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Desulfurization of dibenzothiophene (DBT) by a novel strain *Lysinibacillus sphaericus* DMT-7 isolated from diesel contaminated soil

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Abstract

A new bacterial strain DMT-7 capable of selectively desulfurizing dibenzothiophene (DBT) was isolated from diesel contaminated soil. The DMT-7 was characterized and identified as *Lysinibacillus sphaericus* DMT-7 (NCBI GenBank Accession No. GQ496620) using 16S rDNA gene sequence analysis. The desulfurized product of DBT, 2-hydroxybiphenyl (2HBP), was identified and confirmed by high performance liquid chromatography analysis and gas chromatography-mass spectroscopy analysis respectively. The desulfurization kinetics revealed that DMT-7 started desulfurization of DBT into 2HBP after the lag phase of 24 hr, exponentially increasing the accumulation of 2HBP up to 15 days leading to approximately 60% desulfurization of the DBT. However, further growth resulted into DBT degradation. The induced culture of DMT-7 showed shorter lag phase of 6 hr and early onset of stationary phase within 10 days for desulfurization as compared to that of non-induced culture clearly indicating the inducibility of the desulfurization pathway of DMT-7. In addition, *Lysinibacillus sphaericus* DMT-7 also possess the ability to utilize broad range of substrates as sole source of sulfur such as benzothiophene, 3,4-benzo DBT, 4,6-dimethyl DBT, and 4,6-dibutyl DBT. Therefore, *Lysinibacillus sphaericus* DMT-7 could serve as model system for efficient biodesulfurization of diesel and petrol.

Key words: biodesulfurization; colony forming units; dibenzothiophene; high performance liquid chromatography; *Lysinibacillus sphaericus* DMT-7

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Introduction

Petroleum is a naturally occurring gaseous, liquid or solid mixture which is chiefly composed of hydrocarbons. After carbon and hydrogen, sulfur is characteristically the third most abundant element in petroleum, ranging from 0.05% to 5% in crude oil, but up to 14% in heavier oil (Speight, 1980; van Hamme et al., 2003). Most of the sulfur present in crude oils is organically bound sulfur, with dissolved hydrogen sulfide and elemental sulfur representing minor portions (Sinninghe Damste and de Leeuw, 1990). Organic sulfur compounds including dibenzothiophene (DBT), benzothiophene (BT) and their alkylated derivatives in fossil fuels have been the major cause of worldwide environmental problems including acid rain and air pollution (Kilbane, 1989; Monticello, 2000). With the increasing demands for energy and more stringent environmental policies, deep desulfurization of petroleum is becoming more and more desired. The Environmental Protection Agency of United States has proposed the reduction of the accepted sulfur level of diesel oil from 500 to 15 mg/L (Borngе and Quintero, 2003). The existing hydrodesulfurization (HDS) technology employed in oil refineries to remove sulfur involves catalytic treatment of fuel at high temperature (>300°C) and pressure (>100 atm) (Ma et al., 2002). The massive amount of inorganic sulfur and simple organic sulfur can be removed by HDS, however, up to 70% of the sulfur in the petroleum is in the form of heterocyclic sulfur compounds such as DBT and substituted DBTs (methylated DBTs and benzo-DBTs) that cannot be completely removed by HDS process. Regulations to reduce the sulfur content of fuels for motor vehicles will undoubtedly become increasingly stringent in the future. In these circumstances, biological desulfurization (BDS) has attracted attention as an alternative and complimentary method of treating recalcitrant organic sulfur compounds due to its low cost, mild reaction conditions, and low impact on the environment (McFarland, 1999; Folsom et

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al., 1999; Konishi et al., 2000; Li et al., 2003, 2005; Gray et al., 2003; Yu et al., 2006). Thus DBT and DBTs are generally considered as the sulfur model compounds for biodesulfurization (Monticello, 2000). In developing a cost effective biological desulfurization alternative, promoting selective removal of the sulfur component (attacking C–S bonds) without degrading the non-sulfur (C–C bonds) fuel components will be the most important consideration (Fox, 1993; Kropp and Fedorak, 1998). With DBT as the model compound, research has been focused on microbial strains that can selectively remove sulfur by converting DBT to 2-hydroxybiphenyl (2HBP) using the pathway, known as "4S pathway" (Folsom et al., 1999; Konishi et al., 2000; McFarland, 1999; Monticello et al., 1985; Monticello, 2000). These microbes are expected to be useful as biocatalysts for the biodesulfurization of diesel oil (Suzuki, 1999; Maghsoudi et al., 2000). Biodesulfurization has been studied using *Rhodococcus* sp. (Ohshiro and Izumi, 1999), *Paenibacillus* sp. A11-2 (Konishi et al., 1997), *Pseudomonas* sp. (Setti et al., 1997; Luo et al., 2003), *Corynebacterium* sp. (Maghsoudi et al., 2000; Omori et al., 1992), *Mycobacterium* sp. (Kayser et al., 2002; Li et al., 2003), *Sphingomonas* sp. (Lu et al., 1999), *Bacillus subtilis* WU-S2B (Kirimura et al., 2001), *Gordona* sp. (Rhee et al., 1998; Chang et al., 2000), and *Brevibacterium* sp. (Setti et al., 1999).

In the present study, we describe the isolation and characterization of a novel DBT-desulfurizing bacterium *Lysinibacillus sphaericus* strain DMT-7 from automobile contaminated soil sample. The DMT-7 possesses the ability to remove sulfur from DBT via the sulfur-selective pathways. In addition, DMT-7 is also capable of utilizing broad substrates range such as BT, 3,4-benzo DBT, 4,6-dimethyl DBT (4,6-DMDBT), 4,6 dibutyl DBT as the sole source of sulfur. To the best of our knowledge, this is the first report demonstrating the desulfurization potentials of genus *Lysinibacillus.*

1 Materials and methods

1.1 Chemicals and reagents

DBT, 2HBP, BT, 3,4-benzo DBT, 4,6-DMDBT and 4,6-dibutylDBT (> 98% purity) were purchased from Sigma, USA. Tryptone, peptone, beef extracts, bacto-agar, yeast extracts and staining reagents were obtained from HiMedia Laboratory Pvt. Ltd., India. The general chemicals including sulfur-free constituents of basal salt mineral media (BSM) and solvents of high performance liquid chromatography (HPLC) grade were purchased from Glaxo Pvt. Ltd., India and Merck Pvt. Ltd., India.

1.2 Preparation of media

Sulfur free BSM (pH 7.0) was prepared by dissolving 0.38 g KH_2PO_4, 0.6 g K_2HPO_4, 0.2 g $MgCl_2$, 1.0 g NH_4Cl and 0.05 g $FeCl_3$ in one liter Milli-Q double distilled water and autoclaved. Autoclaved nutrient broth contained 5.0 g peptone, 5.0 g NaCl, 3.0 g beef extracts, 3.0 g yeast extracts per liter double distilled water. All the solid media contained 1.5% agar along with BSM or nutrient broth. The stock solution of DBT (100.0 mg/mL) and 2HBP (5.0 mg/mL) were made in methanol and glucose (10.0 mg/mL) in sterile sulfur free, Milli-Q water. The stock solutions were sterilized by Millipore micro syringe filter assembly (0.45 μm pore size).

1.3 Isolation of DBT desulfurizing bacteria

The subsurface diesel-contaminated soil sample was collected from few oil filling stations situated in Rishikesh, Uttarakhand, India. The diesel contaminated soil was suspended (10%, *W*/*V*) in 100.0 mL of BSMG broth (1% glucose) supplemented with DBT (0.5 mmol/L) as the sole source of sulfur and incubated for 7 days at 37°C under constant stirring at 150 r/min in a incubator shaker (Model CIS-24, Remi, India). After incubation, 2.0 mL culture was withdrawn and re-inoculated in freshly prepared DBT (0.2 mmol/L)-BSMG broth (100.0 mL) for 7 days under conditions as mentioned earlier. Afterwards, 1.0 mL of the culture was taken out and serially diluted in sterile BSM up to 10^{-9}. Inoculum of 100.0 μL of each dilution was spread on BSMG agar plate supplemented with DBT (0.2 mmol/L) as the sole source of sulfur and incubated at 37°C for 72 hr. The DBT-BSMG agar plate (10^{-7} dilution) having well spread bacterial colonies, was used for further desulfurization studies. The seven bigger sized bacterial colonies were aseptically removed and reselected on DBT-BSMG agar plate to obtain their pure cultures. Single colony of each isolate was then inoculated in 10.0 mL nutrient broth and grown for 24 hr at 37°C with constant shaking. The cell suspensions were centrifuged at 8000 r/min for 10 min to obtain cell pellets of each soil isolate. The cell pellets were washed three times with BSM to remove the trace of nutrient broth and suspended in BSM. The optical densities of cell suspensions were adjusted to 1.0, approximately equal to the 10^8 cells per mL. The desulfurization ability of each soil isolate was checked by inoculating 0.1 mL of each soil isolate (approximately 10^7 cells) into the 10.0 mL of BSMG broth supplemented with DBT (0.2 mmol/L) as the sole source of sulfur. The cultures were incubated at 37°C for 7 days with constant shaking at 150 r/min. Simultaneously, the two control experiments were also setup, one which lack DBT or any other sulfur source and the second one that lack soil isolates. To check the viability and growth of isolates, 100.0 μL of each grown culture was taken out and diluted serially up to 0–10^{-20}. Each diluted culture 100.0 μL was spread over DBT-BSMG agar plate and incubated at 37°C for 24 hr. The colonies were counted and CFU/mL was determined for each culture. The respective cultures were then extracted twice with ethyl acetate (1:1, *V*/*V*), and re-extracted twice with acidified ethyl acetate (pH 4.0 was adjusted with 0.1 mol/L HCl) to enhance the recovery of acidic metabolites. The ethyl acetate extracts were vacuum dried on rotary evaporator (Model no. 951, Perfit, Ambala, India) and suspended in 1.0 mL of methanol. The methanolic extracts were subjected to HPLC analysis to check the extent of DBT desulfurization and production of 2HBP). The reverse phase HPLC analysis was performed

using the commercial facility provided by Ozone Pharmaceuticals Limited, Bahadurgarh, Haryana, India (HPLC system: Waters, USA, model no. 600 E pump with Photo Diode Array (PDA) detector). In brief, 20.0 μL of the methanolic extracts were injected into C_{18} column (5 μm particle diameters) and eluted with 80% methanol at flow rate of 1.0 mL/min. Peaks were measured at 254 nm and identified by comparing their retention time with that of purified reference standards namely 2HBP and DBT. Accumulation of 2HBP in the extracts were further confirmed by using the commercial gas chromatography-mass spectroscopy (GC-MS) facility provided by Institute of Himalayan Bioresource Technology (CSIR) Palampur, Himanchal Pradesh, India. The bacterial isolate showing 2HBP productions as evident by the HPLC profile and GC-MS analysis was selected as the DBT-desulfurizing bacteria.

1.4 Identification and characterization of the DBT desulfurizing bacterium DMT-7

The DMT-7 was maintained on DBT-BSMG agar plates. The identification and characterization of the DMT-7 was performed on the basis of the cell and colony morphology, growth characteristics, motility, various staining reactions and various biochemical tests as given by Bergey's Manual of Systematic Bacteriology (Claus and Berkeley, 1986). For molecular characterization, the approximately 1.5 kb 16S rRNA gene fragment was PCR amplified (Model Tpersonal, Biometra, Germany) from DMT-7 genomic DNA using the forward primers 5′-ACCACATGCAAGTGCAACG-3′ and reverse primer 5′-ACGGGCGGTGTGTAC-3′ (Lily et al., 2009). The amplified product was purified by agarose gel electrophoresis and cloned into the pGEM-T vector (Promega Scientific, Santa Barbara, California). Both the strands of clone (approximately 1.5 kb 16S rRNA gene fragment) were sequenced using sequencing facility provided by Bangalore Genei, India. The 16S rRNA gene sequence of DMT-7 was compared to those in GenBank using the Blast Alignment Tool and the phylogenetic tree was constructed employing MEGA 3.1 software using neighbor joining method. The DMT-7 16S rRNA sequence was deposited to the NCBI GenBank, European Molecular Biology Laboratory and DNA Data Bank of Japan libraries.

1.5 Characterization of DBT-desulfurizing ability of DMT-7

All the experiments were set up in triplicates and carried out in dark to minimize the photolytic degradation of DBT. A single colony of DMT-7 maintained on DBT-BSMG agar plate was inoculated in 10.0 mL nutrient broth and grown at 37°C with constant stirring till the A_{600} reaches to 1.0 (1×10^8 cells/mL). The cell culture was centrifuged at 8000 r/min for 10 min and washed three times with BSM. The DMT-7 cell number was adjusted to 10^8 cells /mL in BSMG. To study the desulfurization kinetics, 0.1 mL of DMT-7 BSM suspension (10^7 cells) was re-inoculated in various flasks containing 10.0 mL BSMG supplemented with DBT (0.2 mmol/L) as the sole source of sulfur and incubated at 37°C in incubator shaker at 150 r/min for various time periods (0, 6, 12 hr, 1, 2, 3, 4, 5, 6, 7, 10, 15, 21 and 30 days) along with their respective negative controls lacking the DMT-7 inoculum. At various time points (0, 6, 12 hr, 1, 2, 3, 4, 5, 6, 7, 10, 15, 21 and 30 days), first of all, the CFU/mL was checked to ascertain the viability of DMT-7 as mentioned earlier and then the desulfurized products were extracted and subjected to HPLC analysis for the assessment of residual DBT and its desulfurized product, 2HBP as mentioned above.

1.6 Assessment of the inducibility of DBT-desulfurizing pathway of DMT-7

In order to know whether the DBT-desulfurizing pathway in DMT-7 is inducible or not, the BSM suspension culture of DMT-7 (approximately 10^7 cells) were inoculated separately in two different conical flasks having 50.0 mL of BSMG supplemented with DBT (0.2 mmol/L; induced starter culture) and $MgSO_4$ (0.5 mmol/L; non-induced starter culture) respectively as sole sulfur source and grown at 37°C for 7 days in incubator shaker. Afterwards, the induced and non-induced cultures were harvested, thoroughly washed (three times) with sulfur-free BSM and finally suspended in BSM to adjust the cell number up to 10^8 cell/mL. A 0.1 mL each of DMT-7 induced and non-induced starter cultures were inoculated separately in various flasks containing 10.0 mL DBT-BSMG and grown for different time points (0, 3, 6, 12, 24 hr, 2, 3, 4, 5, 7, 10, 12, 15 days) at 37°C under constant stirring. Afterwards, the respective cultures were subjected to HPLC analysis to determine the effect of induction and the extent of DBT desulfurization in induced and non-induced cultures as mentioned previously.

1.7 Growth of the DMT-7 on various substrates as a sulfur source

The ability of the DMT-7-to metabolize BT, 3,4-benzo-DBT, 4,6-DMDBT and 4,6-dibutylDBT was investigated. The DMT-7 colony was inoculated into a set of tubes, each containing BSMG with 0.2 mmol/L of substrates such as BT, 3,4-benzo DBT, 4,6-DMDBT and 4,6-dibutyl DBT or 0.5 mmol/L $MgSO_4$ (positive control). Another set of tubes containing the medium without a sulfur source was used as a negative control. All the tubes were incubated on the shaker at 150 r/min at 37°C. After 3 days of incubation, 0.1 mL aliquots of bacteria were transferred into a new set of tubes and then incubated for another 7 days. The growth of the DMT-7 was monitored by measuring the OD_{600}.

2 Results and discussion

2.1 Isolation and identification of a DBT desulfurizing bacterium

Numerous bacterial colonies were isolated from diesel contaminated soil by employing the standard culture enrichment techniques using DBT as a sole sulfur source. The seven bacterial colonies with bigger size were checked for production of 2HBP from DBT. The HPLC analysis

of the extracts of all the seven isolates showed that only DMT-7 was able to desulfurize DBT into 2HBP, rest of the six isolates were unable to produce 2HBP, although they were able to degrade DBT to satisfy their need for sulfur (Table 1). Since, the production of 2HBP is the indication of selective cleavage of carbon-sulfur bond in DBT as reported earlier (Izumi et al., 1994; Oldfield et al., 1997), the DMT-7 was selected as DBT desulfurizing bacterium and its desulfurization activity was further characterized.

The cell and colony morphology, various staining reactions and biochemical activities revealed DMT-7 as Gram positive fusiform rod, non-spore forming, non-motile, non-acid fast, catalase positive and did not produce acid from carbohydrate fermentation. The DMT-7 was negative for the utilization of citrate and urea, and production of H_2S, indole, and oxidase (Table 2). The molecular characterization and comparative phylogenetic analysis of PCR amplified 16S rRNA gene fragment of DMT-7 employing MEGA 3.1 software using neighbor joining method to those in GenBank confirmed DMT-7 (NCBI GenBank Accession No. GQ496620), as the closest homologue of *Lysinibacillus sphaericus* strain RG1 (NCBI GenBank Accession No. FJ544252). DMT-7 showed 98.7% and 98.3% homology with *Lysinibacillus sphaericus* strain RG1 (NCBI GenBank Accession No. FJ544252) and *Lysinibacillus sphaericus* (NCBI GenBank Accession No. EU855791), respectively. Therefore, DMT-7 was identified as a novel strain *Lysinibacillus sphaericus* DMT-7 (Fig. 1). To the best of our knowledge, this is the first report demonstrating the ability of genus *Lysinibacillus* in DBT desulfurization.

Table 1 Characteristics and capability of soil bacterial isolates to produce 2HBP as a result of desulfurization of DBT in BSMG-DBT after 7 days

Soil isolate	Morphology	DBT degradation	2HBP production
DMT-7	Fusiform rod shaped, Gram positive	Yes	Yes
BMT637(R)	Rod shaped, Gram positive	Yes	No
BMT4i	Rod shaped, Gram positive	Yes	No
BMT5i	Rod shaped, Gram positive	Yes	No
BMT6	Fusiform rod shaped, Gram positive	Yes	No
DMT628	Coccus, Gram positive	Yes	No
BMT137	Rod shaped, Gram positive	Yes	No
DMT137(R1)	Rod shaped, Gram positive	Yes	No
DMT137(R2)	Rod shaped, Gram positive	Yes	No
DMT128	Coccus, Gram positive	Yes	No

2.2 Characterization of DBT-desulfurizing ability of DMT-7

To study the desulfurization kinetics, the DMT-7 was grown in DBT-BSMG broth for different time periods and the reaction products were purified. The production of 2HBP and the residual DBT in above purified extracts were evaluated by HPLC analysis and plotted against incubation times (Fig. 2). The plot evidently showed that the DMT-7 possesses the ability to desulfurize DBT into 2HBP in time dependent manner. The desulfurized product of DBT, i.e., 2HBP appeared first after the lag phase of 24 hr (5.1 μmol/L 2HBP) and continued to increase up to the 15 days (360 hr) (121.21 μmol/L 2HBP), leading to 60% of the DBT desulfurization, afterwards, its production became stationary during further growth up to 30 days (720 hr) (122.5 μmol/L 2HBP). The accumulation of 2HBP as a metabolite of DBT desulfurization was confirmed by GC-MS analysis. On the other hand, with the 200 μmol/L initial concentration, DBT started disappearing after 6 hr (195.0 μmol/L residual DBT) and continued to decrease exponentially up to 30 days (720 hr) (5.0 μmol/L residual DBT, 97% metabolized). The disappearance of DBT and accumulation of 2HBP is nearly exponential up to the 15 days of growth; however, further growth resulted in the much faster degradation of DBT with negligible enhancement in the 2HBP concentration (Fig. 2). This data was further confirmed by the HPLC profile of the desulfurized products of 15 days of growth, which clearly showed 77%

Table 2 Physical and biochemical characteristics of *Lysinibacillus sphaericus* DMT-7

Test	Result	Test	Result
Morphological characterization		**Biochemical tests**	
Shape and arrangement	Fusiform rod, single	Oxidase	Negative
Capsule	Negative	Catalase	Positive
Gram staining	Positive	Nitrate reduction	Negative
Spore staining	Negative	Litmus milk	Negative, alkaline
Motility	Non-motile	Urease	Negative
Acid fast staining	Non acid-fast	H_2S production	Negative
Culture characterization on agar plates		Methyl Red	Negative
Colonies	White	Vogues proskaeur	Negative
Temperature	Optimum	Citrate utilization	Negative
Growth	Abundant	Indole production	Negative
Form	Circular	**Carbohydrate fermentation**	
Margins	Entire	a. Lactose	Negative
Elevation	Raised	b. Mannitol	Negative
Density	Opaque	c. Sucrose	Negative
Growth on broth		d. Glucose	Negative
Surface growth	None	e. Maltose	Negative
Clouding	Heavy	Starch hydrolysis	Negative
Sediment	Abundant	Gelatin hydrolysis	Negative
		Casein hydrolysis	Negative
		Lipid hydrolysis	Negative

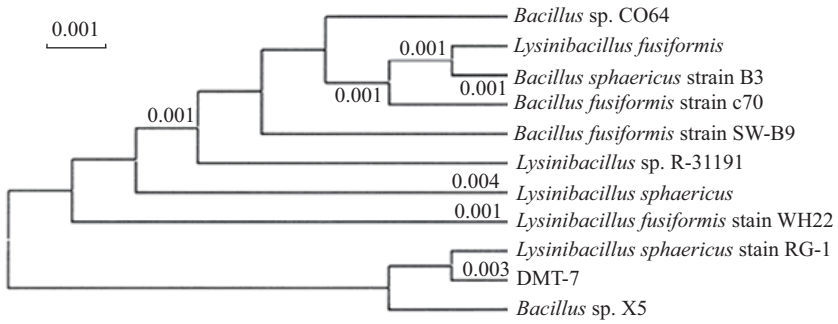

Fig. 1 Phylogenetic tree of strain DMT-7 made in MEGA 3.1 software using neighbor joining method.

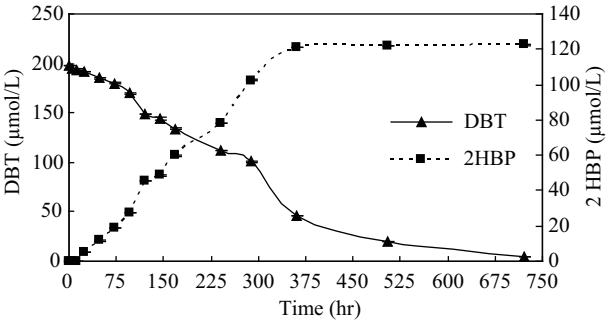

Fig. 2 Desulphurization of DBT to 2HBP by strain DMT-7 with respect to time. Each point represents the average value obtained from triplicate flasks.

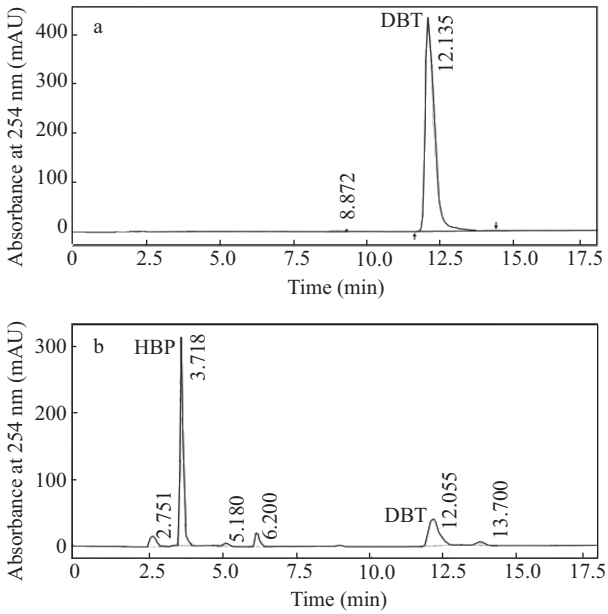

Fig. 3 HPLC profile showing the DBT desulfurization after 15 days of growth. (a) without DMT-7; (b) with DMT-7.

metabolism of DBT, out of which 60% desulfurized to 2HBP as evident by large peak of 2HBP (Fig. 3). It was observed the presence of few small peaks other than 2HBP in the chromatogram which might be an indication of the degradation product of DBT generated by the pathway other than 4S pathway. The growth kinetics of DMT-7 was also studied in the above experiment which revealed an exponential increase in the growth of DMT-7 up to 12 days attaining maxima of 3×10^{20} CFU/mL, thereafter, the viability declined slowly and became almost zero on 30 days of growth. At present it is not clear whether the decrease in the viability of DMT-7 is due to exhaustion of carbon source or due to accumulation of 2HBP at the toxic level. Accumulation of 2HBP above 200 μmol/L was earlier shown to be toxic to the bacterial cells and inhibitory to biodesulfurization (Ohshiro et al., 1996). We have also checked the growth of DMT-7 in the BSMG broth (0.2% $MgSO_4$) containing 2HBP (200 μmol/L) as the source of carbon. Interestingly, the DMT-7 did not show any growth in the 2HBP-BSMG broth instead, the viability was decreased significantly after 24 hr of growth. No growth was observed in the negative control containing DMT-7 inoculum in sulfur free BSM broth.

The time dependent desulfurization of DBT into 2HBP by DMT-7 is in accordance with previous findings showing desulfurization abilities of many bacterial species such as *Rhodococcus erythropolis* IGTS8, *Rhodococcus erythropolis* D-1, *Rhodococcus erythropolis* KA2-5-1, *Mycobacterium* sp. G3, *Rhodococcus* sp. P32C1, *Bacillus subtilis* WU-S2B, *Mycobacterium phlei* WU-F1, *Paenibacillus* sp. All-2 (Kilbane and Bielage, 1990; Oldfield et al., 1997; Li et al., 1996; Ohshiro et al., 1996; Yoshikawa et al., 2002; Okada et al., 2002; Maghasoudi et al., 2001; Kirimura et al., 2001; Furuya et al., 2001; Konishi et al., 1997; Ishii et al., 2000).

2.3 Assessment of the inducibility of DBT-desulfurizing pathway of DMT-7

To check the inducibility of DBT desulfurization pathway in DMT-7, the desulfurization kinetics of induced and non-induced starter cultures were studied at different time points (0, 3, 6, 12, 24 hr, 2, 3, 4, 5, 7, 10, 12, 15 days). The plot of 2HBP production and various incubation time clearly showed the differences in the onset of 2HBP production and the attainment of stationary phase for 2HBP production between the induced and non-induced starter cultures (Fig. 4). In the induced culture of DMT-7, the desulfurization of DBT to 2HBP was detected after the growth of 6 hr (5.2 μmol/L), afterwards, the concentration of 2HBP in the cultures increased linearly up to 107.0 μmol/L on day 5 (120 hr), attaining maximum production of 129.2 μmol/L of 2HBP (65% desulfurization) on day 10 (240 hr) of growth, thereafter, it became stationary. In contrast to the induced culture, the non-induced DMT-7 cultures required prolonged lag phase of 24 hr and 15 days for the accomplishment of maximum DBT desulfurization (60%; 119.5 μmol/L of 2HBP). The shorter lag phase for desulfurization in induced culture is also confirmed

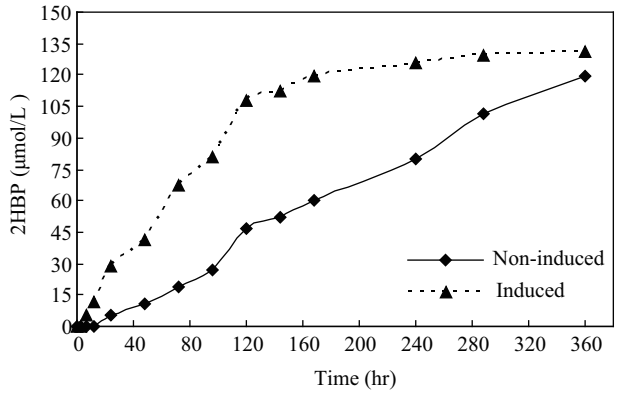

Fig. 4 Desulfurization of DBT to 2HBP by induced and non-induced DMT-7 with respect to time. Each point represents the average value obtained from triplicate flasks.

by the HPLC profile of desulfurized products purified after the 6 hr of growth which evidently showed the presence of 2HBP peak in addition to other smaller peaks (Fig. 5). Therefore, the much shorter desulfurization lag phase and early attainment of stationary phase in induced culture of DMT-7 clearly demonstrated the inducibility of desulfurization pathway in DMT-7. This finding is also supported by previous reports demonstrating the inducibility of desulfurization pathway (Kayser et al., 1993; Ohshiro et al., 1996; Ohshiro and Izumi, 1999).

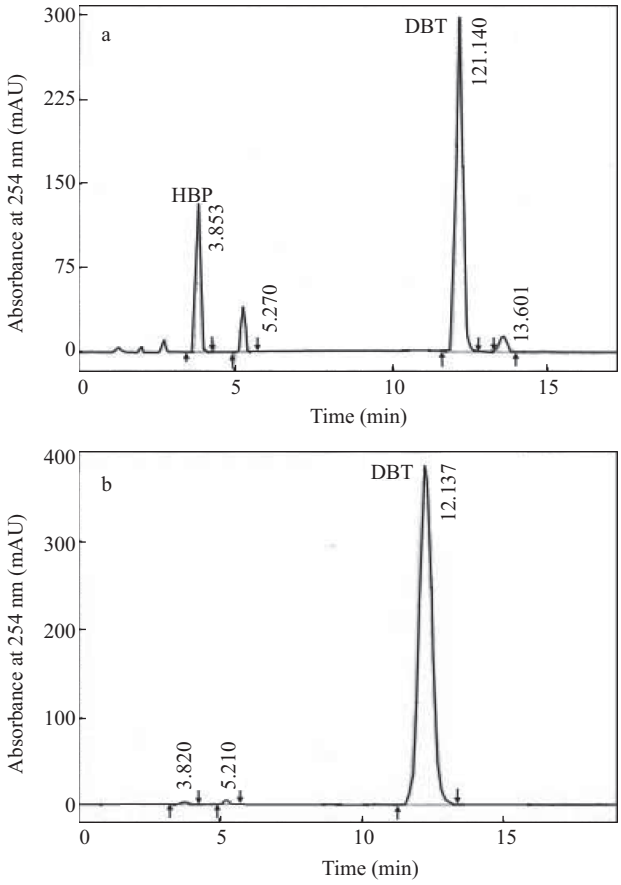

Fig. 5 HPLC profile of the methanolic extracts of DBT desulfurized products after 7 days of DMT-7 growth. (a) induced culture; (b) non-induced culture.

2.4 Growth of the DMT-7 on various organic sulfur compounds

All the organic sulfur compounds listed in Table 3 were examined for their ability to serve as a sole sulfur source for the growth of the DMT-7. The data revealed that BT, 3,4-benzo DBT, 4,6-DMDBT and 4,6-dibutyl DBT were able to support the growth of strain DMT-7, indicating the potential of DMT-7 to desulfurize a wide range of organosulfur compounds.

Table 3 Growth of the DMT-7 on various substrates as a sulfur source

Substrate	Growth (OD_{600}) of DMT-7 after 7 days
BT	1.300 ± 0.090
3,4-benzo DBT	0.911 ± 0.061
4,6-DMDBT	0.822 ± 0.030
4,6-dibutyl DBT	0.817 ± 0.021

3 Conclusions

Present study reports the isolation and characterization of a novel DBT desulfurizing bacteria *Lysinibacillus sphaericus* DMT-7. The results showed that *Lysinibacillus sphaericus* DMT-7 takes 15 days to desulfurize 60% of DBT into 2HBP by an inducible desulfurization pathway. In addition, DMT-7 is able to desulfurize a wide range of organosulfur compounds. Further studies pertaining to optimization of DBT desulfurization activity and characterization of the genes responsible for DBT desulfurization is under investigation. In conclusion, *Lysinibacillus sphaericus* DMT-7 has potential DBT-desulfurization ability and therefore it could serve as model system for efficient biodesulfurization of diesel and petrol.

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