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Selenium Metabolism in a Strain of *Fusarium*

SHADIA E. RAMADAN, A. A. RAZAK,* Y. A. YOUSSEFF, AND N. M. SEDKY

Departments of Botany, Faculties of Science, Al-Azhar University and Ain Shams University, Cairo, Egypt

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ABSTRACT

Fusarium sp. was isolated from Sinai soil at Egypt. It showed tendency to tolerate high concentrations of selenium in the form of sodium selenite up to 3.5% (w/v). The microscopic examination revealed some morphological distortions. However, the fungus was capable to circumvent the toxic effect of selenium. The fungus possesses strong reducing ability as high quantities of elemental selenium were precipitated within the fungal cells as well as on the surface of the fungal hyphae and spores. The presence of selenium increased the cellular contents of carbohydrates, proteins, and lipids. Labeling studies indicate the incorporation of selenite into certain amino acids: selenocysteine and selenocysteic acid. Moreover, the presence of selenium induced the biosynthesis of several types of low molecular weight proteins. The results demonstrated different modes of detoxification of selenium toxicity.

Index Entries: Selenium metabolism; seleno-amino acids; selenotolerant *Fusarium sp.*; selenium and morphogenesis; mineralization of selenium; detoxification of selenium.

INTRODUCTION

Selenium is an element of two faces; at high concentrations its compounds are known to be toxic for plants, animals, and microorganisms

*Author to whom all correspondence and reprint requests should be addressed.

(*1–7*). However, at very low concentrations, selenium is an essential nutrient (*8–14*).

Some microorganisms are able to assimilate relatively high levels of selenium compounds. Selenocysteine and selenomethionine are reportedly synthesized from selenite by several bacteria and fungi (*3,5,15,16*).

The biosynthesis of selenomethionine from selenite by *Escherichia coli* (*17*) and *Candida albicans* (*18*) probably occurs through pathways by which sulphur is incorporated into amino acids.

Penicillium chrysogenum was able to incorporate selenite into selenomethionine and selenocysteine (*5*).

Selenomethionine was active in the first step of protein synthesis in *E. coli* (*19*) and was found to be incorporated into proteins of this and other bacteria as well (*20,21*). Although selenocysteine was detected in acid hydrolysates of protein, it was reported that an extreme care must be taken in interpreting data indicating the presence of selenocysteine in the hydrolysate (*22*).

The role of soil microorganisms in accumulating selenium in addition to its conversion from the soluble inorganic to volatile forms is an important step in the cycling of selenium in the environment and also represents a mechanism of detoxification in seleniferous soils (*23*).

MATERIALS AND METHODS

Chemicals

All chemicals were obtained from Sigma, BDH. But ^{75}Se-sodium selenite was obtained from Radiochemical Centre, Amersham, England.

Organism

A strain of *Fusarium* was isolated from Sinai soil at Egypt. The organism was not fully characterized and for this reason it was given the name *Fusarium sp.*

Preparation of Cell Free Extract

One set of 10 conical flasks of 25 mL capacity were used for each experiment. Five mL, sterilized Oxoid Dox medium was employed for each culturing flask and was inoculated with 0.01 mL spores suspension. Twenty μCI ^{75}Se-sodium selenite was fed to one-d-old culture of *Fusarium sp.*, using an automatic micropipet (Fine Pipette). The pH was adjusted to 6.5 and incubated at 28 ± 2°C for 6 d. The mycelia of each culturing flask was harvested and collected together as well as the filtrate.

The harvested mycelia were homogenized with an approximately equal volume of 60% (v/v) ethanol using a MSE homogenizer. The slurry was centrifuged at 6000 rpm for 10 min. The supernatant was collected and concentrated using Coarse Sephadex G25.

Selenium Determination

Selenium content of the fungus was determined quantitatively using 3,3-diaminobenzidine reagent according to Koval'Skii and ErmaKov (*24*). The absorbance of the developed color was measured at 425 nm using a Spectronic 20 spectrophotometer.

Carbohydrate Determination

Carbohydrate was determined using the anthrone reagent method (*25*). Sucrose was used as a standard carbohydrate.

Lipids Determination

The total lipid determination was carried out using the Phospho-vanillin method (*26*). Cholesterol was used as a standard lipid.

Protein Determination

Protein was determined colorimetrically (*27*) using bovine serum albumin as a standard protein.

Disk Electrophoresis

Polyacrylamide gel electrophoresis was carried out using a Pharmacia power supply EP 400/500 and a Pharmacia Gel electrophoresis GE 2/4 (*28*). 10 × 0.8 cm glass tubes were used for the preparation of the gel rods.

Amino Acids Separation

Amino acids were separated chromatographically on 20 × 20 cm glass plates of cellulose thin layer using two dimensional separation technique according to the method of Bieleski and Turner (*29*).

Identification of amino acids was carried out according to the methods summarized by Razak (*30*).

Radioactivity Assay

A known volume of each gel filtration fraction as well as each gel segment were transferred separately to a vial and counted using a Beckman Biogamma counter.

Radioautography

Radioautographs were prepared from undeveloped chromatograms by superimposing them with Kodak Kodirex X-ray films for two wk in the dark. After developing the X-ray films, one of the duplicate chromatograms was developed by spraying with ninhydrin for identification of the labelled amino acids on the radioautograph, as well as counting the radioactivity of the labeled spots.

RESULTS

Morphological Features of Fusarium *sp. Grown on Selenium Free and 0.5% Sodium Selenite Containing Media*

The fungus grown on selenium free medium heavily giving white cottony like appearance fungal growth with one celled long, straight to slightly curved nonseptate conidia. However, on selenium containing media, the conidia become thicker and shorter, in addition, intercalary lamydospores were noticed (Fig. 1). Nevertheless, the fungal mycelia attained a red coloration. The intensity of the red coloration of the fungal hyphae was increased with increasing sodium selenite concentrations in the medium. This is probably a result of the deposition of elemental selenium within the fungal cells. Moreover, fungal gross growth inhibited with increasing selenium concentration in the culturing media.

Interestingly, the fungus excreted elemental selenium on the outer surface of the mycelia.

Accumulation of Excreted Reduced Selenium Particulates on the Fungal Hyphae

Red coloration of the agar media as well as liquid media was obtained when the fungus was cultivated on selenium containing media. This was interpreted as a biological reduction of selenium compounds into elemental selenium forming colloidal selenium. The reduction of selenium compounds into colloidal selenium in the medium was previously noticed (*3–5,31,32*).

The most interesting observation is the accumulation of a considerable quantity of elemental selenium on the outer surface of the fungus (Fig. 2). It is evidently excreted in order to minimize selenium concentration inside the fungal cells and most likely to avoid the toxic level of selenium inside the fungal cells. This may be a mode of detoxification mechanisms, as was also observed in *Aspergillus terreus, Aspergillus fumigatus,* and *Aspergillus speluneus* (*6*).

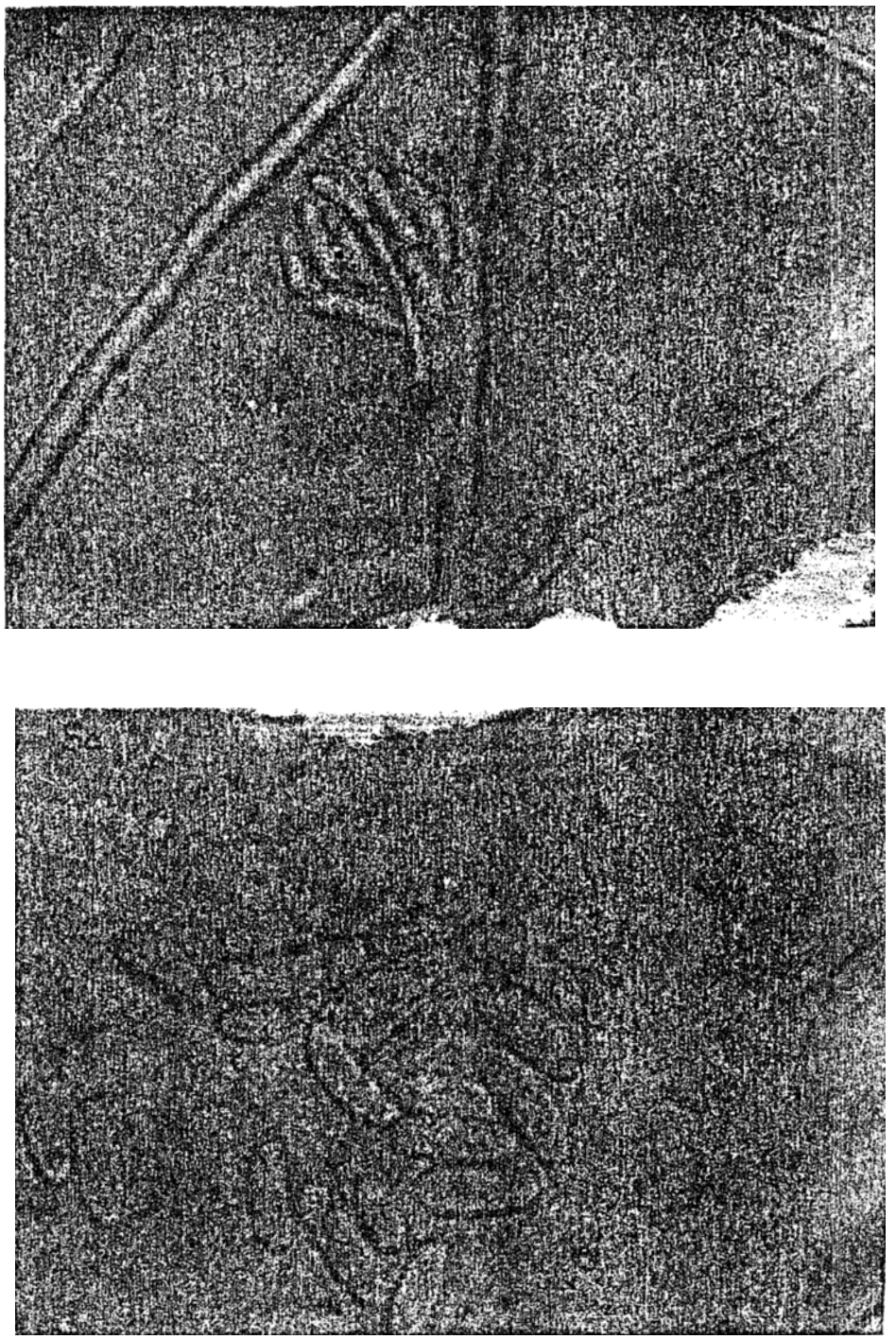

Fig. 1. Microphotographs of *Fusarium sp.* cultivated on selenium free medium (F) and 0.5% sodium selenite containing media (Se) (X = 1250).

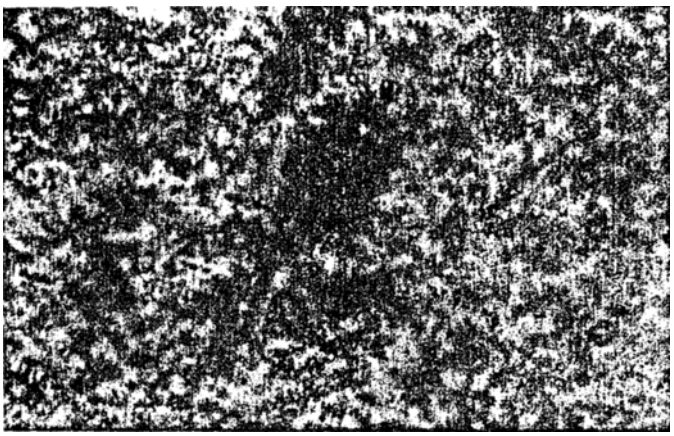

Fig. 2. Microphotograph of selenium particulates collected from the outer surface of the fungal hyphae (X = 1250).

Selenium Accumulation Within Fungal Spores

The foregoing observation on the excretion of selenium particles on the outer surface of the fungal mycelia was also observed on the surface of spores. It was not easy to determine the quantity of selenium within the spores, because of several technical difficulties.

Therefore, spores were subcultured on selenium free and selenium containing media (Fig. 3). Surprisingly, red coloration in the agar growth medium were obtained in both conditions.

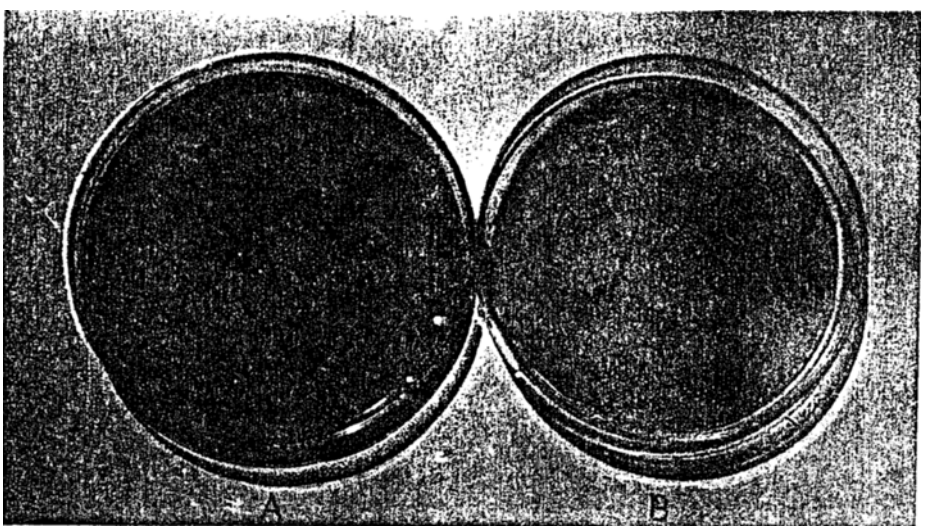

Fig. 3. Subculturing of *Fusarium sp.* spores obtained from the fungus cultivated on selenium containing media, on both selenium containing media 0.5% (A) and selenium free media (control) (B). Red coloration is clearly observed in both media.

A deep red coloration was also observed in the growth medium in selenium containing media, whereas faint red coloration was clearly observed in selenium free media. This observation shows selenium was accumulated within the fungal spores as well.

Uptake of Selenium by Fusarium *sp.*

Data in Table 1 indicate the relatively high absorption of selenium by the fungus. Maximum concentrations of selenium were detected at 1.5 and 1.0% $Na_2Se\ O_3$. Unexpectedly, the lowest absorption of selenium was detected at 2.5%, whereas more selenium was absorbed at higher concentrations 3 and 3.5% $Na_2Se\ O_3$.

These results indicate the irregular pattern of selenium absorption as a function of selenium concentration in the growth media.

Apparently, the absorbed selenium is not dependent on the selenium content in the growth environment. More likely, it depends on factors that influence selenium absorption, such as cell permeability and several other factors.

Determination of Total Carbohydrates, Proteins, and Lipids Content

Data in Table 1 indicate higher levels of carbohydrates, proteins and lipids in the fungal extracts cultivated on selenium containing media than on selenium free media. This increase could be because of osmotic equilibration as well as an activation of the biosynthesis of several adsorbents within the fungal cells.

Table 1
Determination of Selenium, Carbohydrates, Proteins, and Lipids Contents in Cell Free Extract of the *Fusarium sp.* Grown on Dox Liquid Medium Amended with Different Concentrations of Sodium Selenite Incubated for Seven Days at 28 ± 2°C

$Na_2Se\ 0_3$ concentration, % w/v	Selenium content, mg × g^{-1}	Total carbohydrates, mg × mL^{-1}	Total proteins, mg × mL^{-1}	Total lipids, mg × mL^{-1}
0.1	0.820	0.012	1.1	0.6
0.5	1.150	0.240	1.4	1.6
1.0	1.420	0.170	2.5	2.2
1.5	1.450	0.040	1.8	2.6
2.0	0.700	0.015	2.2	2.8
2.5	0.300	0.016	1.3	2.9
3.0	0.370	0.011	1.3	1.9
3.5	0.420	0.011	1.2	2.2

Metabolism of ^{75}Se-sodium Selenite by the Fusarium *sp.*

Amino acid analysis of the fungal content revealed the presence of several amino acids derived from different biosynthetic pathways. However, the detection of sulphur amino acids, cysteine, cystine, methionine, and S-methylcysteine are of specific importance because of the possible incorporation of selenium into these compounds.

Labeling studies indicate the actual incorporation of selenium into certain sulphur amino acids: selenocysteic acid, selenocystine/selenocysteine, as well as another two unidentified compounds, most likely peptides. These results may indicate the ability of the fungus to regulate selenium incorporation into several protein amino acids, consequently minimizing its possible incorporation into proteins.

Disk polyacrylamide gel electrophoresis revealed the biosynthesis of several types of proteins. However, with increasing selenium concentration in the culturing media, higher levels of low molecular weight proteins were detected. Nevertheless, no labeling protein was detected in the separated bands on polyacrylamide gel.

DISCUSSION

Toxic effects of metals on pure cultures of microbes are known to alter cell morphology or metabolism (*5,6,33*). However, some organisms possess very effective tolerance mechanisms and some have the capacity of metabolizing metals in various ways (*34,35*).

The foregoing results show the ability of the studied organism to evolve several mechanisms to avoid selenium toxicity: elemental excretion and accumulation on the outer surface as well as deposition within fungal cells and incorporation into certain free amino acids.

The increase in lipids, carbohydrates, and proteins content in the studied fungus in the presence of different concentrations of selenium is probably a mode of tolerance to the high level of selenium. It could be interpreted as several modes, an equilibrium process or probably a mode of compensation to avoid harmful action on certain cellular compounds belonging to those groups, or more likely, synthesis of several compounds, which would bind the element. The presence of the element presumably also causes a disturbance in the biosynthetic process which leads to the accumulation of carbohydrates, lipids, and proteins and subsequently inhibition of the fungal anabolic activities. This is indicated by the drastic action of selenium on fungal gross growth, as well by its effect on morphological features.

Similar results on selenium action on several other fungi were reported before (*4,6,36*).

The presented results showed that the fungus has strong reducing properties. It was interesting to observe the excretion of elemental sele-

nium on the outer surface of the fungal cells. Such mechanism (mineralization) is considered as a new mode of detoxification.

This finding supports Shrift views (2) that selenium appears to be cycled predominantly via biological pathways.

Similar mineralization of selenium compounds was given by Doran (*37*).

Conclusively, the fungus has evolved several mechanisms to avoid selenium toxicity: a) Mineralization of organic or inorganic selenium compounds inside its cells; and b) Regulation of selenium incorporation into several protein amino acids to avoid its incorporation into proteins.

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