Analysis of the dibenzothiophene metabolic pathway in a newly isolated *Rhodococcus* spp.

Nasrin Akhtar, Muhammad A. Ghauri, Munir A. Anwar & Kalsoom Akhtar

Industrial Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

**Correspondence:** Muhammad A. Ghauri, Industrial Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, PO Box 577, Jhang Road, Faisalabad 38000, Pakistan. Tel.: +92 41 255 0814; fax: +92 41 265 1472; e-mail: maghauri@nibge.org

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**Keywords**

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**Abstract**

Out of 17 samples collected from diverse environments, 110 bacterial isolates of varied characteristics were screened for their dibenzothiophene-desulphurizing activity. A single isolate, Eu-32, originating from a soil sample taken from the roots of a eucalyptus tree, displayed dibenzothiophene-desulphurizing activity. This isolate metabolized dibenzothiophene to 2-hydroxybiphenyl (2-HBP), as detected by HPLC, and was also able to use other organic sulphur compounds as a sole sulphur source. Based on morphological, biochemical and molecular studies, it was found that the organism belongs to the genus *Rhodococcus*, with a maximum of 95% identity to species in this genus for the partial sequence of the 16S rRNA gene. Isolate Eu-32 could desulphurize 0.2 mM dibenzothiophene to 2-HBP in 72 h at a temperature of 30 °C and pH 7.0. The structure and molecular mass of metabolites produced from dibenzothiophene desulphurization were identified by GC-MS, and two sulphur-free products, 2-HBP and biphenyl, were detected in ethyl acetate extract. It was concluded that isolate Eu-32 is a unique desulphurizing biocatalyst that desulphurizes dibenzothiophene through an extended, sulphur-specific degradation pathway with the selective cleavage of C–S bonds.

**Introduction**

Dibenzothiophene has been widely used as a model compound of the polycyclic organic sulphur components of fossil fuels (Calzada *et al.*, 2009). Organic sulphur is characterized by covalent C–S bonds and can be regarded as an element that is integrated into the macromolecular matrix of coal and in organic sulphur compounds present in oil (Klein *et al.*, 1994). The combustion of fossil fuels releases vast amounts of sulphur oxides into the atmosphere and causes serious environmental problems as acid rain (Olmo *et al.*, 2005). To decrease sulphur oxide emissions into the environment, the reduction of the sulphur content in fossil fuels is usually carried out by hydrodesulphurization, which uses high temperature and pressure (Izumi *et al.*, 1994). Various types of sulphur compounds can be removed by hydrodesulphurization, but a number of heterocyclic sulphur compounds are not (Soleimani *et al.*, 2007). Dibenzothiophene is one such recalcitrant organosulphur compound (Kirimura *et al.*, 2002). Biocatalytic desulphurization may offer an attractive alternative due to the mild operating conditions, low costs and greater reaction specificity (Li *et al.*, 2005).

Several aerobic bacteria, such as *Rhodococcus erythropolis* (Gallagher *et al.*, 1993; Izumi *et al.*, 1994; Lee *et al.*, 1995; Ohshiro *et al.*, 1996; Denis-Larose *et al.*, 1997), *Gordona* sp. (Rhee *et al.*, 1998), *Sphingomonas* sp. (Darzins & Mrachko, 2000), *Corynebacterium* sp. (Omori *et al.*, 1992), *Mycobacterium* sp. (Nekozuka *et al.*, 1997; Furuya *et al.*, 2001), *Bacillus subtilis* (Kirimura *et al.*, 2001) *Nocardia* sp. (Rhee *et al.*, 1998) and *Paenibacillus* sp. (Konishi *et al.*, 1997), have been reported to be able to desulphurize dibenzothiophene via a sulphur-specific pathway. Microorganisms utilize different mechanisms for the removal of sulphur from dibenzothiophene (Gupta *et al.*, 2005). Either dibenzothiophene is degraded by cleavage of the C–C bond (Van-Afferden *et al.*, 1990) or a sulphur-specific cleavage of the C–S bond (known as the 4S pathway: Fig. 1) may take place (Gallagher *et al.*, 1993). The latter may be preferable for desulphurization because the carbon skeleton remains intact and the calorific value of the fuel remains conserved (Rashtchi *et al.*, 2009).
In the 4S pathway, dibenzothiophene is oxidized to dibenzothiophene sulphone (DBTO₂) via the formation of dibenzothiophene sulphoxide (DBTO). Then DBTO₂ is transformed to hydroxyphenyl benzene sulphonate (HPBS), and finally this is converted into 2-hydroxybiphenyl (2-HBP) and sulphite (Gallagher et al., 1993; Monticello, 2000).

In this paper, we report a dibenzothiophene-desulphurizing bacterium isolated from a soil sample taken from the roots of a eucalyptus tree, tentatively identified as a Rhodococcus spp. and designated strain Eu-32. We analysed the metabolites produced from dibenzothiophene desulphurization using HPLC and GC-MS techniques. Additionally, the ability of the isolate to use organic sulphur compounds, other than dibenzothiophene, as a sole sulphur source was examined.

**Materials and methods**

**Isolation and cultivation of dibenzothiophene-desulphurizing isolates**

Seventeen different types of samples of soil (five), sea sand (five), liquid (five) and oil sludge (two) were collected for target microbial isolation. Isolation and cultivation were performed using MG medium (Kirimura et al., 2002), containing 5.0 g glucose, 2.0 g KH₂PO₄, 4.0 g K₂HPO₄, 1.0 g NH₄Cl, 0.2 g MgCl₂·6H₂O, 10.0 mL metal solution and 1.0 mL vitamin mixture in 1000 mL distilled water (pH 7.0). The medium was supplemented with 0.54 mM (100 µg mL⁻¹) of dibenzothiophene dissolved in ethanol. Sample aliquots (1 g or 1 mL) were inoculated in liquid MG medium and incubated with shaking at 30 °C for 3–5 days. Then aliquots of turbid cultures were transferred into fresh MG medium. After five subcultivations, the culture broth was appropriately diluted with distilled water and spread onto Luria–Bertani (LB) medium agar plates. Single colonies formed on these plates were again inoculated into liquid MG medium with dibenzothiophene. Enriched cultures were stored in microtubes (1.5 mL capacity) containing 50% v/v glycerol at −20 °C and in slants containing LB/MG medium at 4 °C until required.

**Estimation of bacterial growth and the sulphur content of media**

Bacterial growth was estimated spectrophotometrically using a UV/VIS Spectrometer model T-80 (PG Instruments, UK) by noting OD₆₆₀ nm at regular time intervals. The sulphate sulphur contents were determined using a turbidimetric method (Dodgson, 1961).

**Gibb’s assay for 2-HBP**

Gibb’s assay is a colorimetric method, and was used to detect and quantify accumulated phenolic compounds (2-HBP) in the culture medium using Gibb’s reagent (2,6-dichloroquine-4-chloroimide). The reagent reacts with the aromatic hydroxyl group of 2-HBP to form a blue complex that can be monitored spectrophotometrically. A 150-µL aliquot of medium was removed from a growing culture and mixed with 30 µL of 1 M NaHCO₃ (pH 8). Twenty microlitres of Gibb’s reagent (1 mg mL⁻¹ in ethanol solution) was then added, and the reaction mixture was agitated at room temperature for 15–45 min for full colour development. The absorbance of the reaction mixture was determined at 595 nm and was compared with a 2-HBP-generated standard curve (Konishi et al., 1997).
Analytical methods based on HPLC and GC-MS

Dibenzothiophene and hydroxybiphenyl were quantified using HPLC (Varian, Australia) equipped with a HyperSil C18 column (Thermo Hypersil-Keystone, UK) at 245 nm using standard calibration curves. The mobile phase for HPLC was 60% acetonitrile–water and the flow rate was 1.0 mL min⁻¹. The culture broth (5 mL) was acidified to pH 2.2 with 6 M HCl and extracted with 4 mL of ethyl acetate. The extract was filtered through a 0.2-µm pore size polytetrafluoroethylene membrane filter for HPLC analysis. A portion of the ethyl acetate extract was also used for GC-MS (TRACE GC ULTRA, Thermo Electron Corp.) analysis in full scan and in selective ion mass (SIM) modes using a TR-5 column (0.25 mm i.d. × 30 m length; 5% phenyl polysiloxane). The interpretation of each spectrum was performed by a NIST mass spectral search program (version 2.0d). For GC-MS analysis, samples were concentrated under nitrogen gas before injection.

Phylogenetic analysis of isolate

Genomic DNA was isolated from 15 mL of liquid bacterial culture cultivated in MG medium supplemented with 0.54 mM dibenzothiophene (harvested during the log phase) using the cetyl trimethylammonium bromide method (Ausbuel et al., 1995) and stored at −20 °C until required. PCR amplification of the 16S rRNA gene of isolate Eu-32 was carried out using primers FD1 (AGAGTTT GATCCTGGCTCAG) and rP1 (ACGG[ACT]TACCTTGT TACGACTTT) under the reaction conditions described previously (Akhtar et al., 2008).

The amplified 16S RNA gene fragment of about 1500 bp size was partially sequenced commercially (Macrogen, South Korea). The gene sequences were compared with others in the GenBank databases using the NCBI BLAST (http://www.ncbi.nlm.nih.gov). 16S rRNA gene sequences of selected organisms were obtained from GenBank and aligned with the partial sequence of our isolate using CLUSTALX (Thompson et al., 1997). The aligned sequences were used to construct a distance matrix, after the generation of 100 bootstrap sets, which was subsequently used to construct a phylogenetic tree, by neighbour-joining method, using TRECON software (Akhtar et al., 2008).

Nucleotide sequence accession numbers

The partial 16S rRNA gene sequence for isolate Eu-32 (492 bp) is available in the databases under accession number DQ386111. The accession numbers (in parentheses) of the other 16S rRNA gene sequences used for producing the phylogenetic tree are R. erythreus (X79289); Rhodococcus globulurus (X80619); Rhodococcus marinonascens (X80617); Rhodococcus tukiasuensis (AB067734); Rhodococcus maanshanensis (AF416566) and Escherichia coli (EU130557).

Chemicals

Dibenzothiophene was purchased from Aldrich (Milwaukee). Hydroxybiphenyl and 2,6-dichloroquinone-4-chloroimide were purchased from MP Biomedicals Inc. (France). The model organic sulphur-containing compounds were from Acros Organics.

Results and discussion

Isolation and characterization of the isolate

Out of 17 samples screened for the isolation of organic sulphur-metabolizing bacteria, only one sample, taken from the roots of a eucalyptus tree, showed positive Gibb’s results (indicative of sulphur metabolism) in liquid MG medium having dibenzothiophene as the sole sulphur source. A large number of isolates, on the basis of colony characteristics, were isolated from this enriched (five subcultivations) sample including isolate Eu-32, which was found to use dibenzothiophene as a sole source of sulphur, converting it to 2-HBP as determined by Gibb’s assay and confirmed by HPLC.

The requirement of sulphur by microorganisms is very low in absolute terms (Kilbane, 1990). The natural abundance and the ubiquity of inorganic sulphur, which is the preferred form of sulphur for microorganisms, make it unnecessary for an organism to utilize organic sulphur to meet its growth needs. Therefore, the probability of isolating microorganisms from nature having the ability to utilize organic sulphur is fairly remote (Kilbane, 1990). This could be a possible reason why, out of 110 isolates, only one isolate was found to convert dibenzothiophene to 2-HBP.

By phase-contrast microscopy (Zeiss Axiovert, MC80, Germany), the isolate Eu-32 appeared to be rod–coccus in shape (size ~1–1.5 μm). It formed orange yellow colonies on LB media plates with both a smooth and a rough appearance and was catalase positive and oxidase negative. According to nucleotide BLAST (NCBI, http://www.ncbi.nlm.nih.gov) results for the partial 16S rRNA gene sequence (492 bases, the first nucleotide corresponded to E. coli 16S rRNA gene at position 04), the isolate resembled those in the genus Rhodococcus. The top hit and highest score was obtained with Rhodococcus sp. strain 40 (accession no. AJ002093), with 95% sequence identity to the sequence of isolate Eu-32 (Table 1). Of isolates available in the databases that have been identified to the species level, Eu-32 showed the highest levels of identity to R. fascians and R. yunnanensis. The gaps in BLASTN results were 2% in the case of R. fascians, while they
were 3% in the case of *R. yunnanensis* (Table 1). The percentage identity values for the 16S rRNA gene sequence between isolate Eu-32 and other organic sulphur-metabolizing species with validly published names were < 95% (range 94–91%). To determine the phylogenetic position of isolate Eu-32, the partial 16S rRNA gene sequence was compared with type strains of *Rhodococcus* species retrieved from the GenBank. The tree was rooted with the 16S rRNA gene sequence of *E. coli* as an outgroup. It is evident from the phylogenetic tree (Fig. 2) that isolate Eu-32 and *R. yunnanensis* formed a separate phylogenetic branch along with *R. fascians* and *R. luteus*, a grouping well supported by bootstrapping. Bacteria with 16S rRNA gene similarities of 97.5% and above may be the same species (Stackebrandt & Goebel, 1994). Where there is doubt, DNA–DNA reassociation percentage values can be obtained; a homology value of 70% is deemed to be the minimum for species identity (Wayne et al., 1987). For isolate Eu-32, although it forms a separate clade with *R. yunnanensis* (Fig. 2), the 16S rRNA gene sequence identity is < 97% and may indicate that isolate Eu-32 is a species different from *R. yunnanensis*. For this reason, we have tentatively named it *Rhodococcus* spp. strain Eu-32.

### Table 1. Percentage sequence identity of the partial 16S rRNA gene sequence of isolate Eu-32 with related taxa

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Strain</th>
<th>% Identities</th>
<th>Gaps (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJ002093</td>
<td><em>Rhodococcus</em> sp. Strain 40</td>
<td>95</td>
<td>2</td>
</tr>
<tr>
<td>AB010913</td>
<td><em>Rhodococcus</em> sp. SRB1948-Z40</td>
<td>95</td>
<td>2</td>
</tr>
<tr>
<td>AB180236</td>
<td><em>Rhodococcus</em> fascians</td>
<td>95</td>
<td>2</td>
</tr>
<tr>
<td>DQ985073</td>
<td><em>Rhodococcus</em> yunnanensis</td>
<td>95</td>
<td>3</td>
</tr>
<tr>
<td>AV602219</td>
<td><em>Rhodococcus</em> yunnanensis</td>
<td>95</td>
<td>3</td>
</tr>
<tr>
<td>AF235011</td>
<td><em>Rhodococcus</em> erythropolis</td>
<td>94</td>
<td>4</td>
</tr>
<tr>
<td>AB269261</td>
<td><em>Rhodococcus</em> erythropolis</td>
<td>94</td>
<td>4</td>
</tr>
<tr>
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<td><em>Rhodococcus</em> leuteus, isolate 7Y</td>
<td>94</td>
<td>3</td>
</tr>
<tr>
<td>AJ576249</td>
<td><em>Rhodococcus</em> yunnanensis</td>
<td>94</td>
<td>3</td>
</tr>
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**Fig. 2.** The inferred relationship, based on the partial 16S rRNA gene sequence, of the organic sulphur-metabolizing isolate Eu-32 obtained in this study (in bold) to other bacteria. The tree was rooted with *Escherichia coli*. Scale bar represents the number of inferred nucleotide substitutions per site. Bootstrap values (100 replicates) are shown at the nodes.

**Fig. 3.** Time course of 2-HBP production and dibenzothiophene utilization by growing cells of isolate Eu-32. The isolate was cultivated in MG medium with 0.25 mM dibenzothiophene as the sole source of sulphur.

Desulphurization of dibenzothiophene by growing cells of Eu-32

Preliminary optimization of growth-affecting parameters was carried out at various pH values (4–10) and temperatures (25, 30, 45 °C) (data not shown), which showed isolate Eu-32 to be a neutrophilic mesophile. This isolate displayed a wide pH range for dibenzothiophene desulphurization capacity from 5.0 to 9.0 with an optimum at pH 7.0. Growth yields were reduced away from the optimum pH. The effect of inoculum size on desulphurization of dibenzothiophene was investigated at various cell densities from 2 to 10 g L⁻¹ (with a gap of 2 at each step). The rate of desulphurization was the highest at an inoculum size of 4 g L⁻¹, but it decreased with increasing cell density. This may indicate that oxygen could be a limiting factor for dibenzothiophene degradation by the growing cells. Mohebali et al. (2007) reported that increasing cell concentrations led to a decrease in dibenzothiophene degradation by the formation of cellular flocs, due to the hydrophobic nature of desulphurizing bacteria. The same phenomenon may have been taking place in the case of Eu-32 in that the formation of cellular colloidal material at high cell mass concentrations could ensnare the biodesulphurization activity by lowering the mass transfer of dibenzothiophene and dissolved oxygen availability to the growing bacterial aggregates.

Isolate Eu-32 desulphurized 0.2 mM dibenzothiophene to 2-HBP in 72 h at 30 °C and pH 7.0 (Fig. 3). It is evident that desulphurization of dibenzothiophene and production of 2-HBP were growth dependent and proceeded concomitantly.
with an increase in biomass. The pH decreased as the biodesulphurization proceeded, which may be due to the accumulation of sulphate ions/sulphonic acid in the medium (Fig. 3).

The consecutive oxidation of dibenzothiophene sulphur by bacteria resulted in the release of sulphur, either in the form of sulphite or sulphate (Setti et al., 1999). In the current study, the detected form of sulphur in the culture supernatant consisted only of sulphate. The amount of sulphate detected was small and not stoichiometric to the amount of dibenzothiophene converted to 2-HBP (data not shown). This might be due to the sulfate released being consumed by the bacteria to satisfy their growth requirements and any remaining sulphur being stored by the organisms for future use (Kilbane, 1990). Consequently, very little sulphate sulphur (the released form of sulphur from dibenzothiophene) would be left to be released in the media.

**Metabolic pathway and characteristics of dibenzothiophene desulphurization**

The metabolism of dibenzothiophene by aerobic microorganisms has generally been classified into three types (Gupta et al., 2005): (I) the carbon skeleton of dibenzothiophene is partially oxidized while the C–S bond remains intact (Kodama et al., 1970); (II) dibenzothiophene serves as a source of carbon, sulphur and energy (Van-Afferden et al., 1990); and (III) dibenzothiophene serves as a sole source of sulphur. Moreover, in type III metabolism, dibenzothiophene is desulphurized by the selective cleavage of C–S bond, resulting in the accumulation of 2-HBP (Gallagher et al., 1993; Izumi et al., 1994).

Our study indicated that dibenzothiophene degradation by isolate Eu-32 may belong to type III metabolism, in which C–C bonds remain protected, with 2-HBP as the end product. The conversion of dibenzothiophene to 2-HBP was monitored using chromatographic techniques (HPLC and GC). Analysis of the culture extracts (by HPLC) indicated the presence of dibenzothiophene, DBTO₂ and 2-HBP when compared with chromatographs from the standards. The total GC chromatogram (in selective ion mode) of the ethyl acetate extract from Eu-32 cultures grown on dibenzothiophene as the sole source of sulphur showed the presence of three main peaks with retention times (Rₚ) 15.27, 11.56 and 8.86 min (Fig. 4a). Further analysis (GC-MS) confirmed the presence of 2-HBP in the culture extracts [m/z = 170; Fig. 4c(iii)]. In addition, DBTO₂ was also detected by GC-MS analysis [m/z = 216; Fig. 4c(ii)]. Other than the above-mentioned metabolites of the 4S pathway, DBTO and HPBS were also detected by GC-MS analysis, although their peaks were not very prominent in comparison with the other metabolites.

In another critical experiment, isolate Eu-32 was grown in MG-medium having DBTO₂ and it was found that this isolate could utilize DBTO₂, instead of dibenzothiophene, as the sole source of sulphur, which was confirmed by the presence of 2-HBP in the medium. This result further confirms that DBTO₂ is one of the intermediates in dibenzothiophene metabolism. Therefore, the dibenzothiophene-desulphurizing pathway was found to be identical to the well-documented 4S pathway up to the formation of 2-HBP (Omori et al., 1992; Gallagher et al., 1993; Izumi et al., 1994; Lee et al., 1995; Ohshiro et al., 1996; Denis-Larose et al., 1997; Konishi et al., 1997; Nekozuka et al., 1997; Rhee et al., 1998; Darzins & Mrachko, 2000; Furuya et al., 2001; Kirimura et al., 2001).

**New proposed extended pathway for dibenzothiophene desulphurization**

The mass balance between dibenzothiophene and 2-HBP was not consistent. It is possible that either all the dibenzothiophene was not metabolized by the target microorganisms or some metabolites other than 2-HBP might have been produced in the culture medium. To test this hypothesis, the culture extract was further analysed by GC-MS (in full-scan mode) for the presence of any other metabolites, which had not earlier been documented in the 4S pathway. During this analysis, a further product was detected in the broth, which was identified as biphenyl with mass ions at m/z 154 [Fig. 4c(iv)] and a retention time of 7.07 min (Fig. 4b). This shows that 2-HBP may be further biotransformed to biphenyl by the removal of an OH group. For further confirmation, 2-HBP was used as the sole sulphur source in MG medium. Isolate Eu-32 showed poor growth under these conditions and very little transformation of 2-HBP to biphenyl was observed. When the concentration of 2-HBP was > 0.4 mM, the growth was completely inhibited. Moreover, to assess the effect of high transfer line temperature in GC-MS analysis on the auto conversion of 2-HBP to biphenyl, the authentic standard of 2-HBP was analysed carefully on GC and it only showed the peak for 2-HBP. This confirmed that high temperature does not result in auto formation of biphenyl with the removal of the OH group from the 2-HBP. These results imply that biphenyl may be directly formed from HPBS, and biphenyl could be an additional product of dibenzothiophene desulphurization. Moreover, the mass balance between dibenzothiophene and 2-HBP was almost consistent when biphenyl was also taken into account. It is reported that 2-HBP (the final product of dibenzothiophene) may add to environmental pollution (Ichinose et al., 1999; Chen et al., 2009), but that the formation of biphenyl may partially eliminate the toxic effects of 2-HBP, and consequently lower the polluting impact on the environment. The production of
two extra sulphur-free metabolites, 2-methoxybiphenyl (2-MBP) and biphenyl, in addition to 2-HBP, has been documented by Li et al. (2005) when they targeted dibenzothiophene using Microbacterium strain ZD-M2, while the production of only 2-MBP other than 2-HBP has been reported by Li et al. (2007), Okada et al. (2002) and Chen et al. (2009) using Mycobacterium goodii X7B, Mycobacterium strain G3 and Mycobacterium sp., respectively. However, using Rhodococcus species, this could be the first observation where biphenyl has been formed as another end product of dibenzothiophene desulphurization. Therefore, it can be concluded that isolate Eu-32 desulphurized dibenzothiophene through specific cleavage of the C–S bonds (Fig. 1) and the production of biphenyl along with 2-HBP, indicating that it is a novel organism utilizing a pathway for dibenzothiophene degradation distinct from earlier reports.

Fig. 4. GC chromatograms of the metabolites of dibenzothiophene desulphurization produced by isolate Eu-32. (a) In SIM mode: DBTO$_2$ (15.27 min), dibenzothiophene (11.56 min) and 2-HBP (8.86 min). (b) In full-scan mode: biphenyl (7.07 min), 2-HBP (8.67 min), dibenzothiophene (11.39 min), DBTO$_2$ (14.08 min). (c) GC-MS analysis of desulphurized dibenzothiophene metabolites produced by isolate Eu-32. (c) Dibenzo[b]thiophene (molecular mass 184); (ii) DBTO$_2$ (molecular mass 216); (iii) 2-HBP (molecular mass 170); and (iv) biphenyl (molecular mass, 154). [Note: For GC-MS analysis, the interpretation of each spectrum was performed using the NIST mass spectral search program (version 2.0d). The x-axis of GC-MS chromatograms represents the increasing m/z ratio. The y-axis represents the relative abundance of each ion, which is related to the number of times an ion of that m/z ratio strikes the detector. Assignment of relative abundance begins by assigning the most abundant ion a relative abundance of 100%.]

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Growth specificity of alternate sulphur sources

Many types of organic sulphur-containing compounds can be detected in fossil fuels and are difficult to remove using the conventional hydrodesulphurization treatment (Kirimura et al., 2001). In order to assess the ability of isolate Eu-32 to use sources of sulphur other than dibenzothiophene, the MG medium was supplemented with 0.25 mM of various sulphur compounds as the sole source of sulphur for 6 days (Fig. 5). The isolate produced rich growth (measured by taking OD660 nm) on all the sulphur sources used. However, the growth on thiophene 3-carboxylic acid, p-toluene sulphonic acid and sulphur (25 mg/100 mL) was insignificant in comparison with the other sulphur compounds (Fig. 5). The affinity of the isolate Eu-32 for varied sulphur sources makes it a potentially useful strain for use in the desulphurization of petroleum and its products. Moreover, it may be useful for desulphurization of other dibenzothiophene derivatives, such as alkyl-substituted dibenzothiophenes, which also occur in fossil fuels (Li et al., 2005).

This is the first report of the isolation of organic sulphur-metabolizing bacteria from the soil collected from the roots of a eucalyptus tree. This finding extends our knowledge of the diversity and prevalence of such bacterial types in such environments. In addition to 2-HBP, the formation of biphenyl in the 4S pathway indicates that this is a novel organism following a novel dibenzothiophene desulphurization pathway, in comparison with other biodesulphurizing Rhodococcus species. It is anticipated that due to the wide range of sulphur compounds it is capable of metabolizing, isolate Eu-32 may be a useful desulphurizing biocatalyst for the practical biodesulphurization of fossil fuels.

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