Thermophilic bacteria from Mexican thermal environments: isolation and potential applications

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(Received 30 December 2009; Accepted 6 March 2010)

Extremophiles are microorganisms that possess application possibilities in several industrial fields, including agricultural, chemical, laundry, pharmaceutical, food, petroleum and bioremediation. This work reports the isolation of 19 thermophilic, alkalitolerant and halotolerant bacterial strains from two thermal sites in Veracruz, México: El Carrizal thermal pool and Los Baños hot spring. These strains belong to the Geobacillus, Anoxybacillus and Aeribacillus genera. The strains produce lipases, proteases, and amylases under thermophilic conditions. They may have good potential for application in microbial enhanced oil recovery, since they are thermophilic and halotolerant, produce exopolymers (up to 11.8 mg/mg) and acids, show emulsifying activity (E24 up to 7.5%), and are able to grow in kerosene as carbon source; these strains may also be used in biodesulphurization since they can grow in dibenzothiophene as carbon source; these strains may also be used in biodesulphurization since they can grow in dibenzothiophene producing 2-hydroxybiphenyl under thermophilic conditions (up to 2.9 mg/L).

Keywords: thermophiles; extremoenzymes; microbial enhanced oil recovery; biodesulphurization

Introduction

Extremophiles are a group of microorganisms that thrive in environments previously thought to be hostile to life [1]. These environments may include extremes in physical parameters (temperature, radiation, pressure), geochemical parameters (desiccation, salinity, pH, oxygen species or redox potential), or even biological extremes (nutritional extremes, population density, parasites, prey) [2]. Discovery and research on extremophiles and their enzymes have provided invaluable data and application possibilities in molecular and evolutionary biology, and occupy an important place in the environmental biotechnology industry. Applications span agricultural, biomedical and industrial sectors such as food, laundry, pharmaceutical, petroleum and bioremediation [3–5]. Thermophiles, microorganisms that grow at temperatures greater than 45°C, are among the best studied extremophiles. They have been isolated from hot springs, solfataras, geothermally heated soils, oil reservoirs, and from some mesobiotic environments like soils, composting vegetation, river-, lake-, and sea water [1,2,6]. The enzymes produced by these microorganisms are extremely thermostable and usually resistant to chemical denaturants such as detergents, chaotropic agents, organic solvents and extremes of pH [7]. Performing biotechnological processes at elevated temperature has many advantages: the elevation of temperature is accompanied by a reduced risk of contamination, a decreased viscosity and an increased diffusion coefficient of organic compounds, hence the bioavailability, solubility of organic compounds and reaction rates are improved [7,8]. As a consequence, there is a continuous interest in isolating and characterizing thermophilic bacteria and their enzymes in order to increase the possibilities for their industrial application.

Of great potential could be the application of thermophiles in the petroleum industry, since the bioavailability of less soluble hydrophobic substrates such as polyaromatic and aliphatic hydrocarbons could also be improved dramatically at elevated temperatures. In that respect two processes have attracted attention recently: the microbial removal of sulphur from petroleum fractions, called biodesulphurization (BDS), and the use of microbes down oil wells in order to enhance oil production after primary and secondary recovery procedures (Microbial Enhanced Oil Recovery, MEOR). Although
mesophilic microorganisms have been used in both processes, the application of thermophiles will provide crucial advantages due to the increase in bioavailability and reaction rates, and because thermophiles could survive the harsh environment of the oil reservoir [9]. Nowadays the extremophiles’ research has resulted in the isolation of thermophilic bacteria from a great variety of terrestrial and hot-water environments. In particular, aquatic ecosystems possess an enormous microbial biodiversity which can be explored in order to isolate and discover new microorganisms and biocatalysts [10]. In this work we report the isolation and characterization of thermophilic bacteria from two Mexican geothermal locations in the state of Veracruz, México, and the analysis of some of their potential biotechnological applications.

Materials and methods

Sampling sites

Samples were obtained from the thermal pool ‘El Carrizal’ and from the hot spring ‘Los Baños’. Both sites belong to the hydrothermal system formed by the CTVR in the central part of the state of Veracruz, México, situated in the eastern Trans-Mexican Volcanic Belt, a continental mostly calc-alkaline province of Quaternary age [11]. El Carrizal thermal pool is located at Northern latitude 19°19’00” Western longitude 96°38’40”, 250 m above mean sea level (msl), next to the river Pescados-La Antigua. Los Baños hot spring is located near to the river Actopan, Northern latitude 19°37’42” Western longitude 96°27’28”, 78 m above msl.

Sampling

Soil and water samples (250 mL) mixed with biofilms deposited in submerged rocks were collected in sterile flasks and immediately transported to the lab for enrichment and cultivation in Luria Bertani (LB) medium at pH 6.0° C, 70°C and 150 rpm. El Carrizal water composition was analysed by the National Commission (CNA), México; Los Baños water was analysed at the Geosciences Center (Juriquilla, Qro.) from the National Autonomous University of México (UNAM) (Table 1). and incubated at 60°C for 18 h. Individual colonies were isolated, and streaked onto the agar medium until single, uniform colonies were obtained. The pure isolates were ultimately grown in LB medium until a DO_500nm 0.6–0.8 was reached, and 80% (% v/v) glycerol stocks were prepared and stored at −70°C.

Characterization of the isolates

Isolates were characterized with respect to cell and colonial morphology, Gram reaction, metabolic products (Microscan kit), spore formation, and the effect of salinity, pH and temperature on growth. Temperature and pH growth limits of each strain were established by performing experiments in the range 25–75°C and pH 5–11. Then, optimum temperatures and pHs were found using a 2³ factorial design (T= 40, 55 and 70°C, pH = 7, 7.5 and 8) analysed by the modified Gompertz Bacterium model [13]. Media pHSs were adjusted at the incubation temperature. The influence of salinity on growth was determined by growing cells on LB medium pH 6.0°C 7 containing 0, 0.3, 0.5, 1, 1.5, and 2 M NaCl, at 60°C, 150 rpm for 72 h. Cell growth was determined by optical density at 670 nm. Growth on a single carbon source was tested on a liquid medium containing: carbohydrate 0.5 % (v/v), yeast extract 5 g/L and NaCl 5 g/L, pH 6.5. The different carbon sources were: D-glucose, D-mannose, D-ribose, D-xylene, lactose, mannitol, sucrose, and ramnose [14]. All growth tests were done at 55°C for 48 h.

16S rRNA sequencing and phylogenetic analysis

Phylogenetic analysis was based on 16S rRNA sequencing [15]. The sequences were aligned with those

| Compound | Concentration (mg/L) |
|----------|----------------------|
| El Carrizal | Los Baños |
| CaCO₃ | 232.2 | 40.6 |
| CaSO₄ | 502 | nd |
| MgSO₄ | 325.6 | 379.01 |
| Na₂SO₄ | 66 | 23.4 |
| LiCl | 9.16 | 1.12 |
| KCl | 8.2 | 8.32 |
| NaCl | 61.8 | 36.8 |
| Na₂B₄O₇ | 12.2 | 283.6 |
| SiO₂ | 39.4 | nd |
| Al₂O₃ | 2.4 | nd |
| Parameter | | |
| Temperature (°C) | 39 | 58 |
| pH | 7 | 6 |

*Analysed by the Water National Commission (CNA), México.
**Analysed by Geosciences Center (Juriquilla, Qro.) from the National Autonomous University of México (UNAM).
Screening for enzyme activity

Isolates were evaluated for lipolytic, proteolytic and amylolytic activity. Isolates were screened for lipase activity on agar plates containing Rhodamine B 0.001% (w/v), nutrient broth 0.8% (w/v), NaCl 0.4% (w/v), agar 1% (w/v), and olive oil 3%, in distilled water, pH 5.5 ± 0.5 [17]. Plates were incubated at 55°C for 18 h, and lipase production was identified as an orange halo around colonies under UV light at 350 nm. Screening for proteolytic activity was performed by plating the isolates on an agar medium containing casein 1% (w/v), glucose 1% (w/v), KH₂PO₄ 1 g/L, MgSO₄ 0.2 g/L, pH 5.5 ± 0.5 at 55°C for 24 h. Formation of a clear halo around the colony was an indication of proteolytic activity. Screening for starch hydrolysis activity was performed by plating the isolates on an agar medium containing beef extract 3 g/L and soluble starch 10 g/L, pH 5.5 ± 0.5 and incubating at 55°C for 24 h. Staining of the plates with iodine reagent was carried out to reveal clear halos of starch hydrolysis [18].

Screening for microbial enhanced oil recovery (MEOR) activities

Isolates were evaluated for oil recovery potential with respect to their capability to produce surfactants, gases, acids, and exopolymers.

Growth on kerosene and emulsifying activity

Isolates were grown in the following medium containing kerosene as carbon source [19]: Na₂HPO₄ (2.2 g/L); KH₂PO₄ (1.4 g/L); MgSO₄.7H₂O (0.6 g/L); (NH₄)₂SO₄ (3.0 g/L); yeast extract (1.0 g/L); NaCl (0.05 g/L); CaCl₂ (0.02 g/L); FeSO₄.7H₂O (0.01 g/L); kerosene (20 ml/L); pH 5.5 ± 0.5, at 55°C. Growth was monitored by optical density. Emulsifying activity was assessed by the emulsification index (E-24) measurement [20] as follows: 2 mL of cell-free growth medium (or 2 mL of non-cultivated medium as negative control) were added to 3 mL of kerosene and vortexed at high speed for 2 min. After 24 h, emulsion index (E-24) was calculated as the height of the emulsion layer, divided by the total height, multiplied by 100.

Production of gases and acids

Isolates were grown on Durham tubes containing LB medium with 2% glucose and phenol red (18 mg/L) at 55°C for 24 h. Gas production test was considered positive when a gas bubble was observed at the top of the inverted tube. Acid production was considered positive when the medium colour changed to yellow.

Production of exopolymers

Isolates were grown on a production medium [21] containing: yeast extract (2.5 g/L); glucose (20 g/L); NaCl (1.0 g/L); K₂HPO₄ (5.0 g/L); MgSO₄.7H₂O (0.2 g/L); (NH₄)₂SO₄ (0.6 g/L); pH 5.5 ± 0.5 at 55°C and 150 rpm for 3 days. Cell broth was centrifuged at 15,000 x g for 20 min at 4°C, and cells were washed with distilled water and dried at 105°C until constant weight was reached to determine biomass. The supernatant (or non-cultivated medium as negative control) was mixed with 2 volumes of 95% ethanol and incubated at 4°C for 24 h to precipitate the crude products, then centrifuged at 15,000 x g for 30 min at 4°C, and repeatedly washed with acetone-ether-distilled water (1:1:1). The pellet was dried, and the exopolymer yield was determined by the ratio exopolymer weight/biomass.

Screening for biodesulphurization activity

Isolates were grown on 5 mL of a medium containing: yeast extract (0.25 g/L); FeSO₄.7H₂O (0.001 g/L); CaCl₂.2H₂O (0.001 g/L); MgSO₄ (0.2 g/L); (NH₄)₂SO₄ (2.0 g/L); K₂HPO₄ (4.0 g/L); NaH₂PO₄ (4.0 g/L); and 0.05% dibenzothiophene (DBT) (dissolved in N,N'-dimethylformamide), pH 5.5 ± 0.5 at 55°C and 150 rpm for 12 days. Cell growth was measured by monitoring the optical density at 660 nm. The cultures were centrifuged and the supernatants (or non-cultivated medium as negative control) were subjected to Gibb’s assay to detect 2-hydroxybiphenyl (2-HBP) produced by microbial degradation of DBT [22].

Gibb’s assay

The production of 2-HBP was monitored as follows: 0.150 mL of culture’s supernatant was mixed with 0.03 mL of 1M NaHCO₃ (pH 8). Twenty microlitres of Gibb’s reagent (1 mg of 2,6-dichloroquinone-4-chloroimide per mL in ethanol) was then added, and the reaction mixture was agitated at room temperature for 15 to 45 min for full colour development. The absorbance of the reaction mixture was determined at 595 nm and converted to mg/L by interpolation on a 2-HBP standard curve (1–10 mg/L).

Results

Isolation and characterization of bacterial strains

Inoculation of samples from thermal environments in LB medium at pH 6.0 ± 0.5, 60°C and 150 rpm resulted in
abundant growth after 12 h of culture. Eleven different colonies from El Carrizal and eight from Los Baños were obtained after repeated streaking on LB agar, differing mainly in colony size and cellular shape. Isolates were designated as strains CCR1 to CCR11 (El Carrizal) and DR01-DR08 (Los Baños). Cultures grown on liquid and agar media for 24 h at 55°C and pH 7 were used for characterization. Colonies were circular, punctiform, or irregular, smooth with entire margins, varying in diameter between 1.5 and 8 mm. The isolates were positive for Gram staining and for oxidase test, endospore-forming, and thin rod-shaped varying in cell length (Table 2). As it has been reported for other thermophilic bacteria [23], changes in cell morphology were observed: depending on culture temperature and incubation time strains CCR1, CCR7, CCR8, CCR9, CCR10 and CCR11 occurred as single motile long rods, as aggregates, or as long filaments that formed filamentous cell masses. The isolates were aerobes capable to oxidize carbohydrates. The strains were thermophilic, neutrophilic, and alkalitolerant, growing in a pH 5–11, and at temperatures spanning 27°C to 70°C, with the optimum pH between 6.5 and 7.5, and the optimum temperature between 55°C and 70°C (Tables 2 and 3). Growth rates were fairly rapid, showing doubling times as short as 20 min (CCR5) and 22 min (CCR4). Most of the strains were halotolerant, since they could grow in the presence of up to 3% NaCl (0.5 M).

**Phylogenetic analysis**

The phylogenetic analysis was performed on the most promising isolates based on their potential biotechnological applications (for strains CCR1, CCR2, CCR4, CCR7 and CCR10 we obtained the partial 16S rRNA sequence, and for strains CCR3, CCR11, DR01, DR02, DR03 and DR04, we obtained the complete 16S rRNA sequence). The 16S rRNA sequences obtained were aligned to those available in the EMBL/GenBank database and at the EzTaxon server. Results are shown in Table 4 and Figure 1. The alignment placed strains CCR1, CCR2, CCR7, CCR10 and CCR11 in *Geobacillus* genus, and strains CCR3, CCR4, DR01, DR02, and DR04 in *Anoxybacillus* genus. Strains CCR3, DR01, DR02 and DR04 showed a 99.5–99.6% similarity with *Anoxybacillus kamchatkensis*, a thermophilic facultative aerobic bacterium isolated from the Geyser valley, Kamchatka [24]. Strain CCR4 showed identical 16S rRNA sequence as the type strain of *Anoxybacillus rupiensis*, a thermophilic bacterium isolated from Rupi basin (Bulgaria) [25]. The particular phylogenetic location of strain DR03 let us reclassify it into a new genus in the family *Bacillaceae* as *Aeribacillus pallidus* gen. nov., comb. nov. [26].

**Enzymatic activities**

Isolates were tested for enzymatic activities on agar plates (Table 2 and 3). Eleven strains showed hydrolytic

### Table 2. Morphological and physiological characteristics of El Carrizal thermal pool isolates.

| Character          | CCR1 | CCR2 | CCR3 | CCR4 | CCR5 | CCR6 | CCR7 | CCR8 | CCR9 | CCR10 | CCR11 |
|--------------------|------|------|------|------|------|------|------|------|------|-------|-------|
| Cell size (µm)     | 6    | 4–6  | 6–10 | 10   | 6–12 | 5–9  | 4–14 | 6–12 | 7–13 | 4–8   | 8–14  |
| Motility           | ++++ | +    | −    | ++   | +    | +    | +    | +    | −    | +     | +     |
| Temperature (°C):  |      |      |      |      |      |      |      |      |      |       |       |
| Range              | 40–65| 40–70| 40–70| 40–70| 40–70| 40–70| 40–70| 40–70| 40–70| 40–70 | 40–70 |
| Optimum            | nd   | nd   | 63–70| 62–68| 55–60| nd   | 65–70| 65–70| nd   | 55–60 | 55–60 |
| pH:                |      |      |      |      |      |      |      |      |      |       |       |
| Range              | 7–8  | 7–8  | 5–9  | 5–8  | 7–9  | 5–9  | 7–9  | 7–9  | 7–8  | 7–8   | 7–8   |
| Optimum            | nd   | nd   | 7–7.5| 7–7.5| 7–7.5| nd   | 7–7.5| 7–7.5| nd   | 7–7.5 | 6.5–7 |
| Oxidase            | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     |
| Doubling time (min) |      |      |      |      |      |      |      |      |      |       |       |
| at opt. conditions | nd   | nd   | 69   | 22   | 20   | nd   | 41   | 39   | nd   | 41    | 78    |
| NaCl:              |      |      |      |      |      |      |      |      |      |       |       |
| 0.3 M              | +    | +    | −    | +    | −    | +    | +    | +    | +    | +     | +     |
| 0.5 M              | +    | +    | −    | −    | −    | −    | −    | −    | −    | −     | −     |
| 1–2 M              | −    | −    | −    | −    | −    | −    | −    | −    | −    | −     | −     |
| Production of:     |      |      |      |      |      |      |      |      |      |       |       |
| Proteases          | −    | −    | +    | +    | +    | +    | +    | +    | +    | +     | +     |
| Lipases            | −    | −    | −    | −    | +    | +    | −    | −    | −    | −     | +     |
| Amylases           | −    | −    | −    | −    | +    | +    | −    | −    | +    | −     | +     |
| Growth on:         |      |      |      |      |      |      |      |      |      |       |       |
| Glu/Suc/Arab       | +/−/+| +/−/+| +/−/+| +/−/+| +/−/+| +/−/+| +/−/+| +/−/+| +/−/+| −/+−/−| +/−/+ |
| Ram/Raf/Mel        | −/+−/−| −/+−/−| −/+−/−| −/+−/−| −/+−/−| −/+−/−| −/+−/−| −/+−/−| −/+−/−| −/+−/−| −/+−/−|

Glu=glucose; Suc=sucrose; Arab=arabinose; Ram=rhamnose; Raf=rafinose; Mel=melobiose; nd=non determined.
halos around colonies when grown on casein medium at 55°C, indicating proteases production. Nine strains produced lipases, since an orange halo was observed around colonies under UV light when grown on olive oil and Rhodamine B medium. Eight strains were capable to produce amylases, showing clear halos of starch hydrolysis after staining starch plates with iodine reagent.

**Screening for activities required for microbial enhanced oil recovery**

Isolated strains from El Carrizal thermal pool were analysed for their capability to produce exopolymers, gases, acids, and biosurfactants, and to grow in a medium containing kerosene as a carbon source. Results are shown in Table 4. All the strains produced exopolymers after 72 h at 55°C (0.3–11.8 mg/mg biomass); nine out of 11 strains showed emulsifying activity when grown in a medium containing kerosene (E24= 1.6–7.5%); most of them produced acids but none produced gas. The chemical composition of the acids, exopolymers and biosurfactants remains to be determined.

**Screening for biodesulphurization activity**

All the isolates were capable of growth on a DBT containing medium, especially strains CCR1, CCR7 and CCR10. After 12 days at 55°C, all the microbial cultures were positive to the Gibb’s assay, with the highest production of 2-HBP by strains CCR1, CCR2,
Figure 1. Consensus neighbour-joining phylogenetic unrooted tree (Jules-Cantor model and pairwise deletion option) from sequences of the 16S rRNA gene, encompassing all Aeribacillus, Anoxybacillus and Geobacillus species. The number of GenBank accession sequences is indicated in brackets. The bar represents distance values calculated by MEGA. Bootstrap values (>50%) after 1000 replicates are shown.
CCR3, and CCR9, which showed 2-HBP levels up to 2.8 mg/L (Table 5).

**Discussion**

Enrichment of thermal water from both El Carrizal thermal pool and Los Baños hot spring in LB medium at pH 6.7, 60°C and 150 rpm, resulted in the isolation of 19 thermophilic, alkalitolerant and halotolerant bacterial strains. Phylogenetic analysis placed strains CCR1, CCR2, CCR7, CCR10 and CCR11 as belonging to different species of the *Geobacillus* genus which includes Gram-positive spore-forming rods, neutrophilic, moderately thermophilic and aerobic or facultatively anaerobic species with a temperature range for growth from 37 to 75°C, with an optimum at 55–65°C [27]. Isolates CCR3, CCR4, DR01, DR02 and DR04 were placed in the *Anoxybacillus* genus, which includes Gram-positive spore-forming rods, alkaliphilic or alkalitolerant, thermophilic and aerotolerant or facultative anaerobes, with a temperature range of growth of 35–70°C [28]. The strains showed high similarities (> 98%) with various species of the *Anoxybacillus* genus 

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**Table 5. Analysis of isolated strains for microbial enhanced oil recovery activities and biodesulphurization.**

| Strain | Growth on Kerosene | Gas | Acids | Exopolymers (mg/mg biomass) | Emulsifying activity (E24, %) | DBT degradation 2-HBP (mg/L) |
|--------|---------------------|-----|-------|-----------------------------|-------------------------------|-----------------------------|
| CCR1   | +                   | −   | −     | 11.8                        | 5                             | 2.8                         |
| CCR2   | +                   | −   | +     | 1.5                         | 7.5                           | 2.7                         |
| CCR3   | +                   | −   | +     | 2.2                         | 1.8                           | 2.9                         |
| CCR4   | +                   | −   | +     | 0.31                        | 5                             | 0.7                         |
| CCR5   | +                   | −   | +     | 4.1                         | 1.6                           | 1.3                         |
| CCR6   | +                   | −   | +     | 5                           | 5.3                           | 0.9                         |
| CCR7   | +                   | −   | +     | 5.7                         | 5                             | 1.8                         |
| CCR8   | +                   | −   | +     | 2.2                         | 5                             | 1.3                         |
| CCR9   | −                   | −   | +     | 3.4                         | 0                             | 2.7                         |
| CCR10  | +                   | −   | −     | 3.9                         | 0                             | 1.9                         |
| CCR11  | +                   | −   | +     | 5                           | 3.7                           | 2.1                         |

E24: Emulsification index. DBT: dibenzothiophene. 2-HBP: 2-hydroxibiphenyl.

could be differentiated from *Anoxybacillus* and *Geobacillus* on the basis of DNA G+C content, 16S rRNA gene sequence analysis and fatty acid and polar lipid profiles. From these results, it was proposed that *G. pallidus* (DR03) should be reclassified to *Aeribacillus pallidus* gen. nov., comb. nov. [26]. Isolated strains produced hydrolytic enzymes (proteases, lipases and amylases) under thermophilic conditions (55°C). Thermophilic enzymes are attractive because they are stable and active under conditions comparable to those prevailing in various industrial processes, such as in food processing, laundry, textile and pharmaceutical industries. A thermoalkalophilic lipase produced by strain *Geobacillus thermoleovorans* CCR11 has been already purified and characterized by the research group [29].

The successful application of *in situ* MEOR depends largely on the capability of microorganisms to grow and produce metabolite(s), such as biosurfactants, exopolymers, gas and acids, under the extreme conditions similar to those existing in the oil reservoirs [30]. Although the ideal microorganisms would be extremophiles, since they can grow optimally in extreme temperatures, pressures, pH conditions and salt concentrations, there are few reports on the application of extremophilic organisms in MEOR [9,19,30–36]. In this work we report bacterial strains that may be applied in MEOR, since they are thermophilic and halotolerant and hence could probably withstand reservoir conditions such as high temperature, pressure, salinity and low oxygen. Additionally, isolated strains produced exopolymers after 72 h at 55°C (up to 11.8 mg/mg dry weight of cells), a concentration that lies in the range of other mesophilic fungal and bacterial species, which have shown variable production yields.
(from 0.34 mg/mg dry weight of cells [21] to 700 mg/mg dry weight of cells [33]). With respect to the emulsifying activity, emulsification indexes (E24) found (1.6–7.5 %) were low in comparison to those found with Bacillus subtilis under thermophilic conditions (33–90%) [9] or with the thermophilic Bacillus sp. AB-2 (80–90%) [19]. However, they might be improved by the optimization of some important parameters, such as culture temperature, initial pH of the medium, oxygen supply, nitrogen concentration, and carbon source.

In order to find out if the isolated strains could cleave heterocyclic organosulphur compounds refractory to the desulphurization process in a C-S-bond-targeted fashion at high temperatures, we cultured strains in a medium containing dibenzothiophene (DBT) and analysed the production of 2-HBP at 55°C. The carbon-sulphur (C-S) bond targeted reaction is preferable and ideal for desulphurization because it keeps the remaining hydrocarbon molecules fully active as energy sources without any loss of their thermal units [22]. All the isolates were capable to generate 2-HBP from DBT under thermophilic conditions.

Many mesophilic bacteria capable to degrade DBT in a C-S targeted fashion have been isolated (Rhodococcus erythropolis, Nocardia spp., Agrobacterium sp. MC501, Gordona sp., CYKS1, Klebsiella spp., Xanthomonas spp., Microbacterium and Pseudomonas [36,37]), but few thermophilic bacteria have been reported to do so (Paenibacillus sp. [22,38], Mycobacterium phlei [39–41], and Mycobacterium godii X7B [42]). Biodesulphurization of fossil fuels is attracting more and more attention because such a bioprocess is environmentally friendly. Performing biodesulphurization at high temperatures has many advantages since both the bioavailability of hydrophobic compounds and the reaction rates increases with temperature, and because the process costs could be diminished by avoiding the cooling of petroleum fractions prior to the biodesulphurization reaction. Although work remains to be done regarding the exact mechanisms and the optimal conditions for desulphurization, the CCR strains may be useful candidates for thermophilic desulphurization.

Conclusions

In conclusion, the thermal environments analysed in Veracruz, México, are a good source of extremophilic microorganisms with promising biotechnological potential since we isolated 19 thermophilic and alkali-tolerant bacterial strains that produce hydrolytic enzymes under thermophilic conditions. They may have good potential for application in microbial enhanced oil recovery, since they are thermophilic and halotolerant, produce exopolymers, acids and surfactants, and are able to grow in kerosene as the sole carbon source. Finally they may be used in biodesulphurization since they can grow in dibenzothiophene producing 2-HBP under thermophilic conditions.

Acknowledgements

MSc Dora Luz Pinzón and MSc Citlali Rodríguez acknowledge their scholarships from the Mexican National Council for Science and Technology (Conacyt). This work was supported by grant 431.01-P from the National Council on Technological Education (Cosnet). We gratefully acknowledge Oscar Calahorra Fuertes for his technical advice and Patricia Davis for correcting the manuscript.

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