation time was extended to 5 hours from 2.5 hours for the cells growing in Ni- free YEP medium. 76% of Ni^{+2} was removed in 30 min by the cells grown in YEP containing 1mM Ni^{+2}. We also analyzed the ultrastructural modifications of the cells grown in 1 mM Ni^{+2}. There was a visible thickening of the cell wall and increase in the number of small cytoplasmic vesicles. The plasma membrane appeared irregular compared to smoother contour in control. Vacuoles contained large amounts of electron-dense materials and the size of vacuoles also increased.

Introduction

Contamination of the environment by heavy metals (Cd, Cu, Zn, Ni, Hg and Pb) released from organic chemicals belonging to the different kind of industries is a major health issue. Some of these metals are essential for all organisms at trace amounts but can be toxic at higher concentrations. Nickel (Ni) is needed for growth and acts as a cofactor of several enzymes, such as methyl coenzyme M reductase, CO dehydrogenase and urease in various organisms (6). Ni potentially inhibits synthesis of macromolecules such as RNA and proteins (5) and it is a potent carcinogenic metal as As, Cr and Cd (17, 16, 18). There are many reports on algae, bacteria, fungi or higher plants that remove and/or accumulate large amounts of heavy metals from their external environment (1, 4, 18, 11). A variety of mechanism is known for metal uptake e.g., adsorption to the cell wall, diffusion into the cells, and metabolism-dependent ion transport (2). These processes depend on many factors such as a metal species and the growth conditions of the organisms. There are a few reports on Ni uptake by yeast (7, 2, 9). Joho et al., 1992 (7), isolated a nickel resistant mutant of *Saccharomyces cerevisiae* and in this mutant, more than 70% of internal nickel is distributed in the vacuolar fraction suggesting the vacuole is the detoxifying compartment (7). Although, the route of Ni transport into vacuole is still unknown, it was very recently demonstrated that nickel permease (nic 1) acts as a plasma membrane nickel transporter in the fission yeast (3). It was the first example of this kind of transporter in a eukaryotik organism.

In this paper we demonstrate the growth inhibition and ultrastructural modifications of the fission yeast *Schizosaccharomyces pombe* by Ni ion.

Materials and Methods

Growth assessment

The wild type *Schizosaccharomyces pombe* var. Pombe 972h Lindner (1354) was grown

in a complex medium, YEP (% 1 yeast extract, % 2 peptone, % 2 glucose) medium containing 0.5, 1, 2, 4, and 8 mM $NiSO_4$ at 30 °C, 180 rpm in a shaken flask for 36 hours. The solution of $NiSO_4$ was sterilized separately and added to the culture medium prior to inoculation. Growth was monitored by measuring the optical density every two hours at 600 nm with a Shimadzu UV-160 spectrophotometer.

Uptake of Ni from aqueous solution

The wild type *S. pombe* was grown in Ni free YEP medium at 30 °C, 180 rpm until mid exponential phase (16h), harvested and washed twice with deionized distilled water. Pellet was resuspended at a cell density of approximately 5 mg dry wt/ml (2 x 10^7 cell/ml) in 50mM HEPES/NaOH (pH 6.5) containing 1mM of $NiSO_4$. The mixture was shaken at 30 °C 180 rpm for 2.5h. Aliquots of the cell suspension were withdrawn at 10, 30, 60 and 150 min and the cells were removed from the supernatant by centrifugation at 1000 x g for 5 min. Ni concentrations of the supernatants were measured by atomic adsorbtion spectrometry. The Ni uptake was determined as the amount of Ni (mg) per unit dry weight (g) of cells. The dry weight of cells was determined after the cell pellet had been dried at 80 °C for 3 days.

Ultrastructural analyses

Cells were harvested by centrifugation, washed twice in distilled water and fixed with 2 % glutaraldehyde in 0.1M of phosphate buffer, pH 7.2 for 2-3 hours than post-fixed with 1 % OsO_4 in the same buffer. Pellets were then dehydrated in graded alcohol (60 to 95 % ethanol, 15min for each solution) followed by infiltration with propylene oxide, twice. Samples were embedded in pure epoxy resin at 60 °C overnight. The blocs were cut to a thickness of approximately 70nm (Leica, ultracut R). The sections were post stained with lead citrate and uranyl acetate. 15-40 cells for each sample were selected at random and photographed at final magnification of X 20 000.

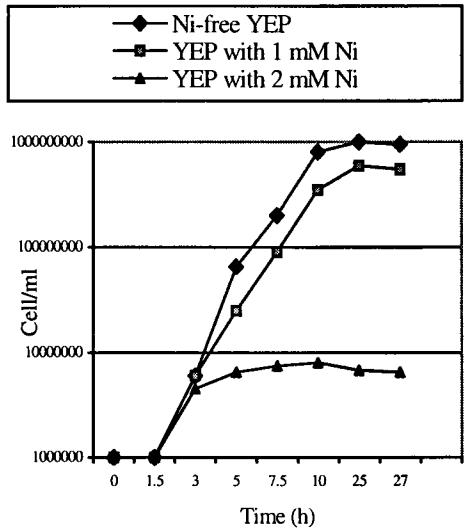

Fig. 1. Effect of Ni on the growth of *S. pombe* cultivated in Ni-free YEP (•) YEP containing 1 mM of Ni (♦) and 2mM of Ni (▪).

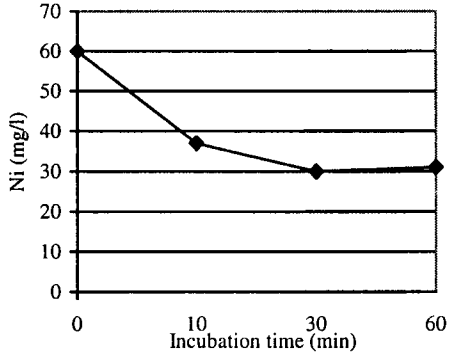

Fig. 2. Time course of Ni uptake by *S.pombe*. Ni concentrations of remaining in the supernatant were measured as described in Materials and Methods.

Results and Discussion

Cells were tolerant against a concentration of 2mM of Ni, but the generation time was extended to 5 hours from 2.5 hours for the cells growing in Ni-free YEP medium. The cells underwent two divisions and went to stationary phase at 10 h. The growth rate of the wild type in medium supplemented with

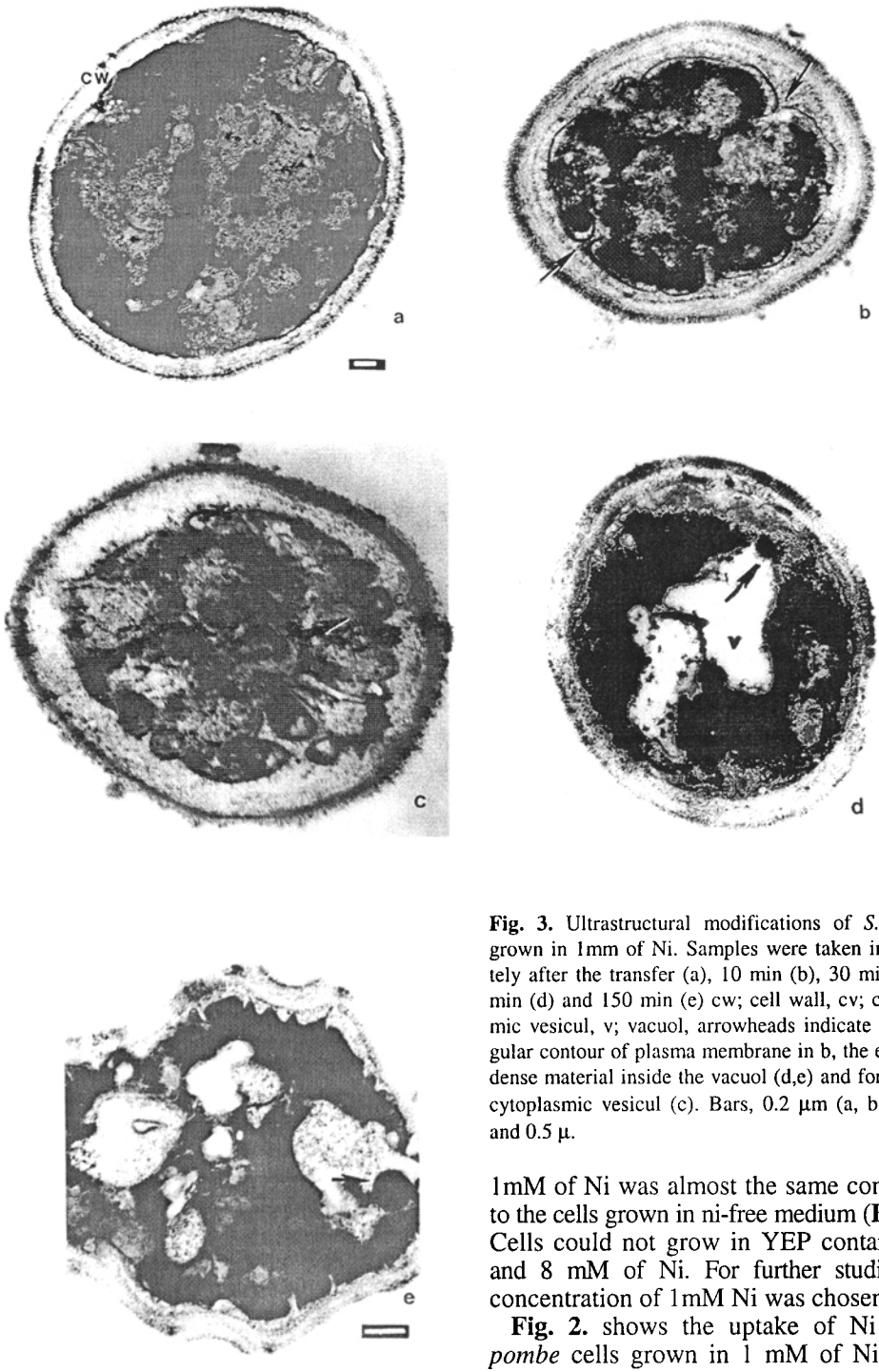

Fig. 3. Ultrastructural modifications of *S. pombe* grown in 1mm of Ni. Samples were taken immediately after the transfer (a), 10 min (b), 30 min(c), 60 min (d) and 150 min (e) cw; cell wall, cv; cytoplasmic vesicul, v; vacuol, arrowheads indicate the irregular contour of plasma membrane in b, the electron-dense material inside the vacuol (d,e) and forming of cytoplasmic vesicul (c). Bars, 0.2 μm (a, b, c,,,, d) and 0.5 μ.

1mM of Ni was almost the same compared to the cells grown in ni-free medium (**Fig. 1**). Cells could not grow in YEP containing 4 and 8 mM of Ni. For further studies the concentration of 1mM Ni was chosen.

Fig. 2. shows the uptake of Ni by *S. pombe* cells grown in 1 mM of Ni. Cells

removed 60 % and 76 % of Ni from the solution within 10 min and 30 min. respectively. It was reported that Ni resistant strain of *Candida guillermondii* strain 13, *C. lusitaniae* strain 16 and 19 removed 80 % of Ni from the solution containing 1 mM of Ni in 1h (9).

Ultrastructural modifications of the cells grown in 1mM of Ni were also observed. There was a visible thickening of the cell wall (**Fig. 3b**) and increase in the number of small cytoplasmic vesicles. The plasma membrane appeared irregular compared to smoother contour in control (**Fig. 3a** and **b**) and cytoplasmic vesicles started to occur (**Fig. 3c**). Vacuoles contained large amounts of electron-dense materials and the size of vacuoles also increased. (**Fig. 3d**). Same kind of ultrastructural reorganizations were also observed in *Hansenula polymorpha* grown in vanadate (10). Vacuole is a compartment known to sequester metal compounds for *S. cerevisiae* (13). Examination of the subcellular distribution of nickel in a Ni resistant strain of *S. cerevisiae*, NOS, grown in 3 mM of Ni showed that 70% of the Ni was distributed in the vacuolar fraction (7). It was also determined that a vacuolar H+-ATPase-negative mutant of *S. cerevisiae* was highly sensitive to Ni ions (12). According to the review of Joho et al. (1995) Ni resistance mechanism for yeast and filamentous fungi might involve (i) inactivation of nickel toxicity by the production of extracellular nickel-chelating substances such as glutathione; (ii) reduced nickel accumulation, probably by modification of a magnesium transport system; (iii) sequestration of nickel into a vacuole associated with free histidine and involving Ni-insensitivity of vacuolar membrane H (+)-ATPase (8). Although the exact mechanism of ni uptake by this yeast is not clarified, it is suggested that Ni is detoxified in the vacuole in *S. cerevisiae* (14).

A complete understanding of the molecular basis of sensitivity to toxic metal ions such as nickel, in lower organisms is expected to provide useful insights in the metal detoxification pathways and diseases related to these pathways in human. In order to understand the molecular mechanism for Ni uptake of *S. pombe* studies are still going on to obtain Ni resistance and sensitive mutants of this yeast by successive cultures in media containing increasing concentrations of Ni and using gene disruption.

Acknowledgements

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