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Full Length Research Paper

Comparison of hexavalent chromium bioaccumulation in five strains of paramecium, *Paramecium bursaria*

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The effects of hexavalent chromium on the cell growth of paramecium, *Paramecium bursaria* were investigated. Five strains (KBW-2, KNN-2, KNN-3, IM-6 and F1-6a) of *P. bursaria* (both symbiotic and algae free) were incubated in lettuce media supplemented with different concentrations of potassium dichromate under LD (12 h light: 12 h dark) conditions. To compare the bioaccumulation of chromium in symbiotic and algae free strains of *P. bursaria*, the concentration of chromium in cell pellet has been analyzed and calculated. The average amount of chromium accumulated in paramecium was ranged from 1.53 to 42.12 pg Cr/cell at concentration-dependent manner after 7-day of incubation. The results show that the symbiotic and algae free strains of *P. bursaria* can accumulate chromium and chromium accumulation ability of symbiotic strains is higher than that of algae free strains. This suggests that interactive effect of paramecium and algal cell was generally found to be synergistic in chromium accumulation for symbiotic paramecia.

Key words: Hexavalent chromium, bioaccumulation, *Paramecium bursaria.*

INTRODUCTION

Knowledge of the aqueous chemistry of metal ions and along with their coordination compounds is very important when assessing the effects of metal ions on biological activity. Toxicity of heavy metals to microbial activity is mainly attributed to soluble metal ions (Sujiarittanonta and Sherrerd, 1981). In aqueous systems, chromium exists primarily in two oxidation states, hexavalent chromium Cr(VI) and trivalent chromium Cr(III). Change in the oxidation states of chromium has a profound effect on the toxicity and bioavailability (Imai and Gloyna, 1990). Hexavalent chromium is being used extensively in variety of commercial processes and unregulated disposal of the chromium containing effluent in both developing and developed countries (Szulczewski et al., 1997).

Although chromium is an essential trace metal ion for living organisms, its elevated level is considered as mutagenic and carcinogenic (Cheung and Gu, 2003). Heavy metal ions can be entrapped in the cellular struc-ture and subsequently biosorbed onto the binding sites present in the cellular structure. This method of uptake is independent of the biological metabolic cycle and known as biosorption or passive uptake. The heavy metal ion can also pass through the cell membrane in the process of cell metabolic cycle. This mode of metal uptake is referred as active uptake. Both active and passive modes of metal uptake are termed as bioaccumulation (Iyer et al., 2004). Since ciliates are unicellular, they can be in very close contact with the environment and thus response intimately to any kind of unfavorable stresses (Cairns, 1974). Ciliates have many advantages as a test organism for investigating environmental pollution (Miyoshi et al., 2003; Madoni et al., 1992 and 1996; Tanaka et al., 2005; Abraham et al., 1997; Salvado et al., 2001; Gutierrez et al., 2003). To pursuing the present studies, *P. bursaria* was selected as a test organism, because of its noticeable abundance in aquatic environment and easy to culture and maintain in laboratory. Having these advantages, *Paramecium* is an important and convenient organism to evaluate the environmental pollution.

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P. bursaria is a unicellular organism, which is widely distributed in aquatic environment. One green paramecium possesses several hundreds of green algae, which are morphologically very similar to algae in the genus *Chlorella*. Since *P. bursaria* can utilize the photosynthetic products supplied by endosymbiotic algae as nutrient (Weis, 1979), the photosynthetic products enable *P. bursaria* to be alive in the starvation condition. Therefore, the culturing green paramecium is much easier than that of other organisms including other protozoa and mammalian cell lines (Takahashi et al., 2005).

The study of *P. bursaria* and its interaction with chromium may be useful for bioremediation of chromium-contaminated environments. The aim of the study was to determine the ability of *P. bursaria* to accumulate chromium. Data on the bioaccumulation of heavy metals by invertebrates are available for lead/cadmium in marine protozoan communities, (Fernandez-leborans and Olalla, 2000) lead/cadmium/copper/zinc in terrestrial invertebrates (Heikens et al., 2001) and organotin in *Artemia fraciscana* (Hadjispyrou et al., 2001). However, no data for chromium accumulation by ciliates have been reported and this led to the present study.

MATERIALS AND METHODS

Stains and culture

Five strains of *P. bursaria* syngen 1, KBW- 2 (mating type IV), KNN-2 (mating type III), KNN-3 (mating type II), IM-6 (mating type I) and F1-6a (mating type IV; described below) were used in this study. The strain KBW-2 was derived from a single cell collected from Biwa-ko Lake (Shiga Prefecture, Japan). The strains, KNN-2 and KNN- 3 were derived from a single cell collected from Kino-kawa River (Wakayama Prefecture, Japan). The strain IM-6 was derived from a single cell collected from Imuta Lake (Kagoshima Prefecture, Japan). The F1-6a was newly produced by hybridization with two stocks, BWK-4 (mating type IV) and KN-15 (mating type I), derived from a single cell collected from Lake Biwa (Shiga Prefecture, Japan) and Kino-kawa River (Wakayama Prefecture, Japan), respectively. The algae free strains of KBW-2, KNN- 2, KNN-3, IM-6 and F1-6a were produced in the laboratory using herbicide paraquat according to Hosoya et al. (1995). Those stains were cultured in lettuce media, containing the bacteria *Klebsiella pneumoniae* as food and growing under LD condition at 23°C following the methods previously described by Hosoya et al. (1995).

Heavy metal salt

Potassium dichromate ($K_2Cr_2O_7$) (Katayama Chemical, Japan) was used as source of chromium (VI). The stock solution of chromium (VI) (20 mM) was made and kept in refrigerator at 4°C until use.

Determination of growth rate

To compare the growth rate of different strains of *P. bursaria* in lettuce media supplemented with various concentrations (0 – 40 µM) of chromium (VI), five stains of *P. bursaria* (both symbiotic and algae free) were incubated for 7-day under the LD condition. The cells were cultured in 12-well microplate (flat bottom and polystyrene-treated plates, Asahi Glass Co. Ltd., Japan). Each well was filled with 2 ml fresh lettuce media containing different concentrations of chromium (VI) without *K. pneumoniae* and each culture was started at an initial density of 1000 cells/ml. After incubation, the number of *P. bursaria* in each well was counted under a binocular microscope (Model C-DS, Nikon, Japan). The growth rate was designated as values relative to the number of cells right after initiation of culture.

Comparative study of chromium bioaccumulation in symbiotic and algae free strains of Paramecia

To compare the chromium accumulation efficiency in symbiotic and algae free strains of Paramecia, five strains (KBW- 2, KNN-2, KNN-3, IM-6 and F1-6a) of *P. bursaria* (both symbiotic and algae free) were treated in lettuce media supplemented with various concentrations (0 – 40 µM) of chromium (VI). The initial cell density was 1000 cells/ml and incubated for 7 days. To quantify the total amount of chromium bioaccumulation in different strains *P. bursaria*, the samples with or without cells were filtered by membrane filters (5 µm pore, ADVANTEC, Japan) and the membranes were washed 3 times with fresh lettuce juice to remove the unbound chromium. After drying the filters at room temperature, they were treated overnight with 2 ml of 0.1N nitric acid (Sigma-Aldrich, Japan). After centrifugation, an aliquot of the supernatant was used to measure the chromium concentration by an atomic absorption spectroscope (AA-220Z, Varian Inc), equipped with a GTA-96 graphite tube atomizer and a hollow cathode lamp for chromium detection.

RESULTS AND DISCUSSION

Effect of chromium (VI) on the cell growth of *P. bursaria*

The cell growth of five strains (KBW-2, KNN- 2, KNN-3, IM-6 and F1-6a) of *P. bursaria* (both symbiotic and algae free) in a lettuce medium supplemented with different concentrations of potassium dichromate is shown in (Figure 1). After 7-day of incubation, the toxicity of chromium was examined on the basis of cell number. The cell numbers decreased tremendously in the pre-sence of potassium dichromate.

Chromium (VI) is the most toxic and mutagenic metal ion in biological systems. Although the toxic effects of chromium on microorganisms and invertebrates have been a topic for researchers over the past few decades (Stasinakis et al., 2002; Madoni et al., 1996; Hadjispyrou et al., 2001; Yap et al., 2004) less information using *P. bursaria* is available. Mortuza et al. (2005) reported that the cell numbers decreased tremendously in the presence of 100 µM potassium dichromate within 24 h, showed acute toxicity to the cell. This result suggests that the sensitivity to chromium in *P. bursaria* is similar among the different strains. The similar observation has reported by Tanaka et al. (2005) for the IC_{50} 5-day value of potassium dichromate ranged from 0.27 to 1.65 µM for *P. bursaria* syngen 1 (KSK-103, mating types-IV) under LD condition.

Comparative study of chromium accumulation in symbiotic and algae free strains of *Paramecia*

To compare the chromium contents in the symbiotic and

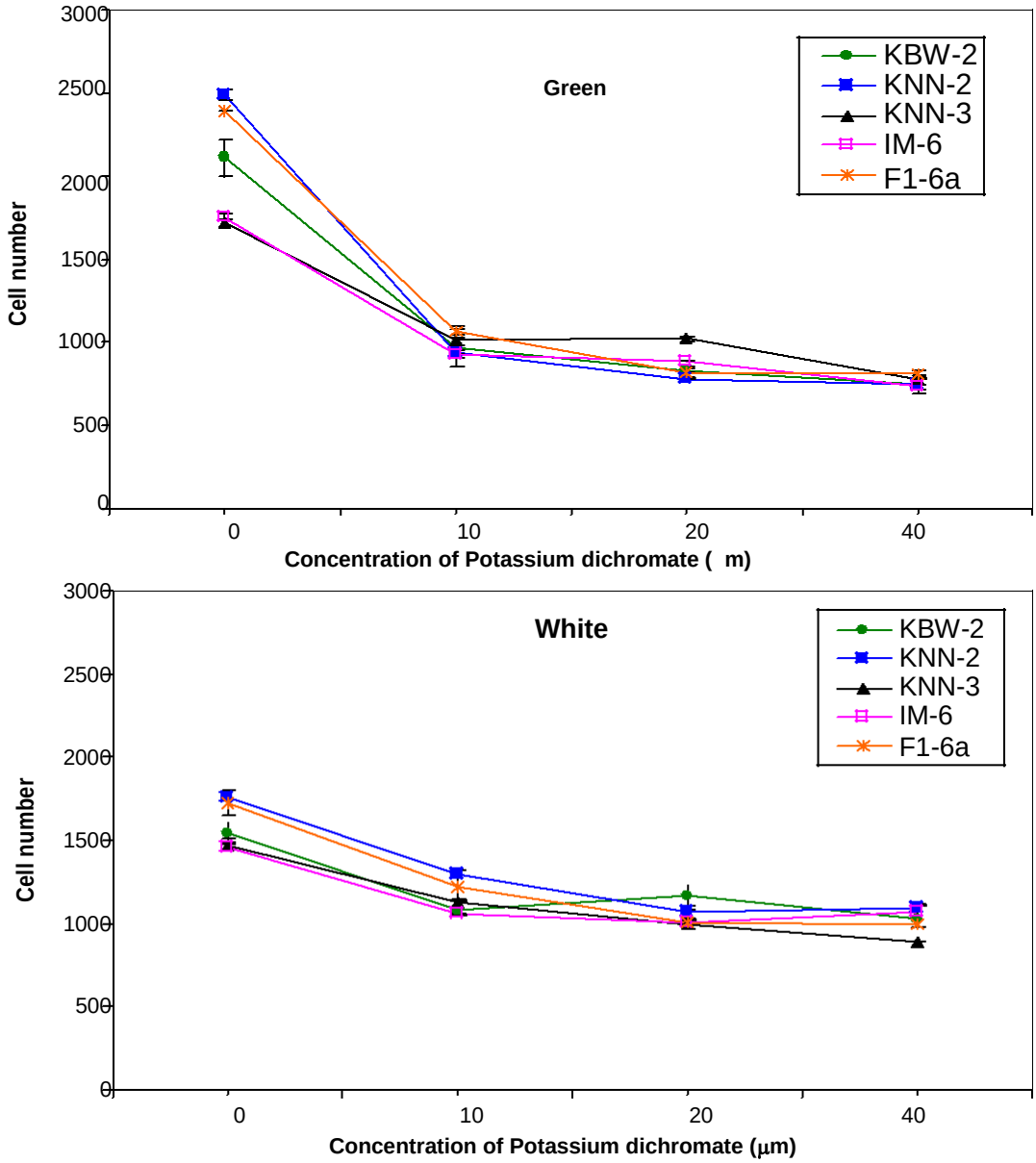

Figure 1. Cell growth of five strains (both green and white) of *P. bursaria* after 7-day exposure to potassium dichromate under LD condition

algae free strains of *P. bursaria*, the amount of chromium in cell pellet has been calculated. The average amount of chromium accumulated in green and white paramecium ranged from 3.37 to 42.12 and 1.53 to 26.25 pg/cell respectively in a concentration-dependent manner (Table 1). The result shows that the symbiotic and algae free strains of *P. bursaria* can able to accumulate chromium and chromium accumulation ability of symbiotic strains is higher than that of algae free strains. Among the green paramecium, F1-6a strain showed the highest accumulation ability (42.12 pg/cell when the Cr (VI) concentration was 40 µM) and followed by KNN-2, KBW-2, KNN-3 and IM-6 strains.

The chromium accumulation ability of green algae is well reported, however, chromium bioaccumulation in ciliates is not available. Gorbi et al. (2001) reported that chromium accumulation was evident after 2 days of treatment in potassium dichromate in two strains of freshwater algae, *Scenedesmus acutus*. Chmielewska and Medved (2001) reported about chromium accumulation abilities of *Cladophora glomerata* in the sewage lagoon. This suggests that interactive effect of paramecium and algae was generally found to be synergistic for all paramecia strain in accumulation of chromium.

Table 1. Bioaccumulation of chromium (VI) in five strains of *P. bursaria* (both symbiotic and algae free) after 7-day treatment with different concentration of potassium dichromate.

P. bursaria		Cr (VI) concentration (0 μm)			Cr (VI) concentration (10 μm)			Cr (VI) concentration (20 μm)			Cr (VI) concentration (40 μm)		
Strains	types	Cell number cells/ml	Total amount of Cr in the pellet (ng)	Amount of Cr/cell (pg)	Cell number cells/ml	Total amount of Cr in the pellet (ng)	Amount of Cr/cell (pg)	Cell number cells/ml	Total amount of Cr in the pellet (ng)	Amount of Cr/cell (pg)	Cell number cells/ml	Total amount of Cr in the pellet (ng)	Amount of Cr/cell (pg)
KBW-2	Green	2111±110	0.17	0.04	967±16	15.1	7.81	833±16	27.78	16.67	745±32	36.2	24.31
	White	1545±63	00	00	1084±39	12.22	3.64	1167±63	24.14	10.88	1028±55	30.08	14.63
KNN-2	Green	2489±31	0.26	0.05	939±86	9.28	4.98	784±8	36.32	23.16	746±47	61.42	41.17
	White	1756±47	00	00	1295±23	14.54	5.61	1073±8	33.12	14.12	1089±16	54.96	25.23
KNN-3	Green	1717±23	00	00	1017±86	7.66	3.77	1022±16	23.33	11.41	784±8	15.54	9.91
	White	1472±40	0.38	0.13	1128±23	6.74	2.99	995±23	11.4	5.73	889±0	13.9	7.82
IM-6	Green	1756±16	0.29	0.08	928±23	6.26	3.37	833±0	13.32	8.00	733±16	16.00	10.91
	White	1461±24	0.12	0.04	1056±00	3.24	1.53	1006±23	7.4	3.68	1072±40	23.36	10.90
F1-6a	Green	2389±00	0.53	0.11	1067±16	14.56	6.82	817±23	41.58	25.45	812±16	68.40	42.12
	White	1722±71	0.38	0.11	1217±86	7.08	2.91	1011±47	26.02	12.87	995±23	52.24	26.25

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