Songklanakarin J. Sci. Technol.
35 (4), 407-414, Jul. - Aug. 2013

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Cr(VI) reduction by cell-free extract of thermophillic *Bacillus fusiformis* NTR9

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Received 2 March 2013; Accepted 29 April 2013

Abstract

Residual chromium compounds in discharged effluents is a serious problem, due to hexavalent chromium or chromate [Cr(VI)] being extremely toxic and showing mutagenic and carcinogenic effects on biological systems. The bacterial enzymatic Cr(VI) reduction can occur and this could be an effective method of detoxifying Cr(VI) polluted effluent. The present study characterized Cr(VI) reductase activity of cell-free extracts (CFE) of thermophilic chromate-reducing bacteria, *Bacillus fusiformis* NTR9. Results showed that the optimum temperature and pH for Cr(VI) reductase activity of CFE was 80°C and pH 7, respectively. The reductase activity remained at 60.34% and 26.44% after 30 minutes of exposure to 70 and 90°C, respectively, suggesting a heat stable enzyme. Moreover, the enzyme was resistant under acidic and neutral condition but its stability was decreased under alkaline condition. The Cr(VI) reductase activity of CFE was enhanced when exposed in Cu^{2+} and Fe^{3+} by 188.19% and 180.38%, respectively. The Cr(VI) reductase activity could be reduced to 72.19% and 8.95% in the presence of Mn^{2+} and Ag^{+}, respectively. Mg^{2+}, Zn^{2+}, As^{3+} and electron acceptors like sulfate and nitrate had no affect on Cr(VI) reductase activity. The external electron donors (glucose, glycerol, citrate, malate, succinate, and acetate, but not NADH) were essential to improve the chromate reductase activity of NTR9 strain. The chromate reductase was mainly associated with the soluble fraction in the cytoplasm of the bacterial cell. The molecular weight of the enzyme was 20 KDa. The results showed that Cr(VI) reductase could be a good candidate for detoxification of Cr(VI) in industrial effluents.

Keywords: Cr(VI) reductase, Cr(VI) reduction, thermophillic chromate-reducing bacteria

1. Introduction

Chromium is one of the most widely used metals in several industries, such as the metal finishing industry, petroleum refining, leather tanning, iron and steel industries, textile manufacturing, pulp production and alloy preparation (Patterson, 1985). The presence of chromium in the environment is of particular importance because the Cr(VI) oxidation state is likely to give carcinogenic and mutagenic species (Langard, 1982). Several treatment approaches have been investigated to remove toxic chromium. In general, biological procedures have been preferred over chemical treatments (Komori *et al.*, 1990) which require either high energy or large quantities of chemicals. Therefore more practical, economic methods are of interest (Srivastava *et al.*, 1986). Recently, microbially-mediated reduction of Cr(VI) represents a potential development not only for detoxification, but also as an essential step towards the ultimate removal of chromium from an aqueous environment (Ohtake *et al.*, 1990; Komori *et al.*, 1990; De Leo and Ehrlich, 1994). Various microorganisms have been reported to reduce Cr(VI) under either aerobic, anaerobic conditions or both such as *Pseudomonas fluorescens* LB300 (Bopp and Ehrlich, 1988) *Enterobacter cloacae* HO1 (Wang *et al.*, 1989) *Escherichia coli* ATCC 33456 (Shen and Wang, 1993) *Agrobacterium radiobacter* EPS-916 (Llovera *et al.*, 1993) *Microbacterium* sp. MP30 (Pattanapipitpaisal *et al.*, 2001) *Achromobacter* sp. Ch-1 (Ma *et al.*, 2007) *Arthrobacter* sp. CR47 (Córdoba *et al.*, 2008) and *Pseudomonas aeruginosa* (Pang *et al.*, 2011). Chromate reduction proceeds correlatively with chromate reductase

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from *Bacillus subtilis* (Garbisu *et al.*, 1998) *P. putida* PRS 2000 (Ishibashi *et al.*, 1990), *Bacillus* sp. (Wang and Xiao, 1995), *E. cloacae* HO1 (Komori *et al.*, 1989), *E. coli* ATCC 33456 (Shen and Wang, 1993) *Pseudomonas ambiguo* G-1 (Suzuki *et al.*, 1992) *Pseudomonas putida* MK1 (Park *et al.*, 2000), *Rhodobacter spaeroides* (Nepple *et al.*, 2000), *Bacillus* sp. ES29 (Camargo *et al.*, 2003) *Thermus scotoductus* SA-01 (Opperman *et al.*, 2008), *Amphibacillus* sp. KSUCr3 (Ibrahmin *et al.*, 2012), *Providencia* sp. G1DM21 (Desai *et al.*, 2008), and *Pannonibacter phragmitetus* LSSE-09 (Xu *et al.*, 2012). However, bacterial enzymes such as hydrogenase (Chardin *et al.*, 2003), nitrogenase (Kwak *et al.*, 2003) and quinine reductase (Gonzáles *et al.*, 2005) have been reported to exhibit chromate reductase activity. Thus, bacterial enzymes responsible for reduction of chromate are more interesting, especially enzymes produced from thermophilic chromate-reducing bacteria which have not been recently reported. The objective of this work was to characterize chromate reductase activity of cell-free extract from *B. fusiformis* NTR9, and localize its activity.

2. Materials and Methods

2.1 Bacterial preparation

B. fusiformis NTR9 was isolated from wastewater samples of a silk weaver factory in Ubon Ratchathani province, Thailand (Pattanapipitpaisal, 2004). The overnight culture was inoculated into Luria-Bertani medium (10 g tryptone, 10 g NaCl, 5 g yeast extract, and 1 L de-ionized water), and incubated with shaking at 40°C and 200 rpm for 24 h.

2.2 Preparation of cell fractionation

Cells were harvested by centrifuged (4,800 x *g*) for 15 min at 4°C, washed twice with de-ionized water, and then suspended in 3-(N-morpholino propanesulfonic acid-NaOH (MOPS-NaOH) buffer, pH 7.0. Cell suspensions were kept in an ice bath and disrupted ultrasonically at 100 W for 30 min. The sonicate was centrifuged at 12,000 x*g* for 15 min at 4°C to pellet unbroken cells. The supernatant was then filtered through 0.45 μM filters (Millipore) to produce cell-free extracts (CFE or S_{12} fraction).

2.2 Chromate reductase activity assay

Chromate reductase activity was performed using a method modified from previous studies (Park *et al.*, 2000; Pal *et al.*, 2005). The reaction mixture (1 ml) contained 0.02 ml of 1.0 μM sodium chromate in 0.78 ml MOPS-NaOH buffer (pH 7.0) and 0.2 ml of enzyme sources (S_{12}, S_{150}, or MF_s). The reaction was kept at 40°C for 30 min unless otherwise stated. The remaining Cr(VI) concentration was quantified colorimetrically at A_{540} using *S*-diphenyl carbazide (DPC) as the complexing reagent. Abiotic control reaction mixtures without the addition of enzyme were also included. Experiments were conducted in triplicate and the mean values were recorded. One unit of enzyme activity was defined as the amount of enzyme that reduced 1.0 μM sodium chromate within 30 min at 40°C. Relative enzyme activity was expressed as percentage of maximum activity taken as 100%.

2.3 Effect of pH and temperature on chromate reductase activity

Optimum pH and temperature for chromate reductase activity were determined for CFE by incubating the reaction mixture at pH values ranging from 4.0 to 10.0 at 40°C and at different temperatures ranging from 28 to 80°C at pH 7.0, respectively for 30 min.

2.4 Effect of pH and temperature on chromate reductase stability

The enzyme pH and heat stability of CFE were determined by exposing to MOPS-NaOH buffer at pH values ranging from 4.0 to 10.0 at room temperature (28°C) for 30 min and at different temperatures ranging from 40 to 90°C at pH 7.0, respectively for 30 min and cooled in an ice bath when appropriate.

2.5 Effect of metal cations, electron donors and electron acceptors

Chromate reductase activity of CFE was determined in the presence of 1 mM of various metal ions ($CuCl_2$, $FeCl_2$, $MgCl_2$, $MnSO_4$, $ZnSO_4$, AgCl, and KH_2AsO_4), electron donors (0.5 mM NADH, 0.5 mM sodium acetate, 1.0 % glycerol, 0.5 mM glucose, 0.5 mM trisodium citrate, 0.5 mM magnesium malate, and 0.5 mM sodium succinate), and electron acceptors (1 mM $NaNO_3$ and 1 mM $NaSO_4$). The experiments were preformed at 40°C and pH 7.0, using MOPS-NaOH buffer.

2.6 Localization of chromate reductase activity

CFE was prepared as described above. Ten milliliters of the CFE was then centrifuged at 150,000 x*g* for 60 min at 4°C to obtain a supernatant as soluble fraction (S_{150} fraction) and a membrane pellet. The membrane pellet was re-suspended in 10 ml MOPS-NaOH buffer (pH 7.0) and was used as membrane fractions (MF_s). All fractions were assayed for chromate reductase activity as described above and protein content was determined according to Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as standard. Specific enzyme activity was defined as unit chromate reductase activity per milligram protein.

2.7 Purification of chromate reductase

Ammomium sulfate was added to the CFE at 50-80% saturations with continuous stirring for 2 h. The mixture was

kept at 4°C overnight and then centrifuged at 10,000 x*g* for 30 min. The fraction with the highest activity was dialyzed against MOPS-NaOH buffer (pH 7.0) and applied to a DEAE Sepharose CL-6B column. Selected fractions with the highest chromate reductase activity were analyzed for molecular mass by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and MALDI-TOF mass spectrometry and for protein identification by LC/MS spectrometry.

3. Results and Discussion

3.1 Effect of pH and temperature on chromate reductase activity

To determine the optimum pH, the chromate reductase activity of CFE was measured in pH values ranging from 4.0 to 10.0. As shown in Figure 1, significant chromate reductase activity was observed in a wide pH range, 4.0 to 7.0, with an optimum pH at pH 7.0 (0.55 units). At neutral pH, trivalent chromium from reduction reaction will change to oxide and hydroxide and then immediately precipitate (Rai *et al.*, 1987; Xu *et al.*, 2005). However, the chromate reductase activity of CFE was decreased when increasing the pH values (8.0 to 10.0). The change in the pH brings about conformational changes in the structure of the CFE (Bailey and Ollis, 1986). The result suggests that cell fraction could probably be applied in detoxification of chromate- containing wastewater with neutral and acidic pH. Many other researchers reported the optimum pH value to be neutral such as for *Rhodobacter sphaeroides* (Nepple *et al.*, 2000), *Providencia* sp. UTDM 314 (Thacker *et al.*, 2006), *Pseudomonas* sp. G1DM21 (Desai *et al.*, 2008) *Amphibacillus* sp. KSUCr3 (Ibrahmin *et al.*, 2012), *Pannonibacter phragmitetus* LSSE-09 (Xu *et al.*, 2012), *Steptomyces* sp. (Polti *et al.*, 2012), and *Steptomyces griseus* (Poopal and Laxman, 2009). However chromate reductase from other strains showed the highest activity at acidic and alkaline pH; pH 5.0 for *P. putida* MK1 (Park *et al.*, 2000); pH 6.0 for *Bacillus* sp. (Elangovan *et al.*, 2006); pH 6.3 for *Thermus scotoductus* SA-01 (Opperman *et al.*, 2008); pH 6.5 for *Halomonas* sp. TA-04 (Focardi *et al.*, 2012) and pH 8.6 for *Pseudomonas ambigua* G-1 (Suzuki *et al.*, 1992).

The optimum temperature of chromate reductase activity by CFE was determined by exposure at various temperature ranges from 28 to 80°C at pH 7.0. The CFE showed higher activity within an extensive temperature range, with an optimum temperature as high as 80°C (0.67 units) (Figure 2). The activity decreased rapidly when the temperature was increased further (data not shown) which was probably due to heat denaturation. Similar observation was reported on soluble reductase of *P. putida* MK1, which showed highest activity at the same temperature (80°C) but different optimum pH (pH 5.0) (Park *et al.*, 2000). An optimum of chromate reductase activity at high temperature was also reported. For example, the optimum temperatures of chromate reductase activity from *Bacillus firmus*, *T. scotoductus* SA-01, and *P. ambigua* G-1 were 70, 65, and 50°C, respectively (Sau *et al.*, 2010; Opperman *et al.*, 2008; Suzuki *et al.*, 1992). Several strains had optimum temperature in the range of 28-40°C: 28°C for *Holomonas* sp. TA-04 (Focardi *et al.*, 2012) and *S. griseus* (Poopal and Laxman, 2009); 30°C for *R. sphaeroides* (Nepple *et al.*, 2000) *Pseudomonas* sp. G1DM21 (Desai *et al.*, 2008) *Streptomyces* sp. (Polti *et al.*, 2010); 37°C for *Providencia* sp. (Thacker *et al.*, 2006) and *P. phragmitetus* LSSE-09 (Xu *et al.*, 2012); and 40°C for *Amphibacillus* sp. KSUCr3 (Ibrahmin *et al.*, 2012).

3.2 Effect of pH and temperature on chromate reductase stability

CFE was incubated in MOPS-NaOH buffer (pH 7.0) at various temperatures for 30 min and, its activity determined at 40°C. The enzyme was stable in the range of temperature;

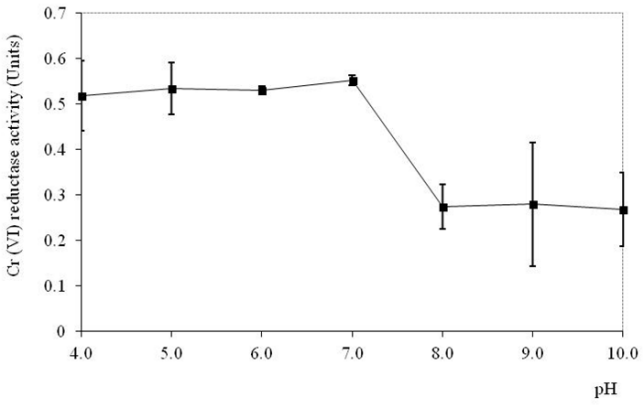

Figure 1. Effect of pH on the activity of chromate reduction by CFE of *B. fusiformis* NTR9, determined in MOPS-NaOH buffer (pH 4.0-10.0) with initial concentration of 1.0 µM Cr(VI) at 40°C for 30 min. Data represent mean of three separate experiments, and error bars indicate standard deviation

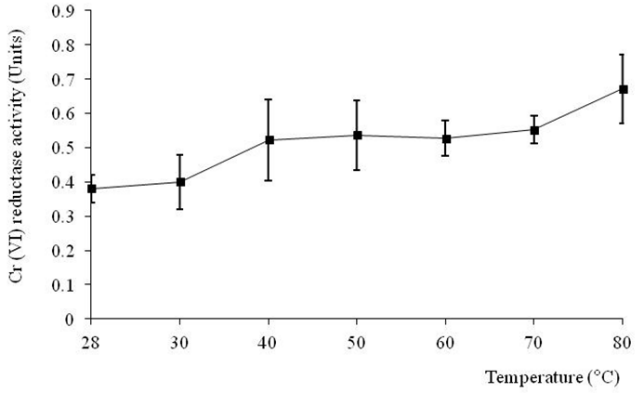

Figure 2. Effect of temperature on the activity of chromate reduction by CFE of *B. fusiformis* NTR9, determined in MOPS-NaOH buffer (pH 7.0) with initial concentration of 1.0 µM Cr(VI) at various temperature (28-80°C) for 30 min. Data represent mean of three separate experiments, and error bars indicate standard deviation.

however further increase of temperature resulted in loss of stability. The activity of 60.34 and 26.44% was still retained at 70°C and 90°C, respectively (Figure 3). The result indicated that CFE of thermophilic *B. fusiformis* NTR9 exhibited heat stability. Similarly, soluble chromate reductase of *P. putida* MK 1 (Park *et al.*, 2000) and *P. ambigua* G-1 (Suzuki *et al.*, 1992) were heat stable. As seen in Figure 4 alkaline pH condition drastically decreased the enzyme stability (14.75%, at pH 9.0) while acidic and neutral pH condition slightly reduced the stability with retention of 97.70% (pH 4.0) and 62.26% (pH 7.0).

3.3 Effect of metal cations, electron acceptors and electron donors

The effect of different metal cations, electron acceptors and electron donors on the chromate reductase activity of the CFE of *B. fusiformis* NTR9 was determined as exhibited in Figures 5-6. Amongst the metal ions test, Cu^{2+} ion markedly stimulated chromate reductase activity by 88.19%. Similarly, enhancement of chromate reductase activity by Cu^{2+} has been also observed in *Bacillus* sp. ES29 (Camargo *et al.*, 2003) *Bacillus* sp. (Elangovan *et al.*, 2006) *Pseudomonas* sp. G1DM21 (Desai *et al.*, 2008) *Amphibacillus* sp. KSUCr3 (Ibrahim *et al.*, 2012) *Halomonas* sp. TA-04 (Focardi *et al.*, 2012) and *P. phragmitetus* LSSE-09 (Xu *et al.*, 2012). Cu^{2+} is a transition metal and is a prosthetic group for many reductase enzymes. The role of Cu^{2+} in stimulation of chromate redustase could be related to its main function as a protective agent for electron transport, as a single electron redox center, and as a shuttle for electrons between protein subunits (Camargo *et al.*, 2003; Abe *et al.*, 2001). In addition, chromate reduction of *B. fusiformis* NTR9 showed higher activity under anaerobic than aerobic condition (Pattanapipitpaisal, 2004). It is possible that Cu^{2+} is indirectly involved in the protection of chromate reductase from O_2, for oxygen sensitive enzymes (Ettinger, 1984; Xu *et al.*, 2012). However, Cu^{2+} did not affect the enzyme activity of *Desulfovibrio vulgaris* (Lovley and Phillips, 1994) or *Pseudomonas* sp. CRB5 (McLean and Beveridge, 2001), whereas, Cu^{2+} reduced the membrane associated chromate reductase activity of *Enterobacter cloacae* HO1 (Ohtake *et al.*, 1990), and the soluble chromate reductase activity of *P. putida* MK1 (Park *et al.*, 2000) and *B. sphaericus* AND303 (Pal *et al.*, 2005). Fe^{3+} substantially stimulated the activity of the CFE of *B. fusiformis* NTR9 by 80.38%. In contrast, Fe^{3+} inhibited chromate reductase activity of *P. phragmitetus* LSSE-09 (Xu *et al.*, 2012) but showed no significant effect on the relative activity of *Bacillus* sp. ES29 (Camargo *et al.*, 2003). Zn^{2+} did not exhibit any significant effect on the reductase activity. Similar results were also observed in the case of *Bacillus* sp. ES29 (Camargo *et al.*, 2003) and *Amphibacillus* sp. KSUCr3 (Ibrahmin *et al.*, 2012). However, Zn^{2+} inhibited chromate reductase activity of *P. putida* MK1 (Park *et al.*, 2000), *T. scotoductus* SA-01 (Opperman *et al.*, 2008), *Bacillus* sp. (Elangovan *et al.*, 2006), and *Halomonas* sp. TA-04 (Focardi *et al.*, 2012). Mg^{2+} showed no significant effect on the chromate reductase activity of the CFE of *B. fusiformis* NTR9 as in the case of *Bacillus* sp. ES29 (Camargo *et al.*, 2003), *Bacillus* sp. (Elangovan *et al.*, 2006), and *Amphibacillus* sp. KSUCr3 (Ibrahim *et al.*, 2012). On the other hand, Mg^{2+} stimulated chromate reductase activity of the CFE of *Pseudomonas* sp. G1DM21 (Desai *et al.*, 2008) and *T. scotoductus* SA-01 (Opperman *et al.*, 2008). Mn^{2+} inhibited chromate reductase activity by 27.10%. Inhibition of chromate reductase activity by Mn^{2+} has been observed in the case of *P. phragmitetus* LSSE-09 (Xu *et al.*, 2012) and *T. scotoductus* SA-01 (Opperman *et al.*, 2008), while chromate reductase activity of the CFE of *Bacillus* sp. ES29 was slightly stimulated by Mn^{2+} (Camargo *et al.*, 2003). Ag^{+} strongly inhibited the chromate reductase activity by 91.05%. Several salts have been reported

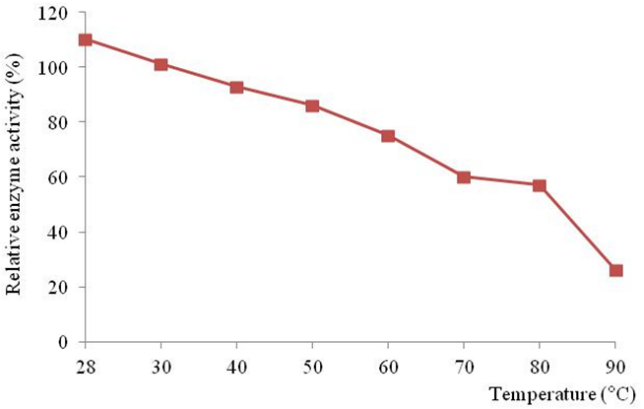

Figure 3. Temperature stability of CFE from *B. fusiformis* NTR9, incubated at various temperature (28-70 °C) at room temperature (28 °C) for 30 min. The activity was determined in MOPS-NaOH buffer (pH 7.0) with initial concentration of 1.0 µM Cr(VI) at 40 °C. Data represent mean of three separate experiments.

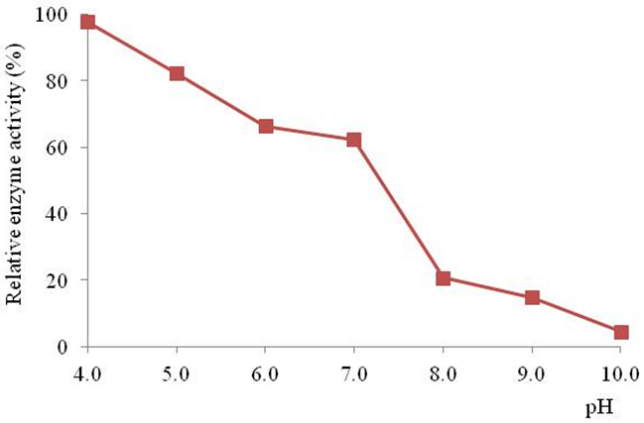

Figure 4. pH stability of CFE from *B. fusiformis* NTR9, incubated at various pH (4.0-10.0) for 30 min. The acitivity was determined in MOPS-NaOH buffer (pH 7.0) with initial concentration of 1.0 µM Cr(VI) at 40°C. Data represent mean of three separate experiments.

to decrease the chromate reductase activity in *Bacillus* sp. (Elangovan *et al.*, 2006) and *Pseudomonas* sp. G1DM21 (Desai *et al.*, 2008). Strong inhibition of Cr(VI) reduction by Ag^{+} was characterized as noncompetitive (Ishibashi *et al.*, 1989). However, Ag^{+} did not affect the chromate reductase activity in *Bacillus* sp. ES29 (Camargo *et al.*, 2003). As^{3+} showed no significant effect on the chromate reductase activity of the CFE of *B. fusiformis* NTR9 as in the case of *P. putida* MK1 (Park *et al.*, 2000).

In the reaction mixtures supplemented with electron acceptors like sulfate and nitrate, it was found that neither of them exhibited significant effect on the enzyme activity as shown in Figure 5. Similar results were also observed in the case of *P. putida* PRS2000 (Ishibashi *et al.*, 1990), *E. coli* ATCC33456 (Shen and Wang, 1993) and *Bacillus* sp. (Wang and Xiao, 1995). However, sulphate inhibited the chromate reductase activity of *P. putida* MK1 (Park *et al.*, 2000), *A. radiobacter* EPS-916 (Llovera *et al.*, 1993), *Ent. cloacae* HO1 (Wang *et al.*, 1989) and *Comamonas testosterone* VMC-2 (Cooke *et al.*, 1995). It has been reported that sulphate is a competitive inhibitor of chromate transport (Cervantes *et al.*, 2001) and can inhibit the chromate reductase activity noncompetitively (Park *et al.*, 2000).

The effect of external electron donors on the chromate reductase activity of the CFE of *B. fusiformis* NTR9 was investigated in the presence of glucose, glycerol, citrate, malate, succinate and acetate. As shown in Figure 6, chromate reductase activity was significantly improved in the reaction mixtures supplemented with most tested electron donor by 61.90-77.14%, but in the presence of NADH, the activity was slightly decreased by 12.38%. Other researchers have also reported that many bacteria do not require NADH as an electron donor for their chromate reductase activity, for example *Comamonas testosterone* VMC-2 (Cooke *et al.*, 1995), *Enterobacter cloacae* HO1 (Wang *et al.*, 1989), *Agrobacterium radiobacter* EPS-916 (Llovera *et al.*, 1993), *Bacillus* sp. (Wang and Xiao, 1995), and *Pseudomonas* sp. CRB5 (McLean and Beveridge, 2001). On the other hand, NADH has been reported to enhance chromate reductase activity in several bacteria (Bopp and Ehrlich, 1988; Ishibashi *et al.*, 1990; Suzuki *et al.*, 1992; Shen and Wang, 1993; Campos *et al.*, 1995; Oh and Choi, 1997; Garbisu *et al.*,1998; Park *et al.*, 2000; Nepple *et al.*, 2000; Camargo *et al.*, 2003; Thacker *et al.*, 2006; Desai *et al.*,2008; Opperman *et al.*, 2008; Focardi *et al.*, 2012; Xu *et al.*, 2012). The result suggested that external electron donors, except NADH, were essential to improve the chromate reductase activity of NTR9 strain. Even through its heat stability was the same as that of soluble reductase of *P. ambigua* G-1 (Suzuki *et al.*, 1992) and *P. putida* MK1 (Park *et al.*, 2000), these strains could reduced chromate only in an aerobic condition and thus required NADH as electron donor. On the other hand *B. fusiformis* NTR9 could reduced chromate under anaerobic better than aerobic condition and so it is not necessary to use NADH as electron donor. This strain can use chromate, organic compounds or other endogenous electron donor reserves.

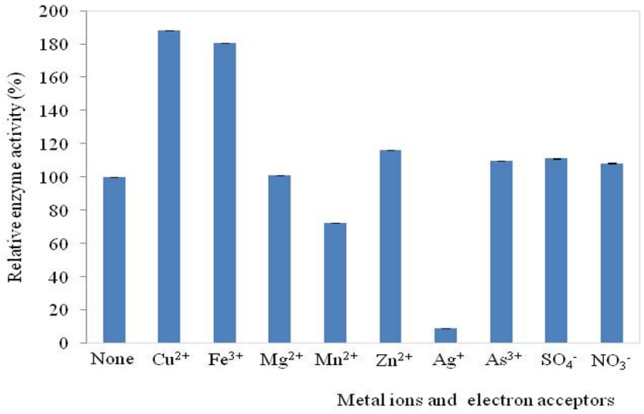

Figure 5. Effect of metal ions and electron acceptors on the activity of chromate reduction by CFE of *B. fusiformis* NTR9, determined in MOPS-NaOH buffer (pH 7.0) with initial concentration of 1.0 µM Cr(VI) at 40°C for 30 min. Data represent mean of three separate experiments.

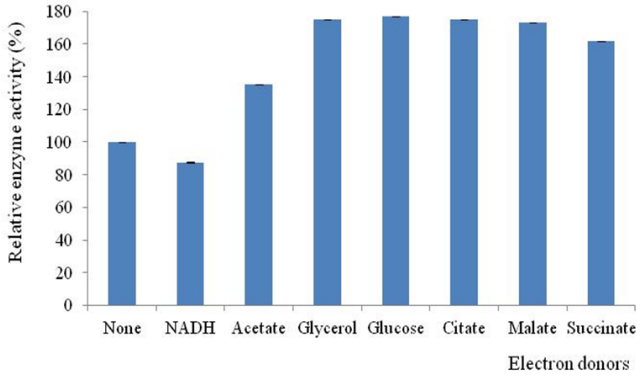

Figure 6. Effect of electron donors on the activity of chromate reduction by CFE of *B. fusiformis* NTR9, determined in MOPS-NaOH buffer (pH 7.0) with initial concentration of 1.0 µM Cr(VI) at 40°C for 30 min. Data represent mean of three separate experiments.

3.4 Localization of chromate reductase activity

To identify the localization of chromate reductase activity, various sub-cellular fractions were prepared by ultracentrifigation into cell-free extract (S_{12}), soluble fraction (S_{150}) and membrane fractions (MF_s). As shown in Table 1, chromate reductase activity was found in both S_{12} and S_{150} fraction and most of the activity was in the soluble fraction, while the membrane fraction showed no activity. Thus, the location of the enzyme is restricted to the cytoplasm or, possibly, the periplasm. The molecular mass of the native protein responsible for chromate reductase activity was estimated on SDS-PAGE to be 20 kDa. For protein identification using LC/MS, it was found that this protein is similar to ribosome recycling factor of *Bacillus* sp. NRRL B-14911, which was a molecular weight of 20,741.60. The result suggests that chromate reductase of *B. fusiformis* NTR9 is soluble chromate reductase as reported for *P. putida* PRS

Table 1. Localization of Cr(VI) reductase activity in cell fractions of *B. fusiformis* NTR9

Cell fraction	Cr(VI) reduct ase activity (Units)	Total Protein (mg/ml)	Specific activity (U/mg protein)
cell-free extract (S_{12})	0.57	5.04	0.113
soluble fraction (S_{150})	0.72	5.04	0.143
membrane fractions (MF_s)	0.01	1.08	0.009

2000 (Ishibashi *et al.*, 1990), *P. ambigua* G-1 (Suzuki *et al.*, 1992), *E. coli* ATCC33456 (Shen and Wang, 1993), *D. vulgaris* (Lovley and Phillips, 1994), *Bacillus* sp. (Wang and Xiao, 1995), *P. aeruginosa* (Oh and Choi, 1997), *B. subtilis* (Garbisu *et al.*, 1998), *P. putida* MK1 (Park *et al.*, 2000), *R. sphaeroides* (Nepple *et al.*, 2000), *Pseudomonas* sp. CRB5 (McLean and Beveridge, 2001), *Bacillus* sp. ES29 (Camargo *et al.*, 2003), *Providencia* sp. (Thacker *et al.*, 2006), *T. scotoductus* SA-01 (Opperman *et al.*, 2008), *Pseudomonas* sp. G1DM21 (Desai *et al.*, 2008), and *P. phragmitetus* LSSE-09 (Xu *et al.*, 2012).

4. Conclusion

Cr(VI) reductase activity of CFE of thermophilic chromate-reducing bacteria, *B. fusiformis* NTR9 has an optimum temperature and pH of 80°C and pH 7, respectively. It is a heat stable enzyme. Moreover, the enzyme was resistant in acid and neutral condition but its stability was decreased in alkaline condition. The Cr(VI) reductase activity of CFE was enhanced when exposed in Cu^{2+} and Fe^{3+}, while Mg^{2+}, Zn^{2+}, and As^{3+} had only a slightly stimulating effect on the activity. The Cr(VI) reductase activity was inhibited by Mn^{2+} and Ag^{+}. Sulfate and nitrate had no effect on Cr(VI) reductase activity. The CFE did not require NADH as an electron donor for Cr(VI) reductase activity. The chromate reductase is mainly associated with the soluble fraction in the cytoplasm of the bacterial cell. The molecular weight of the enzyme was 20 kDa.

Acknowledgement

The authors acknowledge the National Research Council of Thailand for project funding. Facilities were provided by the Faculty of Science, Ubon Ratchathani University.

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