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Uptake of Radionuclides by Some Fungi

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Mycobiota including *Alternaria alternata*, *Fusarium* verticillioides and *Aspergillus pulverulents* were tested for their ability to uptake radiocobalt (Co-60) and radiocesium (Cs-137) from radionuclide containing medium. *A. alternata* was the most efficient fungal species for uptake of radioisotopes, followed by *A. pulverulents*, whereas *F. verticilliodies* came in the last rank. The conditions of radioisotope uptake were optimized such as the form of the fungal organism either spores or mycelium, inoculum age and pH of growing medium. Furthermore the total pigments of the tested fungi were extracted and tested for their ability to bind with radioisotope, where melanin of *A. alternata* produced about 60% for radioisotope uptake out of total added radioisotope radioactivity. Moreover, transmission electron microscopic examination of radioisotope exposed spores showed high precipitation of melanin granules in the spore wall and within the cell as comparing to untreated spores.

KEYWORDS: Fungi, Melanin, Radiocesium, Radiocobalt, Radionuclide wastes

The level of radioactive wastes and hazardous solution that were originated through the using of radioisotopes accentuate a real problem for the environment safety. Therefore, the treatment of such wastes before its disposal is very important; where the restriction regulatory rules are applied throughout the world to keep the environment clean as possible.

Many chemical analytical techniques have been applied to get ride of such low and intermediate radioisotope wastes at industrial level in many countries. The newly developed methods were included electrodialysis, dialysis, osmosis, ultrafiltration, and flotation (IAEA 1992). However, evaporation, ion exchange process and chemical treatments are well established and applied in industrial field (Hang, 1994; Voget, 1994).

Bioremediation of these radioisotope wastes is recently developed, whereas the treatments using the chemical tools are time consuming and costly. The process depends upon the ability of some microorganisms to biouptake of radionuclide from the surrounding waste solution (Baeza *et al.*, 1998; Kuwahara *et al.*, 1998; Mahmoud and Abou Zeid, 2002; Sugiyama *et al.*, 2000; Zherko *et al.*, 1993). This technique is simple and need low running cost (Cherry, 1997; Krumpholz *et al.*, 1999). Laboratory studies on yeasts and fungal biomass have shown effective uptake of uranium, leading to the biological treatment of metal contaminated effluents (McLean *et al.*, 1998a). Zhdanova *et al.* (2000) found that extensive fungal growth on the walls and other building constructions in the inner parts of the shelter of the damaged fourth unit of the Chernobyl power plant in 1997~98. The mycobiota comprised 37 species of 19 genera. In the sample studies on *Trapelia involuta* lichen, there was a strong correlation between the localization of melanin and high concentration of uranium (McLean *et al.*, 1998b). The mechanism of radionuclide bioaccumulation is poorly understood in microorganisms and lichenized fungi. From this point of view, the present work has been designed to answer the question: is there any relationship between fungus efficiency to uptake the radiocesium (Cs-137) and radiocobalt (Co-60) in melanized and nonmelanized fungi. Also, the factors affecting the radionuclide uptake efficiency were optimized.

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Materials and Methods

Fungi. *Fusarium verticillioides* AUMC 2652.1 was obtained from Mycological center, Assuit University, Egypt, *Alternaria alternata* and *Aspergillus pulverulents* were isolated from soil and identified according to the conventional method used in fungi identification.

Medium. Czapek's medium was used for growing of the fungi during all experiments.

Fungal inoculum preparation. Mycelial disks (~15 mm) in diameter were used as inoculums for inoculating the Czapek's liquid culture at specified incubation period. Three replicates of Erlenmeyer flasks were prepared each containing 50 ml of Czapek's medium were sterilized and inoculated with (~15 mm) in diameter from 14 days old fungal culture of fungi under study.

Radionuclides. A determined amount of radioactive waste solution stimulate labeled with carrier free Cs-137 and Co-60 radionuclides was added to the culture (710 Bq./ml; 576 of Co-60 and 134 of Cs-137) and then left

static at 28°C. After a definite incubation period, an aliquot volume of the supernal solution was withdrawn and analyzed radiometerically using Multichannel Analyzer PCA-A (Oxford instrument Inc., USA). Assays have been carried out in Middle Eastern Regional Radioisotopes, Center for Arab countries, Atomic energy authority, Egypt. The activity of radionuclide uptake to evaluate the efficiency of the biological process was calculated according to the following formula:

Uptake percent = $[(Ao - X)/Ao] \times 100$

Where, Ao = the initial radionuclide added
X = the remained activity in the solution

Factors affecting radionuclide uptake. Inoculums were taken after 1, 3, and 7 days as starter in order to inoculate the Czapek's broth with labeled carrier free Cs-137 and Co-60 radionuclides (650 Bq./ml; 576 of Co-60 and 134 of Cs-137) radionuclides. Also, fungal inoculum form in shape of spore suspension or mycelial was tested. The 15 mm fungal disks were obtained and homogenized in sterile distilled water with 1% tween 80 for emulsification. The homogenate was filtered in order to separate the spores from the mycelia. Then, the spores equivalent to one fungal disk (15 mm in diameter) was used to inoculate the Czpaek's broth and all culture were left for one week at 28°C. To study the effect of pH value on the activity of radionuclide uptake, the pH of the broth was adjusted to the definite value before sterilization.

Effect of pigments in radionuclide uptake. Melanin pigments were extracted according to Gadd (1982). The extracted alkaline melanin was acidified to pH 2.0 with concentrated HCl to precipitate melanin. Then the precipitated melanin was washed with three changes of distilled water and dried at 20°C. Seven mg melanin pigments that produced by inoculation of the Czpaek's broth with 15 mm disk of *A. pulverulents* and 10 mg of *A. alternata* which were produced from the same inoculum size (15 mm), each of melanin pigments was dissolved in 0.5 ml of 0.1 M NaOH, then 1.5 ml of 5 mM potassium phosphate buffer, pH 5.7 was added and mix well and added to18 ml of distilled water at the same amount of radionuclides (total of 35.5×10^3 Bq.; 28.8×10^3 of Co-60 and 6.7×10^3 of Cs-137). The solutions were stirred for one h (Gadd and de Roome, 1988). Latter the whole solution was centrifuged and the radioactivity was measured. No melanin was found in *F. verticillioides* AUMC 2652.1, but its pigment in the form of naphthoquinone was extracted (Steyn *et al.*, 1978) and used in the same way like as melanin.

Transmission electron microscope. Under a safety conditions and careful handling portion of *A.alternata* mycelial mat of exposed culture to normal either Cobalt chloride or cesium chloride at 3 mM concentration and that of control were fixed at room temperature in 2% glutaraldehyde in potassium phosphate buffer at pH 7.0 for 2 h, followed by postfixation in 2% (w/v) osmium tetraoxide buffered in 5 mM sodium cocadylate buffer at pH 6.5 for 40 min. After fixation the mycelia mat was washed overnight in appropriate buffer, dehydrated at room temperature in acetone and embedded in low viscosity epoxy resin at 65°C (Spurr, 1969). Sections were prepared and stabilized on copper grids, stained with lead acetate and examined by a Gol 100 CX electron microscope (Ellis and Griffith, 1974).

Results and Discussion

The ability of fungal organism to remove the radionu-

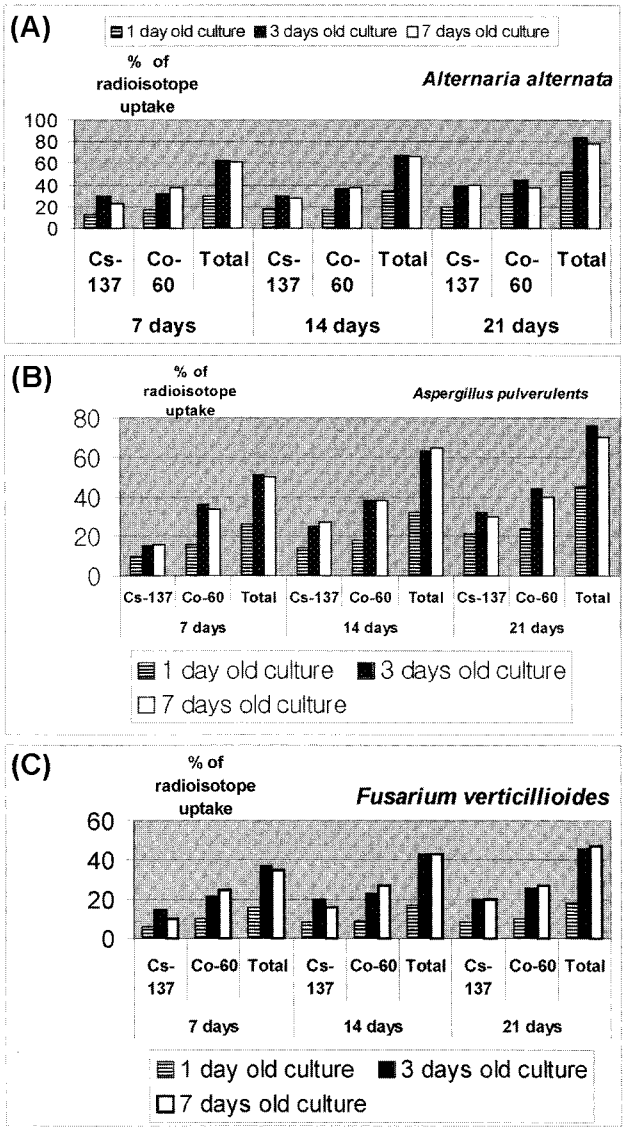

Fig. 1. Effect of start inoculum age of the studied fungi (A: *Alternaria alternata*, B: *Aspergillus pulverulents*, and C: *Fusarium verticillioides*) on the Co-60 and Cs-137 uptake percentage.

clides from the surrounding medium during the extended time periods was investigated (Fig. 1: A, B and C). Samples from grown medium were collected at different time intervals and radiometrically analyzed. Reaction rate of radionuclide uptake was covered over a period extending from 1 to 7 days. There is a pronounced difference in radionuclide total biouptake percentage as a function of incubation period. *A. alternata* was produced 84 percentage (the highest total uptake) which was achieved after incubation for three weeks with a starter inoculum age of three days old. On the other hand, *A. pulverulents* was came in the second rank of radionuclide total uptake with three days age starter, which produced 76% of total uptake followed with 70% of radionuclide uptake after 7 days. However, *F. verticillioides* AUMC 2652.1 has nearly the same percent of total radionuclide uptake with the same age starter inoculum.

The effects of fungal inoculum forms either the mycelial or the spore suspension in the uptake efficiency were tested (Table 1). Increasing the incubation time increased the percentage of radioisotope uptake either with the mycelia or the spores. The obtained data indicated that the percentage of isotope uptake was comparable for both the mycelial and the spore's form. The radioisotopes uptake by fungi can be either solubilized or immobilized depending upon the need of the fungal organism. The high uptake of Co-60 may be due to the sorption of soluble cobalt onto immobilized microorganisms. However, the low uptake of Cs-137 may be due to the effect of the solubilization action of microorganism on the absorbed cesium. A close behavior was observed in the literature previous reported by (Cherry, 1997). It was found that fungi have the ability to synthesize cobaltamins (Bilgrami *et al.*, 1978). Consequently, the high ability of the tested fungi to uptake Co-60 more than Cs-137 could be also explained the essentiality of cobalt to fungi (Marston, 1952). The results in this study suggest that melanin of *A. pulverulents* and *A. alternata* might play a role in the radioisotopes uptake and clearing of the wastes. Gadd (1993) has stated that melanin is formed in non-lichenized fungal hyphae as a response to a wide range of environmental stresses, including metal contamination. Furthermore, McLean *et al.* (1998b) have suggested that the pigment of *Trapelia* apothecia lichen is melanin or melanin like pigments. In the samples which they studied, there was a strong correlation between the localization of melanin and high concentration of uranium. Ellis and Griffith (1974) have also showed that melanin has a high adsorption capacity for uranium. Zhdanova *et al.* (2000) realized that the comparison of the species growing under severe and relatively weak radioactive contamination in building construction of the shelter of the damaged fourth unit of the Chernobyl Nuclear Power Plant (1997~1998), revealed a dominance of melanin-containing species in heavily contaminated sites.

The pH-value of the growing medium produced a pro-

Table 1. Effect of inoculum type on the radioisotope (Co-60 and Cs-137) uptake

Fungus	Inoculum form	% of radioisotope uptake								
		7 days			14 days			21 days		
		Cs-137	Co-60	Total	Cs-137	Co-60	Total	Cs-137	Co-60	Total
Fusarium verticillioides	Mycelia	15	21	36	20	23	43	22	25	47
Alternaria alternata	Mycelia	25	32	57	32	35	67	36	46	82
Aspergillus pulverulents	Mycelia	15	35	50	25	37	62	34	40	74
Fusarium verticillioides	Spores	17	22	38	19	24	43	23	25	48
A. alternata	Spores	27	35	62	31	37	68	39	45	84
A. pulverulents	Spores	18	25	43	25	30	55	32	36	68

Table 2. Effect of pH vales of the growing medium on the uptake percentage of Co-60 and Cs-137 radionuclides

Fungus	PH	% of radioisotope uptake								
		7 days			14 days			21 days		
		Cs-137	Co-60	Total	Cs-137	Co-60	Total	Cs-137	Co-60	Total
Fusarium verticillioides	4	6	10	6	10	14	24	10	15	25
	6	16	20	36	21	23	44	22	25	47
	8	6	6	12	8	6	14	8	9	17
Alternaria alternata	4	19	21	40	24	24	48	20	40	60
	6	29	30	59	31	36	67	38	44	82
	8	13	16	29	21	16	37	15	37	52
Aspergillus pulverulents	4	18	12	30	20	23	43	25	31	56
	6	14	37	51	26	38	64	33	43	76
	8	8	12	20	10	23	33	22	23	45

Table 3. Efficiency of pigment purified from tested fungal organisms for sorption of radioisotopes (Co-60 and Cs 137)

Organism	Total pigements con. and its type (mg)	% of radioisotope uptake		
		Cs-137	Co-60	Total
Fusarium verticillioides	4 naphthoquninone	6	9	15
Alternaria alternata	14 melanin	24	35	59
Aspergillus pulverulents	9 melanin	18	27	45

found effect on the biological activities of the tested fungi (Table 2). The highest percentage of radioisotope uptake was achieved at pH 6.0 for all the tested fungal organisms. Both pH 4 and 8.0 are not suitable for radioisotope uptake. This may be due to the effect of pH values of the surrounding medium on the transport of nutrients or the nutrient solubility. Also, pH value may affect the permeability of the cell membrane, internal pH of the mycelium and also the enzymes activities which in turn affect the uptake of the different elements from the growing medium. Efficiency of extracted and purified melanin of both *A. alternata* and *A. pulverulents* was tested for Co-60 and Cs-137 uptake after 1 hr of incubation. The data presented in Table 3 showed that 60% of both radionuclides was uptakeded by melanin in case of *A. alternata* and *A. pulverulents*. However, about 40% of radioisotope uptake was carried out by fungal mycelial growth. In the case of *Fusarium verticillioides* AUMC 2652.1, about 30% of radionuclide uptake was achieved by naphthoquinone produced by the fungus. The process of radioisotope bioaccumulation is not well understood in microorganisms and need additional work. Melanins from natural sources contain free carboxyl and phenolic hydroxyl groups, and also there may be amino acid type binding sites (Froncisz *et al.*, 1980). Carboxyl or hydroxyl groups of melanin may be the main binding sites but this may depend upon the pH and type of melanin (Larsson and Tjalve, 1978; Froncisz *et al.*, 1980). It looks more likely that melanins bind radioisotopes and thereby decrease the toxicity of these isotopes to the fungal cells. The ultra section of *A. alternata* condium treated with radioisotopes examining it (Fig. 2b) indicated a high precipitation of melanin in the spore cell wall and between the septation walls inside aleuriospore. However, there is less melanin contents for control spores. In a pilot study by Yakubu and Dudeney (1986), pellets of *A. niger* were used successfully in a fluidized-bed reactor for the removal of uranium, a process 14 times more effective than a commercial concentrate metals from solution.

In conclusion, the dark colored fungi in manipulating the treatment of radioactive liquid wastes are good candidates. The process seems to be simple and not to need much cost. Also, further work need to look for the most proper way for formulation of these dark colored fungi as a bioreactor for large scale application.

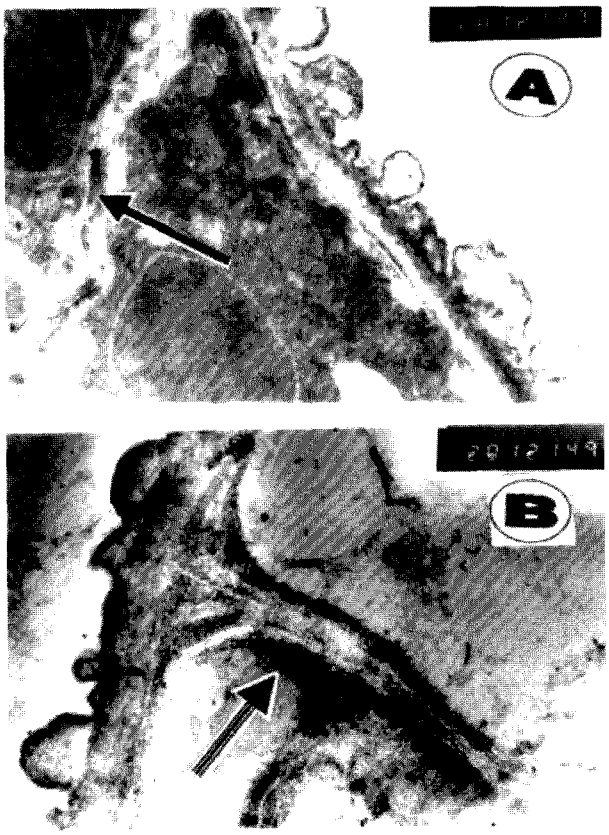

Fig. 2. Transmission electron micrograph for *Alternaria alternata* aleuriospore grown on Czapek's medium under the effect of 3 mM concentration of either cobalt chloride and cesium chloride and that of the control. Arrow in (A) indicateshe low concentration of melanin deposit within the spore or on the spore septation. However arrow in (B) indicates the high precipitation of melanin on spore wall septa and also, there is a large deposit of melanin granules inside the spore

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