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ORIGINAL PAPER

Responses of a novel salt-tolerant *Streptomyces albidoflavus* DUT_AHX capable of degrading nitrobenzene to salinity stress

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Abstract A novel salt-tolerant strain DUT_AHX, which was capable of utilizing nitrobenzene (NB) as the sole carbon source, was isolated from NB-contaminated soil. Furthermore, it was identified as *Streptomyces albidoflavus* on the basis of physiological and biochemical tests and 16S ribosomal DNA (rDNA) sequence analysis. It can grow in the presence of NaCl up to 12% (w/v) or NB up to 900 mg/l in mineral salts basal (MSB) medium. The exogenously added osmoprotectants such as glycin, glutamic acid, proline, betaine and ectoine can improve growth of strain DUT_AHX in the presence of 10% (w/v) NaCl. NB-grown cells of strain DUT_AHX in modified MSB medium can degrade NB with the concomitant release of ammonia. Moreover, crude extracts of NB-grown strain DUT_AHX mainly contained 2-aminophenol 1,6-dioxygenase activity. These indicate that NB degradation by strain DUT_AHX might involve a partial reductive pathway. The proteins induced by salinity stress or NB were analyzed by native-gradient polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate (SDS)-PAGE. In NB-induced proteins de novo, 141 kDa protein on the native-gradient PAGE gel was excised and electroeluted. Furthermore, enzyme tests exhibit the 2-aminophenol 1,6-dioxygenase activity of purified 141 kDa protein is 11-fold that of the cell-free extracts. The exploitation of strain DUT_AHX in salinity stress will be a remarkable improvement in NB bioremediation and wastewater treatment in high salinity.

Keywords Nitrobenzene · Degradation · *Streptomyces* · Salinity stress · Induced proteins

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Introduction

Hyper-salinity chemical industrial wastewater usually contains a large number of chemicals such as nitroaromatic compounds, phenol, aniline, dyes and so on. Nitroaromatic compounds are relatively rare in nature but widely used in the chemical industry for the production of dyes, resins, pesticides, herbicides, explosives, and other useful materials (Spain 1995). Many of these compounds are highly toxic even at low concentrations and recalcitrant to biodegradation. As the simplest nitroaromatic compound, nitrobenzene (NB) is one of the top 50 industrial chemicals produced in the United States (Storck et al. 1996) and one of seven nitroaromatic compounds on the U.S. Environmental Protection Agency's list of priority pollutants (Keith and Telliard 1979).

Microbial degradation and mineralization of NB are important processes during the treatment of NB wastewater. The bacteria capable of degrading NB, such as *Pseudomonas pseudoalcaligenes* (Nishino and Spain 1993), *Comamonas* sp. (Nishino and Spain 1995; Zhao and Ward 1999), *Acidovorax delafieldii* (Zhao and Ward 1999), *Pseudomonas putida* (Park et al. 1999; Li et al. 2007) and *Mycobacterium chelonae* (Oh et al. 2003), have been isolated from contaminated soils and wastewater in the past decades. Moreover, NB is known to be metabolized by aerobic bacteria through either a partial reductive pathway characterized by the release of ammonia (Nishino and Spain 1993; Park et al. 1999) or an oxidative pathway characterized by the release of nitrite (Nishino and Spain 1995). The partial reductive pathway, described in *Pseudomonas pseudoalcaligenes* JS45, leads to the formation of 2-aminophenol and some enzymes involved in NB catabolism have been purified and characterized (Somerville et al. 1995; Lendenmann and Spain 1996; He and Spain 1998; He et al. 1998; He et al. 2000). In *Pseudomonas putida* HS12 (Park and Kim 2000, 2001), *Pseudomonas putida* ZWL73 (Zhen et al. 2006; Xiao et al. 2006) and *Comamonas* sp. CNB-1 (Wu et al. 2006) which were all capable of degrading 4-chloronitrobenzene and NB, NB catabolic gene clusters were investigated at the molecular and biochemical levels. The oxidative pathway, described in *Comamonas* sp. JS765, converts NB to catechol (Nishino and Spain 1995).

However, in general industrial wastewater containing NB also consists of high inorganic salts (Patil and Shinde 1989; Yao et al. 2003; Li et al. 2006). When sodium concentration is above 3 g/l, the growth of most microorganisms is inhibited (De Baere et al. 1984). As a result, the traditional biological treatments may be out of action in high salinity.

So far, little is reported on the ability of *Streptomyces* species to degrade NB in high salinity. Although environmental-stress-induced modifications of protein synthesis have been observed in bacteria such as *Anabaena* (Apte and Bhagwat 1989; Iyer et al. 1994), *Halomonas* (Mojica et al. 1997) and *Listeria* (Duché et al. 2002), there are no reports on environmental-stress-induced proteins in *Streptomyces*. Therefore, exploiting the salt-tolerant bacteria will be a great enhancement in the conventional biotreatment and bioremediation systems.

In this paper, a novel *Streptomyces albidoflavus* DUT_AHX capable of utilizing NB as a sole carbon source was isolated and identified. The effects of salinity stress on growth and NB degradation of strain DUT_AHX were investigated. Furthermore, the protein synthesis patterns induced by salinity stress and NB were examined by native-gradient polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate (SDS)-PAGE.

Materials and methods

Organism and growth conditions

The microorganism was isolated from NB-contaminated soil by an enrichment culture and repeatedly streaking culture technique. It was grown in the mineral salts basal (MSB) medium containing 100 mg/l NB dissolved ultrasonically as the sole carbon source. The MSB medium contained 7 g/l $Na_2HPO_4 \cdot 12H_2O$, 1 g/l KH_2PO_4, 10 mg/l $CaCl_2 \cdot 2H_2O$, 1 mg/l $FeCl_3$, 20 mg/l $MgSO_4 \cdot 7H_2O$, 1 g/l $(NH_4)_2SO_4$. The modified MSB medium without $(NH_4)_2SO_4$, which contained 100 mg/l NB as the sole carbon and nitrogen sources, was used to examine the release of ammonia and nitrite. The complex medium was used to investigate the effect of various osmoprotectants on growth of the strain. The complex medium contained 10 g/l glucose, 7 g/l $Na_2HPO_4 \cdot 12H_2O$, 1 g/l KH_2PO_4, 10 mg/l $CaCl_2 \cdot 2H_2O$, 1 mg/l $FeCl_3$, 20 mg/l $MgSO_4 \cdot 7H_2O$, 1 g/l $(NH_4)_2SO_4$, 100 g/l NaCl. The Luria-Bertani (LB) medium consisted of 10 g/l tryptone, 5 g/l yeast extract and 10 g/l NaCl. The pH of the media above was adjusted to 7.0. When required, the media were supplemented with 15 g/l agar and different NaCl concentrations as described in the text.

Identification of bacteria

Microorganism isolated from the enrichment culture was identified by the standard procedures described in *Bergey's Manual of Systematic Bacteriology* (Buchanan and Gibbons 1984). The effect of NaCl (1–15%, w/v) on growth of the strain in LB medium was investigated. The ability to utilize other substrates as the sole carbon source was tested on MSB medium plates. The total DNA was isolated by the modified SDS method (Sambrook and Russell 2001).

The G + C content of the DNA was determined by the thermal denaturation method (Marmur and Doty 1962), with *E. coli* K-12 as the reference.

A PCR was also performed in order to amplify the 16S ribosomal DNA (rDNA) of strain DUT_AHX. The total DNA was used as PCR template and 27f and 1522r universal primers were used as PCR primers (Lane 1991). The purified PCR product was directly sequenced on an ABI model 3730XL automatic DNA analyzer by using BigDye Teminator V3.1 Kit (Applied Biosystems, USA). The 16S rDNA sequence analysis was performed with BLAST program at National Center for Biotechnology Information. The 16S rDNA gene sequence was aligned with previously published sequences by the multiple-sequence alignment program CLUSTALX 1.83 (Thompson et al. 1997). The phylogenetic tree was constructed by the neighbor-joining method in MEGA 3.1 software (Kumar et al. 2004). The topology of the phylogenetic tree was evaluated by bootstrap resampling with 1000 replicates.

Growth and NB degradation

The effect of different NB concentrations (100–1000 mg/l) on growth and NB degradation of strain DUT_AHX in MSB medium at 30°C and 150 rpm for 48 h was investigated. Cultures without cells and cell cultures without NB served as the controls. The effect of NaCl (1–15%, w/v) on growth and NB degradation of strain DUT_AHX in MSB medium at 30°C and 150 rpm for 24 h was tested. Cell cultures without NaCl served as the control. The effect of various osmoprotectants (0.2%, w/v), viz. glycin, glutamic acid, proline, betaine and ectoine on growth of strain DUT_AHX was detected in complex medium at 30°C and 150 rpm for 48 h. The cell cultures without osmoprotectants served as the control.

Preparation of cell-free extracts

Cultures with 100 mg/l NB in LB medium and cultures with 100 mg/l NB in MSB medium were separately incubated at 30°C and 150 rpm for 48 h. Cultures without NB in LB medium served as the control. Cultures with different NaCl concentrations (1%, 7% and 12%, w/v) in LB medium were cultivated at 30°C and 150 rpm for 48 h. Cultures without NaCl in LB medium served as the control.

Cells from different cultures were harvested by centrifugation (8,000×*g*) for 10 min at 4°C, washed twice and resuspended in cold fresh 0.02 M phosphate buffer (pH 7.2). After cells were disrupted by an ultrasonic disruptor CPX750 (20% amplitude, Cole-Parmer, USA), unbroken cells and cell debris were removed by centrifugation (60,000×*g*) for 60 min at 4°C. The pellets were discarded, and the supernatant fluids were concentrated on a YM10 membrane (Amicon, Danvers, USA) in a stirred cell ultrafiltration device 8050 (Amicon, Danvers, USA) at 4°C.

Protein gel electrophoresis

The samples including equal amounts of total proteins were determined by native-gradient PAGE with a 5 to 12% (w/v) linear gradient resolving gel and a 5% (w/v) stacking gel, and SDS-PAGE with a 12% (w/v) resolving gel and a 5% (w/v) stacking gel (Sambrook and Russell 2001). The high molecular weight (HMW) native protein marker for PAGE was purchased from Amersham Pharmacia Biotech (USA) and the low molecular weight (LMW) protein marker for SDS-PAGE was purchased from TaKaRa Biotech (Dalian, China). After electrophoresis, gels were stained with Coomassie brilliant blue R-250 and destained for visualization of molecular weight standards (Sambrook and Russell 2001). The area containing the interested proteins on the native PAGE gel was excised and then electroeluted on a Protein Gel Eluter DYCP-43 (LiuYi, Beijing, China) (Weldingh et al. 2000).

Enzyme assays

2-aminophenol 1,6-dioxygenase activity was measured by monitoring the increase in the absorbance of the 2-aminophenol ring cleavage product at 380 nm (Nishino and Spain 1993). Catechol 1.2-dioxygenase and catechol 2,3-dioxygenase were determined as described previously (Schraa et al. 1986). Reaction mixtures contained 2-aminophenol or catechol (0.3 μmol), sodium phosphate (29.5 μmol, pH 7.2), and cell extracts (0.3–1.0 mg of protein) in a final volume of 3 ml at room temperature.

Analytical methods

NB was determined by measuring the maximum absorbance at 268 nm using an UV–visible

spectrophotometer V-560 (JASCO, Japan) via the Beer–Lambert law. The relationship between absorbance and concentration was linear in NB concentration range of 0–30 mg/l. Cell concentration was detected by measuring the optical density (OD) at 600 nm. Nitrite and ammonia releases were measured by standard methods (Gerhardt et al. 1994). Protein was measured by Bradford assay (Bradford 1976) with bovine serum albumin as the protein standard. All experiments were conducted three times, each with triplicate and the mean values of the data were presented.

Results and discussion

Identification of strain DUT_AHX

After 2 months of enrichment, strain DUT_AHX capable of utilizing NB as a sole carbon source was isolated by repeatedly streaking culture. Strain DUT_AHX developed well on several media including glucose-asparagine agar, inorganic salt-starch agar, yeast-malt extract agar, LB agar and Sauton agar. It grew moderately on glycerol-asparagine agar, oatmeal agar, Gause No. 1 agar and Gause No. 2 agar (Table 1). Highly branched aerial mycelium and substrate mycelium were well developed on LB agar (Fig. 1). At maturity, the aerial mycelium formed short, straight to *Rectiflexibiles* spore chains. Smooth-surfaced oval spores were non-motile and borne in chains on the tip of the aerial mycelium. The physiological and biochemical characteristics were showed in Table 2. Strain DUT_AHX grew well in LB medium at 25°C, 30°C, 35°C and 40°C but did not grow at 20°C or 45°C. The optimum temperature for growth was 30°C (Fig. 2). Strain DUT_AHX grew well in LB medium containing NaCl up to 10% (w/v); the weak growth was observed in the presence of 12% (w/v) NaCl and no growth was observed in the presence of 15% (w/v) NaCl (Fig. 3). Strain DUT_AHX was examined for growth on MSB medium plates with various compounds as the sole carbon source (Table 3). Strain DUT_AHX was also able to utilize 2-aminophenol, catechol, phenol, toluene, lignin and azure B as growth substrates. The DNA G + C content of strain DUT_AHX was 70.4 mol%. The nearly complete 16S rDNA gene sequence (1443 nt) of strain DUT_AHX (GenBank No. DQ409080) was compared to that of *Streptomyces* species deposited in the GenBank database. The closest phylogenetic relative of strain DUT_AHX was *Streptomyces albidoflavus* (16S rDNA gene sequence identity was 99%, Fig. 4). Therefore, the strain is identified as a *Streptomyces albidoflavus* and

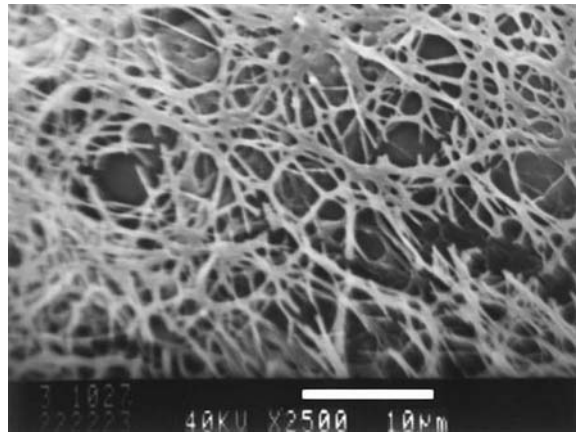

Fig. 1 Scanning electron micrograph of *Streptomyces albidoflavus* DUT_AHX

Table 1 Cultural characteristics of strain DUT_AHX on different media

Medium	Aerial hypha	Substrate mycelium	Soluble pigment
Glucose-asparagine agar	Lotus-white	Mustard-yellow	None
Glycerol-asparagine agar	Hawksbill-yellow	Pomegranate-yellow	None
Inorganic salt-starch agar	Mustard-yellow	Smoky black	None
Yeast-malt extract agar	Dust-grey	Bamboo-brown	Clove-brown
LB agar	Greyish white	Grey	Grey
Oatmeal agar	Dust-grey	Bamboo-brown	Clove-brown
Gause No. 1 agar	Cheese-yellow	Mustard-yellow	None
Gause No. 2 agar	Pale yellow	Pale yellow	None
Sauton agar	Lotus-white	Withered green	Shark-cyan

Table 2 Phenotypic properties of strain DUT_AHX

Characteristic	Reaction	Characteristic	Reaction	Characteristic	Reaction
Spore	Oval	Tyrosinase	−	Glycerol	+
Spore chain	*Rectiflexibiles*	Nitrate reduction	+	Inulin	−
Spore surface	Smooth	Cellobiose	+	Raffinose	−
Optimal growth temp	30°C	Inositol	−	Rhamnose	−
Melanin production	−	Mannose	+	Melibiose	+
Aerobiosis	+	Mannitol	+	Melizitose	−
Gram stain	+	Sorbose	−	Maltose	+
Motility	−	Sorbitol	−	Ribose	+
Gelatin liquefaction	−	Sucrose	−	Arabinose	+
Milk peptonization	+	Lactose	+	Trehalose	+
Hydrogen sulphide	−	Xylose	+	D-Galactose	+
Starch hydrolysis	+	Fructose	+	D-Glucose	+

+, Positive; −, negative

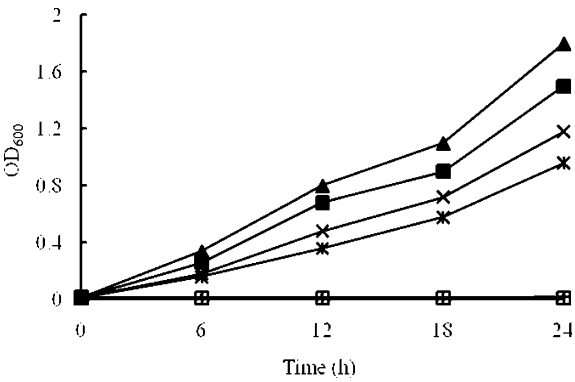

Fig. 2 Growth of *Streptomyces albidoflavus* DUT_AHX in LB medium at different temperature. +, 20°C; ×, 25°C; ▲ 30°C; ■, 35°C; *, 40°C; □, 45°C

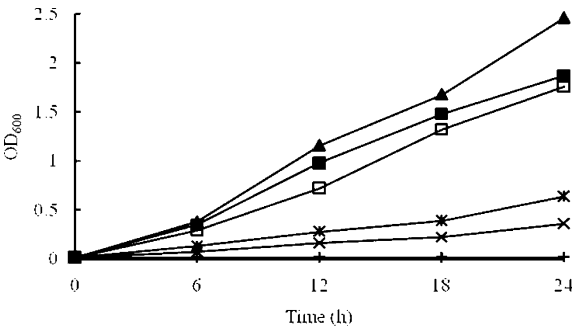

Fig. 3 Growth of *Streptomyces albidoflavus* DUT_AHX in LB medium with different NaCl concentrations. ▲, 1% NaCl; □, 5% NaCl; *, 10% NaCl; ×, 12% NaCl; +, 15% NaCl; ■, control

Table 3 Growth of strain DUT_AHX on various compounds as the sole carbon source

Substrates	Growth	Substrates	Growth
Benzene	−	Aniline	−
Chlorobenzene	−	Benzoate	−
2-Aminophenol	+	Catechol	+
3-Aminophenol	−	Phenol	+
Picolinic acid	−	Toluene	+
Lignin	+	Azure B	+

+, Growth; −, no growth

designated strain DUT_AHX on the basis of these phenotypic properties and the 16S rDNA gene sequence anlaysis.

Growth and NB degradation

The effect of different NB concentrations on growth and NB degradation of strain DUT_AHX was examined. As shown in Fig. 5, strain DUT_AHX was unable to grow in MSB medium with 1000 mg/l NB.

Since industrial wastewater containing NB also consists of high inorganic salts which can influence the performance of biological process, the effect of salinity on growth and NB degradation of strain DUT_AHX in MSB medium with additive NaCl was tested (Fig. 6). As shown in Fig. 6, strain DUT_AHX grew well in the absence of NaCl and could also grow in the presence of NaCl up to 12% (w/v) in MSB medium. When NaCl concentrations increased from 5

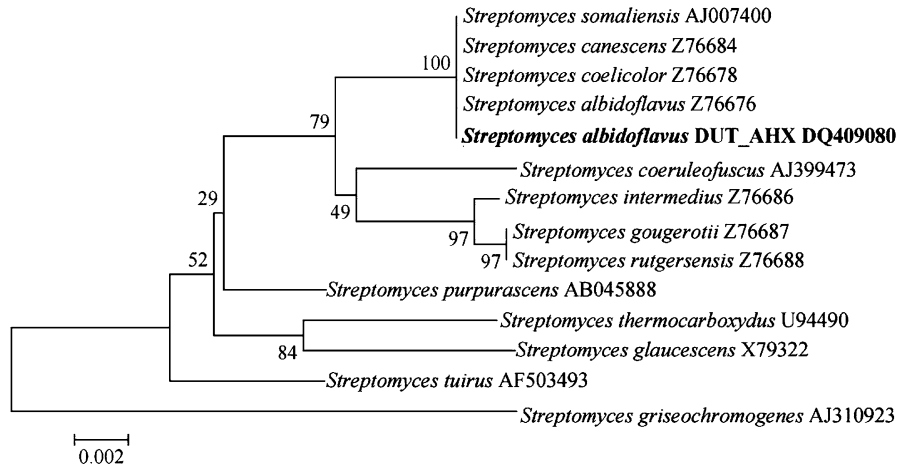

Fig. 4 Phylogenetic tree of *Streptomyces albidoflavus* DUT_AHX

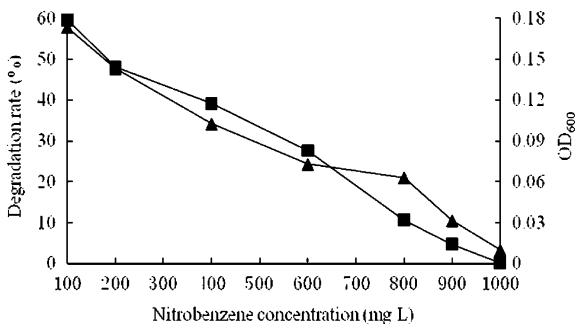

Fig. 5 Effect of NB concentrations on growth and NB degradation of *Streptomyces albidoflavus* DUT_AHX. ■, degradation rate; ▲, biomass

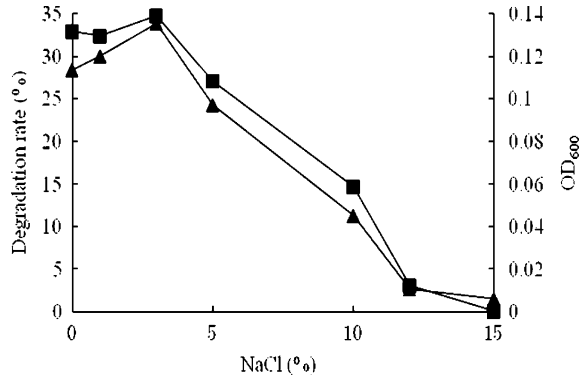

Fig. 6 Effect of NaCl concentrations on growth and NB degradation of *Streptomyces albidoflavus* DUT_AHX. ■, degradation rate; ▲, biomass

to 15% (w/v), the growth rate and NB degradation rate of strain DUT_AHX gradually decreased. Replacement of NaCl by KCl did not cause significant changes in the growth pattern (data not shown). Strain DUT_AHX can tolerate moderately NaCl but does not require NaCl for the growth. Hence, it is not halophilic but halotolerant (Vreeland 1987).

Subsequently, the effect of exogenously added osmoprotectants on growth of strain DUT_AHX was investigated at growth inhibiting salinities (Fig. 7). Some amino acids such as glycin, glutamic acid and proline improve growth of strain DUT_AHX better than some amino acid derivatives such as betaine and ectoine. This suggests that the presence of exogenous compatible solutes can partially relieve the inhibiting effect of high salinities on the growth. Therefore, the accumulation of common compatible solutes under high salinity stress may be a universal mechanism of adaptation.

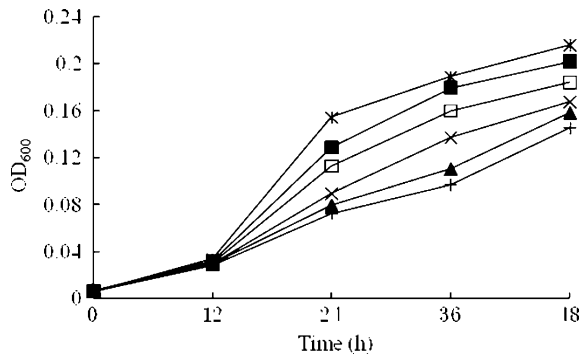

Fig. 7 Effect of added osmoprotectants on growth of *Streptomyces albidoflavus* DUT_AHX in complex medium with 10% NaCl. *, glycin; ■, glutamic acid; □, proline; ×, betaine; ▲, ectoine; +, control

Strain DUT_AHX was grown in modified MSB medium which contained 100 mg/l NB as the sole carbon and nitrogen sources. As shown in Fig. 8, NB rapidly decreased with culture time and cell density gradually increased. Ammonia was detected but no nitrite was detected in the culture medium. This indicates that NB degradation by strain DUT_AHX may involve a partial reductive pathway (Nishino and Spain 1993; 1995; Park et al. 1999).

Enzyme activities in cell-free extracts

The activities of key enzymes involved in catabolic pathway of NB degradation by strain DUT_AHX were investigated. As showed in Table 4, cell-free extracts in MSB medium contained activities of 2-aminophenol 1,6-dioxygenase, catechol 1,2-dioxygenase and catechol 2,3-dioxygenase, but the activity of catechol 2,3-dioxygenase was low. The cell-free extracts of cells grown in LB medium exhibited none of three enzyme activities. These results imply that enzymes of NB degradation are induced by NB in MSB medium and NB degradation by strain DUT_AHX may involve a partial reductive pathway (Nishino and Spain 1993; Nishino and Spain 1995; Park et al. 1999).

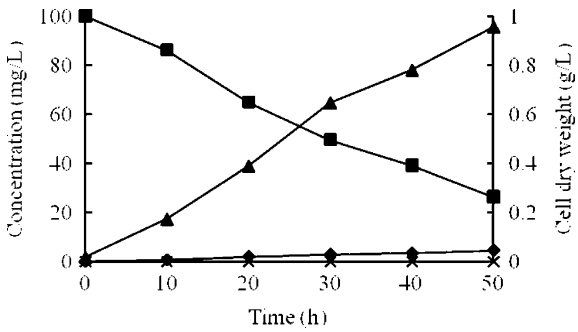

Fig. 8 Degradation of nitrobenzene by *Streptomyces albidoflavus* DUT_AHX in modified MSB medium. ■: nitrobenzene; ▲: cell dry weight; ◆: ammonia; ×; nitrite

Table 4 Enzyme activities in cell-free extracts of *Streptomyces albidoflavus* DUT_AHX

Enzymes	Specific activity (μmol/min/mg of protein)
2-Aminophenol 1,6-dioxygenase	0.514
Catechol 1.2-dioxygenase	0.008
Catechol 2,3-dioxygenase	0.012

Protein synthesis profiles under various salinity conditions

Since growth and NB degradation of strain DUT_AHX were inhibited in MSB medium with 7 and 12% (w/v) NaCl, 7 and 12% (w/v) NaCl were adequate to create stressful conditions. In high salinity, most of metabolic activities may be shut off. As a result, the growth is inhibited in high salinity. Exposure of strain DUT_AHX to salinity stress resulted in alterations in protein syntheses patterns (Figs. 9 and 10). There were three prominent types of modifications, which several proteins were declined, certain proteins were enhanced and some proteins were induced de novo. Synthesis of a set of proteins such as 440, 395, 194, 183, 162, 125,

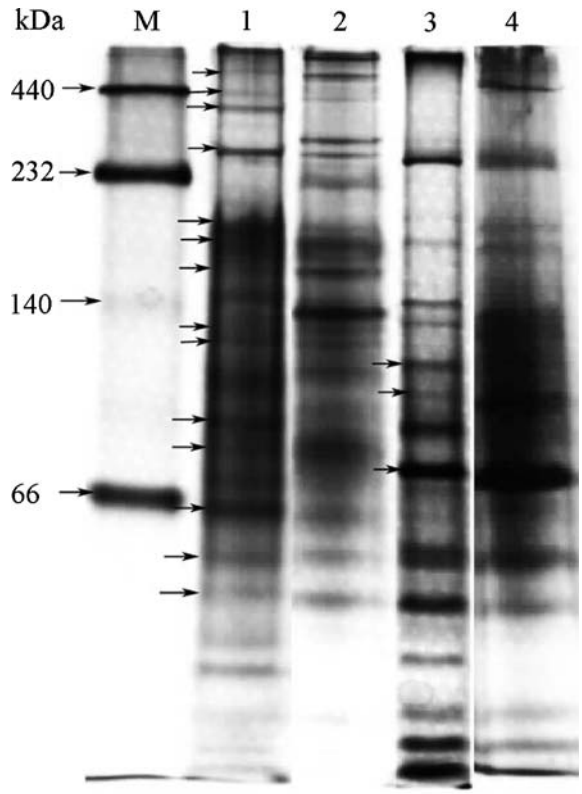

Fig. 9 Effect of external NaCl concentrations on protein synthesis of *Streptomyces albidoflavus* DUT_AHX visualized by native-gradient PAGE. Lane M: HMW native marker; Lane 1: none of NaCl; Lane 2: 1% NaCl; Lane 3: 7% NaCl; Lane 4: 12% NaCl

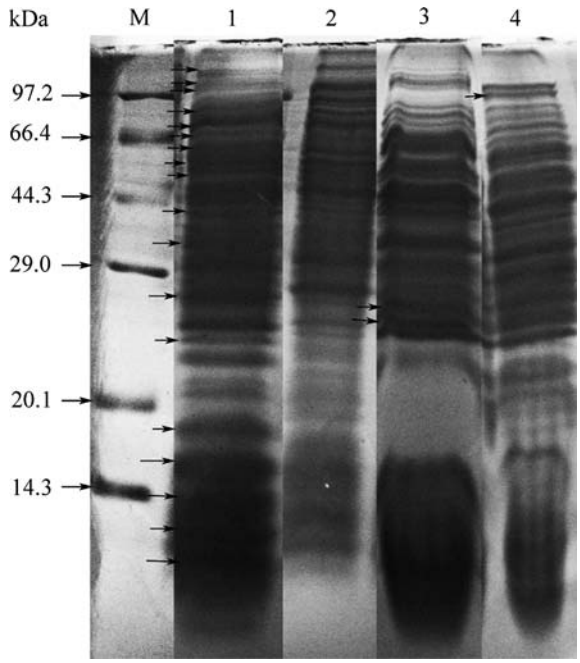

Fig. 10 Effect of external NaCl concentrations on protein synthesis of *Streptomyces albidoflavus* DUT_AHX visualized by SDS-PAGE. Lane M: LMW marker; Lane 1: none of NaCl; Lane 2: 1% NaCl; Lane 3: 7% NaCl; Lane 4: 12% NaCl

111, 82 and 57 kDa by native-gradient PAGE and 115, 106, 86, 77, 27, 24, 18, 16, 14, 12 and 9 kDa by SDS-PAGE significantly declined. Salinity specifically enhanced the synthesis of a set of proteins such as 484, 276, 133, 91, 42 and 29 kDa by native-gradient PAGE and 99, 69, 65, 59, 54, 41 and 34 kDa by SDS-PAGE. Salinity also induced de novo synthesis of another set of proteins such as 118, 105 and 78 kDa by native-gradient PAGE and 98, 26 and 25 kDa by SDS-PAGE. These indicate the salinity stress-induced proteins can be induced externally.

Protein synthesis profiles induced by NB

The synthesis of proteins in MSB medium with NB, exhibited 319, 270, 183, 141, 129, 65, 52, 37 and 21 kDa by native-gradient PAGE and 73, 65, 59, 51, 37, 34, 27, 19 and 14 kDa by SDS-PAGE (Figs. 11 and 12). These proteins were not synthesized in LB medium with NB and without NB. Furthermore, they might be induced specifically by NB in the process of NB degradation in MSB medium. The common proteins induced by salinity stress or NB

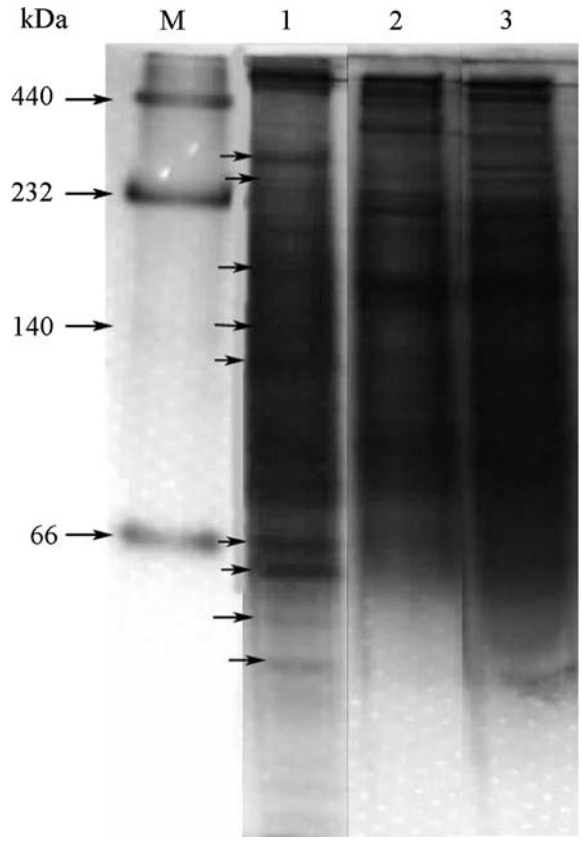

Fig. 11 NB-induced protein synthesis pattern of *Streptomyces albidoflavus* DUT_AHX grown in different medium visualized by native-gradient PAGE. Lane M: HMW native marker; Lane 1: MSB medium with NB; Lane 2: LB medium with NB; Lane 3: LB medium without NB

were 183 kDa by native-gradient PAGE and 65, 59, 34, 27, 14 kDa by SDS-PAGE. This indicates that a certain commonality may exist between responses to different environmental stresses such as salinity, heat, osmotic, heavy metals, organic solvents but individual stresses can also induce a unique response.

So far, some enzymes involved in NB catabolism have been purified and characterized (Somerville et al. 1995; Lendenmann and Spain 1996; Takenaka et al. 1997; He and Spain 1998; He et al. 1998; Wu et al. 2005; Liu et al. 2007). As shown in Table 5, 141 kDa protein by native-gradient PAGE may be 2-aminophenol 1,6-dioxygenase which native mass is 140 kDa from strain JS45 and AP-3 or 130 kDa from strain CNB-1. The interested proteins of 319, 270, 183, 141 and 129 kDa on the native PAGE gel were

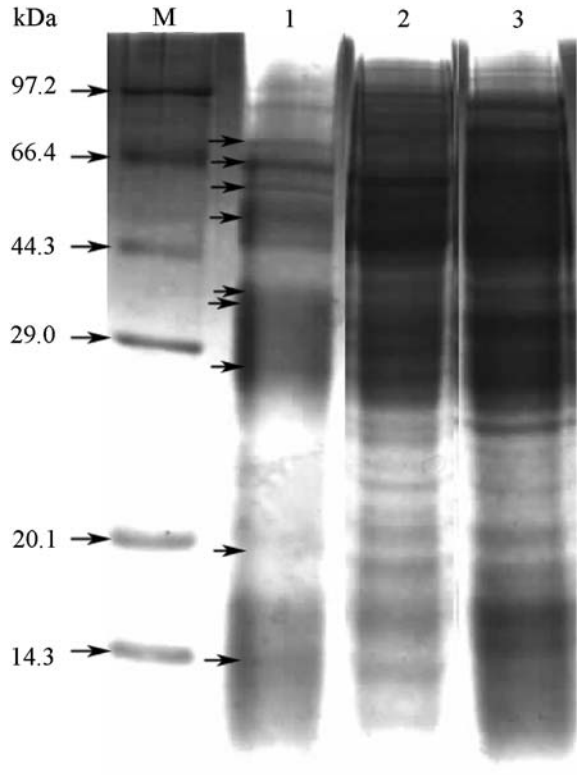

Fig. 12 NB-induced protein synthesis pattern of *Streptomyces albidoflavus* DUT_AHX cells grown in different medium visualized by SDS-PAGE. Lane M: LMW marker; Lane 1: MSB medium with NB; Lane 2: LB medium with NB; Lane 3: LB medium without NB

separately excised, electroeluted and then used for enzyme assays. This result shows that 141 kDa protein possesses 2-aminophenol 1,6-dioxygenase activity (5.825 μmol/min/mg of protein), which is approximately 11-fold that of cell-free crude extracts. This implies that 141 kDa protein may be 2-aminophenol 1,6-dioxygenase of strain DUT_AHX induced by NB.

Conclusions

Strain DUT_AHX capable of degrading NB was identified as a *Streptomyces albidoflavus* by physiological and biochemical tests and 16S rDNA sequence analysis. Strain DUT_AHX could grow and degrade NB in the presence of NaCl up to 12% (w/v) or NB up to 900 mg/l. The exogenously common compatible solutes such as glycin, glutamic acid, proline, betaine and ectoine could improve growth of strain DUT_AHX in the presence of 10% (w/v) NaCl. Furthermore, the protein synthesis patterns of strain DUT_AHX in salinity stress were analyzed by native-gradient PAGE and SDS-PAGE. The proteins induced were 484, 440, 395, 276, 194, 183, 162, 133, 125, 118, 111, 105, 91, 82, 78, 57, 42 and 29 kDa by native-gradient PAGE and 115, 106, 99, 98, 86, 77, 69, 65, 59, 54, 41, 34, 27, 26, 25, 24, 18, 16, 14, 12 and 9 kDa by SDS-PAGE. The proteins induced by NB were 319, 270, 183, 141, 129, 65, 52, 37 and 21 kDa by native-gradient PAGE and 73, 65, 59, 51, 37, 34, 27, 19 and 14 kDa by SDS-PAGE. These suggest that there are certain common responses between different environmental stresses. Moreover, 141 kDa protein purified from the native PAGE gel exhibited 2-aminophenol 1,6-dioxygenase activity, which is approximately 11-fold that of cell-free crude extracts. This suggests that *Streptomyces albidoflavus* DUT_AHX may possess a partial reductive pathway of NB catabolism. Hence, strain DUT_AHX has a remarkable potential for

Table 5 The molecular mass of enzymes purified and characterized in the degradation of nitrobenzene

Enzyme	Native mass (kDa)	Denatured mass (kDa)	Source
Nitrobenzene nitroreductase	30	33	*Pseudomonas pseudoalcaligenes* JS45
2-Aminophenol 1,6-dioxygenase	140	35.39	*Pseudomonas pseudoalcaligenes* JS45
2-Aminophenol 1,6-dioxygenase	130	33.38	*Comamonas* sp. CNB-1
2-Aminophenol 1,6-dioxygenase	140	32.40	*Pseudomonas* sp. AP-3
2-Aminomuconic 6-semialdehyde dehydrogenase	160	57	*Pseudomonas pseudoalcaligenes* JS45
2-Aminomuconate deaminase	100	16.6	*Pseudomonas pseudoalcaligenes* JS45
2-Aminomuconate deaminase	30	28	*Comamonas* sp. CNB-1

application in hyper-salinity industrial wastewater treatment systems.

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