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Nickel resistance mechanisms in yeasts and other fungi

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SUMMARY

This review describes nickel toxicity and nickel resistance mechanisms in fungi. Nickel toxicity in fungi is influenced by environmental factors such as pH, temperature and the existence of organic matter and other ions. We describe resistance mechanisms in nickel-resistant mutants of yeasts and filamentous fungi which were obtained by exposure to a mutagen or by successive culture in media containing increasing concentrations of nickel ion. Nickel resistance may involve: (1) inactivation of nickel toxicity by the production of extracellular nickel-chelating substances such as glutathione; (2) reduced nickel accumulation, probably by modification of a magnesium transport system; (3) sequestration of nickel into a vacuole associated with free histidine and involving Ni-insensitivity of vacuolar membrane H^+-ATPase.

INTRODUCTION

Nickel is virtually ubiquitous [11] and is a trace element in most plants and animals. In some organisms it is an essential constituent as a co-factor of some enzymes [32]. At present there has been an increasing introduction of nickel into the environment as a result of mining and smelting or industrial pollution. In fact, nickel-tolerant bacteria and green algae were isolated from highly polluted domestic and industrial wastes [22,67] and metal-contaminated lakes [71]. Although nickel is still not a widespread contaminant in the biosphere, the environmental hazards of nickel exposure are also a serious problem concerning the metal-working industry [64].

Microorganisms have been used to monitor some aquatic pollutants [10,17,31,53] and in the laboratory can also be induced to evolve a number of different mechanisms to cope with a toxic environment, including high concentrations of heavy metals, by experimental manipulation. The purpose of acquiring metal-tolerant microorganisms is for the removal of metal ions from a polluted environment as well as to provide a biological understanding of the adaptation of living organisms to an extreme environment. Therefore, many studies of metal toxicity and tolerance mechanisms in microorganisms have been carried out. However, despite a number of reviews of metal resistance mechanisms in yeasts and other fungi [4,24,25,65], there is little information concerning the Ni^{2+} ion. The present short review is focused on Ni^{2+} toxicity and resistance mechanisms in yeast and other fungi.

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HEAVY METAL DETOXIFICATION MECHANISMS IN YEASTS AND OTHER FUNGI

Most of the heavy metal resistance mechanisms in filamentous fungi and yeasts rely on effective sequestering of toxic metals within the cell, because there are many metal-sensitive targets involving cellular metabolism. Therefore, metal resistance mechanisms may be divided into two categories: 1) a reduced accumulation of the metal ion by cells as a result of the excretion of metal-chelating substances [58] or by a defect in the specific transport system [13,50]; 2) a change in the intracellular distribution of the ion by binding to specific intracellular molecules, e.g. metallothionein in the case of copper [30] or cadmium [34], to the cell wall in the case of mercury [60] or copper [27], to cellular particulates in the case of manganese [9] and to the vacuole [73] or mitochondria in the case of cobalt [18].

NICKEL TOXICITY TOWARDS GROWTH

Nickel, as well as other heavy metal ions, is an effective fungicide. Somers [69] reported that the fungitoxic order of metal salts against spore germination of *Alternaria tenuis* and *Botrytis fabae* depended on the exponential relationship with their electronegativity. These results indicated that nickel and cobalt were less toxic to fungi than were mercury, silver and copper.

However, metal toxicity in fungi is also influenced by environmetal factors. Among the environmental factors that can cause a decrease in nickel toxicity, are changes in pH or the existence of other ions and organic matter, which can lead to the formation of a metal-complex [5,7,63]. Protheroe et al. [62] reported that nutrient broth and its constituents involving peptone and yeast extract markedly reduced the adsorption of nickel onto the cell surface of *Saccharomyces cerevisiae*. The inhibitory effect of nickel on the growth of *S. cerevisiae* was

decreased by the addition of amino acids such as aspartate or histidine in the medium, causing the reduced accumulation of nickel ion by the cells [37]. Furthermore, Babich et al. [8] found that when *Aspergillus niger* was grown in a Ni-supplemented medium, inhibition of mycelial growth rates was reduced in the presence of Cd^{2+} at a non-inhibitory concentration.

On the other hand, the toxicity of nickel to the growth of fungi was enhanced synergistically by the presence of cadmium in *Trichoderma viride* [8] or by copper at an acidic pH in *Aspergillus flavipes* and *Candida krusei* [7]. From the above results, it is clear that when assessment of heavy metal toxicity towards fungi is carried out, environmental factors should be taken into account.

NICKEL TOXICITY TOWARDS CELLULAR METABOLISM

Ni is a trace element functioning as an essential constituent of several enzymes such as hydrogenase, methyl co-enzyme M reductase, CO dehydrogenase and urease in bacteria and plants [32]. However, it is well known that higher concentrations of Ni^{2+} ions interact with many cellular components such as organic acids [15], nucleotides [12], amino acids [48,51] and phospholipids [33] and result in disturbance of physiological and biochemical processes in fungi, including yeasts. Mohan and Sastry [56] reported that Ni^{2+} toxicity in *Neurospora crassa* was displayed as a derangement in carbohydrate metabolism, and large amounts of pyruvate were excreted from the cells into the culture medium. Furthermore, there is also some information on the disturbance of iron or organic acid metabolisms in *A. niger* [2]. In these processes, the toxicity of Ni^{2+} resulted from interference with the normal metabolism of essential trace elements because of the recovery, in many cases, from its inhibitory effect on the addition of Mg^{2+} or Fe^{3+} [1].

The fact that large amounts of intracellular pyruvate were excreted from *N. crassa* at higher Ni^{2+} concentrations [56], may also indicate alteration of membrane integrity, because there are some reports of the toxic effect of Ni^{2+} on the cell membrane, resulting in the release of K^+ from lichen cells [14] or the formation of large plasmolysed spheroplasts in bacteria [16]. In *S. cerevisiae*, however, the direct effect of Ni^{2+} on cell membrane permeability was less than that of Cu^{2+}, Cd^{2+} and Zn^{2+} [26,35]. Ni^{2+} was also a potent inhibitor of macromolecular synthesis such as RNA and proteins in *S. cerevisiae* [36] as well as in *Escherichia coli* [29].

NICKEL RESISTANCE MECHANISMS

Extracellular Ni^{2+} detoxification

Murata et al. [57] reported that transformants carrying the methylglyoxal resistance gene, obtained by cloning from the yeast genomic library of *S. cerevisiae*, showed resistance to several heavy metal ions such as Cd^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+}. This multiple resistance to chemicals depended on the excretion of large amounts of glutathione into the medium. Because heavy metal ions including nickel generally have a great affinity for compounds with a thiol group such as glutathione, it was suggested that the reduced toxicity of heavy metal ions on yeast was established by the non-enzymatic formation of extracellular metal–glutathione complexes. A similar metal-resistance mechanism was found by Murphy et al. [58] with Cu^{2+}-tolerant *A. niger, Penicillium spinulosum, Verticillium psalliotae* and *Poria placenta*, which excreted a large amount of oxalate into a Cu-supplemented medium and detoxified the Cu by the formation of an extracellular Cu–oxalate complex. However, there is no information for extracellular Ni^{2+} detoxification of Ni^{2+}-resistant fungi.

Reduced nickel accumulation

The nickel ion was taken up by the magnesium transport system in various microorganisms [6,59,66] but not in the bacterium *Bradyrhizobium japonicum* [72], because magnesium ion antagonized the entry of Ni^{2+} into the cells. In *S. cerevisiae*, nickel is also taken up actively by the cells through a magnesium transport system [23]. Joho et al. [39] reported that a nickel-resistant strain of *S. cerevisiae* exhibited decreased accumulation of Mg^{2+}. This demonstrates that the nickel-resistant mutant may have evolved resistance by decreasing the rate of entry of Ni^{2+} through a magnesium transport system. Nickel-resistant mutants with reduced Ni^{2+} accumulation in fungi and bacteria often show co-resistance to Co^{2+} [28,54]. A magnesium transport system was also suggested to participate in the uptake of Co^{2+} as well as Ni^{2+} by the microorganisms [59].

Another possible mechanism of the reduction of intracellular nickel concentration involves an energy-dependent ion-specific efflux, which is found in the nickel-resistant bacterium *Alcaligenes eutrophus* [68]. However, there is no evidence for such a nickel efflux mechanism in fungi.

Participation of the vacuole in the sequestration of Ni^{2+}

The fungal vacuole is often described as an important organelle for the cell in the regulation of intracellular pH [74], ions [49] and the storage of metabolites [43]. In a storage capacity, the cellular histidine pool in *S. cerevisiae* could increase approximately 250 times with the addition of histidine at 0.1 mg ml^{-1} to a glutamate-medium and 87% of the total histidine pool was found in the vacuolar compartment [52]. Vacuolar function defective mutants, which show deficiency in transport systems [19], changes in H^+-ATPase activity [21] and incomplete morphological development [44], could not grow under slightly-stressed conditions involving excess concentrations of basic amino acids, Ca^{2+}, Mg^{2+} and heavy metal ions [44], osmotic change [47] and carbon starvation [43]. This evidence suggests that the vacuole in yeast cells may play an important role not only in intracellular homeostasis for metabolites, pH and the control of ions such as Ca^{2+} in the cytosol but also as a defence system against temporary environmental stress.

The intracellular regulatory mechanisms for homeostasis in the cytosol are mainly assisted by the specific transport system which uses an electrochemical proton gradient, resulting from the participation of the vacuolar membrane ATPase. The vacuolar H^+-ATPase has been isolated and partially purified

from *S. cerevisiae, Saccharomyces carlsbergensis* and *N. crassa* [3,45]. In its sensitivity to some chemicals such as NO_3 and bafilomycin, the vacuolar H^+-ATPase is different from the mitochondrial or plasma membrane ATPase. Moreover, the vacuolar membrane ATPase was more sensitive to heavy metal ions such as Cu^{2+}, Ni^{2+}, Hg^{2+}, and Zn^{2+} than the plasma membrane ATPase [3].

Joho et al. [41] reported that the vacuolar membrane ATPase isolated from a Ni-resistant strain of *S. cerevisiae* was less sensitive to Ni^{2+} than that from the parental strain. A characteristic of the Ni-resistant *S. cerevisiae* was the enhanced sequestration of Ni^{2+} from the cytosol into the vacuolar compartment, although the vacuole was also the main compartment for Ni^{2+} in the parental strain. The accumulation of Ni^{2+} ions into the vacuole of the Ni^{2+}-resistant yeast was accompanied by an increase in the level of the histidine pool, which has a great affinity for Ni^{2+} [38]. Furthermore, histidine-rich cells grown in medium containing a high concentration of histidine, but not lysine- or arginine-rich cells, showed preferential sequestration of Ni^{2+} in the vacuole [40]. This evidence may indicate that histidine, localized mainly in the yeast vacuole, could assist in changing the chemical state from free Ni^{2+} to a Ni^{2+}-histidine complex, probably causing enhancement of the accumulation capacity for Ni^{2+} ion in the vacuole. Similar evidence was reported by White and Gadd [73] with *S. cerevisiae* in that a Co^{2+}-resistant strain obtained by training with repeated culture under elevated Co^{2+} concentrations increased Co^{2+}-accumulation in the vacuole. In this case, it was also suggested that the sequestration of Co^{2+} ions from the cytosol to the vacuolar compartment overcame its entry from the environment.

Other Ni-resistant mechanisms

Joho et al. (unpublished data, 1994) recently isolated a Ni-resistant mutant of *S. cerevisiae* by mutagenesis after UV irradiation. The mutation conferring Ni^{2+} resistance in the yeast was a single gene and recessive. The Ni^{2+}-resistant mutant was crossed with a vacuolar-defective strain and sporulated. When the tetrads were analyzed, some vacuolar-defective segregants showed Ni-resistance despite considerable Ni^{2+} accumulation by the cells. Accordingly, Ni-resistance in the newly isolated Ni^{2+}-resistant mutant of *S. cerevisiae* appeared to rely not on vacuolar function but on a novel Ni^{2+}-resistant mechanism. Kumar et al. [46] reported that a Ni-resistant strain of *N. crassa* isolated by repeated subculture on agar slants containing high concentrations of Ni^{2+} showed hyperaccumulation of Ni^{2+} from the medium, resulting in the sequestration of 90% of Ni from the medium containing 120 mg L^{-1} Ni^{2+}. The Ni-resistant *N. crassa* appears to be a suitable organism for the removal of Ni^{2+} from a polluted environment. However, there is still no information on the resistance mechanism in the Ni-resistant *N. crassa*.

CONCLUDING REMARKS

It is obvious that current knowledge of Ni^{2+} resistance mechanisms of fungi and yeasts is mainly limited to physiological and biochemical approaches. Therefore, we need analysis at the molecular level involving gene manipulation. Furthermore, if the removal of Ni^{2+} ions from a polluted environment using metal-tolerant fungi is to be attempted, the study of heavy metal transport across the vacuolar membrane should not be neglected because of the high storage capacity for ions in the vacuole. As one device for modification of the metal-accumulating activity by the yeast vacuole, overexpression of a Cd^{2+}-resistant gene (*HMT1*) as the specific transporter in the vacuolar membrane of *Schizosaccharomyces pombe* may prove a powerful approach for compartmentation studies [61]. In a similar approach for overexpression of metal resistance conferring genes in *S. cerevisiae*, there are some reports of the following: 1) the overproduction of the Cu-binding protein, metallothionein, produced by the *CUP1* gene [20]; 2) the product of the *COT1* gene encoded a Co-binding protein, a 48 kDa, which is found in mitochondrial membrane fractions [18]; and 3) the *ZRC1* gene which conferred resistance to Zn^{2+} and Cd^{2+} [42].

Finally, naturally-occurring microorganisms in polluted environments often show co-resistance between metal ions [67,70]. The study of multiple heavy metal-resistant mechanisms in fungi also appears to be an important problem in some polluted environments involving two or more toxic metals. Some metal-resistant yeasts, however, are often more sensitive to other metal toxicants than the parent strain, when they were acquired by mutagen treatment or repeated subculture on an agar plate containing a heavy metal [36,55]. Therefore, we may also need to pay attention to metal-resistant yeasts and filamentous fungi obtained in the laboratory by experimental manipulation.

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