African Journal of Biotechnology Vol. 8 (10), pp. 2218-2223, 18 May, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Isolation and molecular genetic characterization of a yeast strain able to degrade petroleum polycyclic aromatic hydrocarbons

Abd El-Latif Hesham[1],* Saad A. Alamri[2], Sardar Khan[3], Motamed E. Mahmoud[4] and Hashem M. Mahmoud[5]

[1]Genetics Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.
[2]Biological Science Department, Faculty of Science, King Khalid University, Saudi Arabia
[3]Department of Environmental Sciences, University of Peshawar, Peshawar, Pakistan.
[4]Department of Animal Hygiene, Animal behavior, Faculty of Veterinary Medicine, Assiut University, Egypt.
[5]Soils and Water Department, Faculty of Agriculture, Assiut University, Assiut, Egypt

Accepted 16 February, 2009

Polycyclic aromatic hydrocarbons (PAHs) belong to a class of toxic environmental pollutants and PAH exposure causes public health risks and raises environmental concerns. Identification of the key microorganisms that play a role in pollutant degradation processes is relevant to the development of optimal *in situ* bioremediation strategies. In the current study, three yeast strains were isolated from oil-contaminated soil by enrichment technique in mineral basal salts (MBS) medium supplemented with phenanthrene as a sole carbon source. Out of these, strain AH70 was selected for PAHs degradation, because of its fast growth on agar plate coated by PAHs as sole source of carbon and energy. The yeast was identified by molecular genetics technique based on sequence analysis of the variable D1/D2 domain of the large subunit (26S) ribosomal DNA. Subsequent 26S rRNA gene sequencing showed 100% base sequence homology and it was identified as *Candida viswanathii*. The degradation of PAHs by this yeast was confirmed by GC-MS analyses. The yeast was capable of degrading a mixture of low and high molecular weight PAHs and degradation efficiency was found as 89.76% for naphthalene, 77.21% for phenanthrene, 60.77% for pyrene and 55.53% for benzo(a)pyrene at the end of 10 days.

Key words: Yeast, PAHs pollutant, 26S rRNA gene.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment and released into the environment from anthropogenic sources such as gas manufacturing plants, combustion process, petroleum spillage and timber treatment plants (Juhasz and Naidu, 2000). Many PAHs are highly toxic, mutagenic, carcinogenic and teratogenic in nature; exposure to PAHs represents public health risks and raises environmental concerns (Mastrangelo et al., 1996; Goldman et al., 2001; Khan et al., 2008). Even though higher molecular weight (HMW-PAHs) such as those containing four or more benzene rings are considered to be responsible for potential hazards to environment and human health (Anonymous, 1984), lower molecular weight (LMW) types such as naphthalene and phenanthrene (containing 2 and 3 benzene rings, respectively), are known to have health effects that though are comparatively mild could be potentially hazardous (Klaasen, 2001).

Biodegradation using microorganisms is usually the preferred and major route of PAH removal from contaminated environment because of some inherent advantages such as its cost effectiveness and comparatively higher cleanup (Pothuluri and Cerniglia, 1994; Jacques et al., 2008). Microorganisms were found to degrade PAHs via either metabolism or co-metabolism. Co-metabolism is important for the degradation of mixtures of low and high-molecular weight PAHs (Cerniglia, 1992; Pan et al., 2004;

*Correspondence author. E-mail: hesham_egypt5@yahoo.com. Fax: +2088-2331384.

Hesham et al. 2006). A large number of bacteria capable of degrading PAHs, have been isolated (Santos et al., 2008), but a few authors had reported the growth of yeast with phenanthrene (Romero et al., 1998; Pan et al., 2004; Hesham et al., 2006).

Previous studies also demonstrated that the HMW-PAHs could not be utilized as carbon sources for yeasts such as *Saccharomyces cerevisiae*, *Debaromyces hansenii, Candida lipolytica, C. albicans C. guilliermondii, C. maltosa* and *Pichia anomala* (Cerniglia and Crow, 1981; McGillivray and Sharis, 1993; Zinjarde and Pant, 2002; Pan et al., 2004).

Typically, this study focuses on PAH-degrading yeast. The yeast is usually identified by the appearance of the cellular morphology, fermentation, and assimilation of carbon source and nitrogen source (Yarrow, 1998; Barnett et al., 2000). This conventional method needs evaluation of some 60 - 90 tests for correct species identification, and the result are sometimes confused by strain variation. This process is complex, laborious, time consuming, lack discriminatory power and misidentify-cation occurs frequently. In contrast, the identification methods by molecular genetics techniques are rapid, easy and more precise for yeast identification. Among such techniques, identification of yeasts based on sequence analysis of D1/D2 of large subunit (LSU) 26S rRNA gene which, has been established as most reliable way in the identification of yeasts (Kurtzman and Robnett, 1998) and has also been applied to study the phylogeny of different yeast groups (Boekhout et al., 1994; Kurtzman and Robnett, 1998). Therefore, the sequencing of the D1/D2 domain is increasingly being used to identify yeasts (Phaff et al., 1999; Hong et al., 2001; Scorzetti et al., 2002) and according to Frutos et al. (2004) it is accepted universally as the main tool for yeast taxonomy. Databases of the D1/D2 sequences are now available for all currently recognized ascomycetous and basidiomycetous yeasts (Kurtzman and Robnett, 1997; Kurtzman and Robnett, 1998; Guffogg et al., 2004). This extensive available database makes the task of species identification much easier (Kurtzman, 2001; Starmer et al., 2001; Wesselink et al., 2002) and could serve as reliable and practical criteria for identification of most known yeasts (Abliz et al., 2004).

The objectives of this study were to isolate and classify the PAH-degrading yeast, which are able to degrade PAHs, at the genus and species levels by sequencing of the gene coding for 26S rRNA gene.

MATERIALS AND METHODS

Chemicals

The PAHs used were naphthalene, phenanthrene, pyrene and benzo(a) pyrene, were of highest purity (Acros Organic Co.).

Isolation of PAH-degrading yeast by enrichment technique

Three PAH-degrading yeast strains were isolated from the oil contaminated soil by enrichment culture technique (Hesham et al., 2006). Briefly, 1 g soil was suspended in 10 ml sterile water and added to a mineral basal salts (MBS containing, per litre of distilled water, 1000 mg $(NH_4)_2SO_4$, 800 mg K_2HPO_4, 200 mg KH_2PO_4, 200 mg $MgSO_4 \cdot 7H_2O$, 100 mg $CaCl_2 \cdot 2H_2O$, 5 mg $FeSO_4 \cdot 7H_2O$, pH 5 - 6 and 1 ml of vitamin solution sterilized by filtration) medium supplemented with 0.2% phenanthrene at 27°C at 150 rpm for 10 days. When growth was visible, enrichment was continued by serially sub-culturing several times in the same medium, using a 10% inoculum from the previous culture. Yeast strains were isolated from MBS agar plates coated with phenanthrene as the sole carbon source (Romero et al., 1998).

Genomic DNA extraction

Cells used for DNA extraction were grown for approximately 24 h at 25°C in 50 ml YM broth medium contains, per liter of distilled water, 10 g glucose, 5 g peptone, 3 g malt extract, 3 g yeast extract and 20 g agar for solid medium on a rotary shaker at 200 rpm and harvested by centrifugation. The cells were washed once with distilled water, re-suspended in 2 ml of distilled water, and the suspension was divided into two 1.5 ml micro centrifuge tubes. After centrifugation, the supernatant was decanted from the micro centrifuge tubes, and the packed cells were used for DNA extraction which was performed according to procedures described by Harju et al. (2004).

Identification and genetic characterization

In order to identify and determine the correct phylogenetic position of the isolate, sequence analysis of the variable D1/D2 domain of the large subunit (26S) ribosomal DNA was performed. This procedure is recognized as a reference method for identify and comparison of phylogenetic relationships among ascomycetous yeasts (Kurtzman and Robnett, 1998). DNA was isolated and fragments containing about 600 - 650 bp at the 5' end of the 26S rDNA were amplified using primers: NL-1 (5'-GCA TAT CAA TAA GCG GAG GAAAAG-3') and NL-4 (5'-GGT CCG TGT TTC AAG ACG G-3'). The polymerase chain reaction (PCR) reactions were performed in a final volume of 50 µl containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, each dNTPs at a concentration of 0.2 mM, 1.25 IU of Taq polymerase, each primer at a concentration of 0.2 mM and 1 µl of DNA template. The amplification was carried out by PCR under the following conditions: initial denaturation at 95°C for 5 min, followed by 36 cycles at 94°C for 2 min, 52°C for 1 min, 72°C for 2 min; final extension at 72°C for 7 min and holding at 4°C. The amplified product was then purified and sequenced.

The 26S sequence of AH70 isolate was used for a BLAST search in the EMBL/GenBank database. The 26S sequence of the isolate was further aligned and compared to published 26S rRNA sequences searched with the taxonomy browser of the National Center for Biotechnology Information (NCBI; Bethesda, MD) and retrieved from GenBank.

Degradation of PAHs by strain AH70

For preparation of PAH stock solutions, 100 mg from the each selected PAH was dissolved in 10 ml acetone, added to 50 g Tween 80 in 50 ml distilled water, ultrasonicated, and the suspension diluted with water to make 200 ml (Andres et al., 1999).

About 5 ml batch cultures grown in 40 ml sample tubes with screw caps and Teflon-lined septa. The isolate was pre-cultivated at 150 rpm at 27°C in the following basal medium (BM): KH_2PO_4 1 g, $(NH_4)_2SO_4$ 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, yeast extract 0.5 g, distilled water 1000 ml, pH 5 - 6, supplemented with 10 g glucose (Yang et

Figure 1. Amplified DNA of the D1/D2 domain with primer pair NL1 and NL4. Lane M represents marker, 1000, 900, 500, 300, 200, and 100 bp; Lanes 1 - 2 are PCR products amplified from selected yeast strain AH70, and lane C represents negative control.

al., 2003). Cells were harvested by centrifugation, washed twice with sterile BM and inoculated into the modified BM supplemented with sole or mixtures of PAHs as the carbon source. The inoculum was controlled to approx. 2.4 × 108 cells ml−1. Heat- killed cells (autoclaved) and non-inoculated flasks were used as negative and blank controls. Cultures were grown in the dark with tumble-shaking at 150 rpm and 27°C (McNally et al., 1999). All experiments were carried out in triplicates

GC-MS analysis

Five ml hexane was added to each culture, shaken horizontally for 2 h at 150 rpm and approx. 2 ml of the hexane layer was placed in an autosampler vial, crimp-sealed with a Teflon-lined cap, and then stored at -20°C until analysis (Leblond et al., 2001). PAH concentrations were analyzed by gas chromatography interfaced with mass spectrometer detector (GC/MS), equipped with a 5973 mass selective detector under the selected ion-monitoring (SIM) mode. An HP-5 silica fused capillary column (60 m × 0.25 mm inner diameter × 0.25 µm film thickness) was used with helium as the carrier gas at a constant flow rate of 1 ml min^{-1}. The GC oven temperature was programmed from 50°C (2 min) to 200°C (2 min) at 10°C min^{-1}, then to 290°C at 20°C min^{-1} and held for 8 min. The injector and detector temperatures were 280 and 290°C, respectively. Mass spectra were acquired at the electron ionization mode with an electron multiplier voltage of 1906 eV. For identification, SIM mode was carried out using the molecular ions selective for individual PAHs. Chromatographic data were collected and processed using GC Chemstation software.

Nucleotide sequence accession number

The partial 26S ribosomal DNA sequences of strain AH70 reported in this paper has been deposited in the DDBJ, EMBL, and GenBank databases under accession number EU328289.

RESULTS AND DISCUSSION

Isolation of PAHs degrading yeast by enrichment technique

Culture on agar media still remains as the main approach for isolation of yeasts from natural habitats. Three different colonies of the yeasts were isolated on MBS-agar plates coated by phenanthrene, as a LMW-PAH, as sole source of carbon and energy. Although all the strains were able to grow on MBS-agar plates coated by pyrene as a HMW-PAH, the strain AH70 exhibited faster growth than others. Therefore, it was selected as PAH degrader.

The previous studies demonstrated that the micro-organisms, bacteria or yeasts, which isolated on naphthalene as the sole carbon source were unable to grow on anthrancen, phenanthrene, fluranthene, pyrene or chrysene, whereas microorganisms isolated on any of the other five compounds were able to grow on at least one of the other substrates (Aitken et al., 1998; Pan et al., 2004; Hesham et al., 2006). On the other hand, the use of phenanthrene as a sole carbon source has been taken as a best way for selection of HMW-PAH degrading yeast strains (Hesham et al., 2006).

Identification and genetic characterization

As shown in (Figure 1), the size of the amplified D1/D2 region was 600 bp for the yeast isolate, which is the expected size of the D1/D2 variable domain of the large subunit. Genetic analysis was performed by a BLAST search on the EMBL/GenBank database. BLAST analysis of the 26S rRNA gene sequence of the isolate, AH70, was revealed it to be a perfect match with that of *C. viswanathii* type strain. The alignment and comparison of the 26S sequence of the isolate to the published 26S rRNA sequences belonging to five reference strains of phenotypically close species of Candida confirmed the 100% correspondence to the *C. viswanathii* type strain and revealed identity rates <95% with respect to *Candida lodderae*, *C. tropicali*, *C. neerlandi*, and *Candida albicans* type strains (Figure 2). In the 26S rDNA sequence result, as shown in Figure 3 the numbers of base substitutions between the isolate AH70 and *C. viswanathii* were zero (100% similarity).

The sequencing of the D1/D2 of the large-subunit 26S ribosomal DNA is now widely accepted as a standard procedure for yeast identification (Kurtzman and Robnett, 1998; Phaff et al., 1999; Hong et al., 2001; Scorzetti et al., 2002; Frutos et al., 2004). It was also found that molecular methods based on the sequences of the 26S rDNA, D1/D2 domain and the ITS region are rapid and precise compared with the physiological method for the

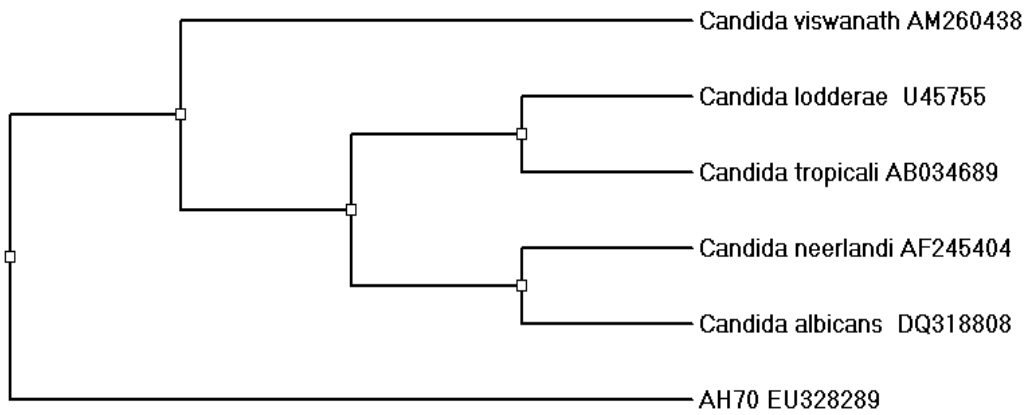

Figure 2. 26S sequence-based phylogenetic tree showing the relationships between the PAHs degrading isolate (AH70) and the type strains of 5 yeast taxa, including *Candida viswanathii* and phenotypically close species.

```
Query  17   CCTTAGTAGCGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGGCTCTTTCAGAGT  76
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CCTTAGTAGCGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGGCTCTTTCAGAGT  60

Query  77   CCGAGTTGTAATTTGAAGAAGGTATCTTTGGGCCTGGCTCTTGTCTATGTTTCTTGGAAC  136
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CCGAGTTGTAATTTGAAGAAGGTATCTTTGGGCCTGGCTCTTGTCTATGTTTCTTGGAAC  120

Query  137  AGAACGTCACAGAGGGTGAGAATCCCGTGCGATGAGATGACCCAGGTCCGTGTAAAGTTC  196
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  AGAACGTCACAGAGGGTGAGAATCCCGTGCGATGAGATGACCCAGGTCCGTGTAAAGTTC  180

Query  197  CTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAGTGGGTGGTAAATTCCATCTAAA  256
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAGTGGGTGGTAAATTCCATCTAAA  240

Query  257  GCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACAGTGATGGAAAGATGAAAAGAAC  316
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACAGTGATGGAAAGATGAAAAGAAC  300

Query  317  TTTGAAAAGAGAGTGAAAAAGTACGTGAAATTGTTGAAAGGGAAGGGCTTGAGATCAGAC  376
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  TTTGAAAAGAGAGTGAAAAAGTACGTGAAATTGTTGAAAGGGAAGGGCTTGAGATCAGAC  360

Query  377  TTGGCATTTTGCATGTTGCTTCTTCGGGGGCGGCCTCTGCGGTTTGTCGGGCCAGCATCA  436
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TTGGCATTTTGCATGTTGCTTCTTCGGGGGCGGCCTCTGCGGTTTGTCGGGCCAGCATCA  420

Query  437  GTTTGGGCGGCAGGACAATCGCGTGGGAATGTGGCACGGCCTCGGCTGTGTGTTATAGCC  496
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  GTTTGGGCGGCAGGACAATCGCGTGGGAATGTGGCACGGCCTCGGCTGTGTGTTATAGCC  480

Query  497  CGCGTGGATACTGCCAGCCTAGACTGAGGACTGCGGTTTATACCTAGGATGTTGGCATAA  556
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CGCGTGGATACTGCCAGCCTAGACTGAGGACTGCGGTTTATACCTAGGATGTTGGCATAA  540

Query  557  TGATCTTAAGTCGC  570
           ||||||||||||||
Sbjct  541  TGATCTTAAGTCGC  554
```

Figure 3. Sequence alignment of the isolate AH70 against D1/D2 of 26S rDNA sequence data of *Candida viswanathii* showing no base substitutions.

Table 1. Calculated mass balance of PAHs in liqued culture (n = 3).

PAH	No. of rings	Initial concentration (mg/kg)	Remaining (mg/kg)	Degradation (%)
Naphthalene (LMW)	2	1.79	0.097	89.76 %
Phenanthrene (LMW)	3	1.89	0.244	77.21%
Pyrene (HMW)	4	1.96	0.464	60.77%
Benzo(a)pyrene (HMW)	5	1.98	0.566	55.53%

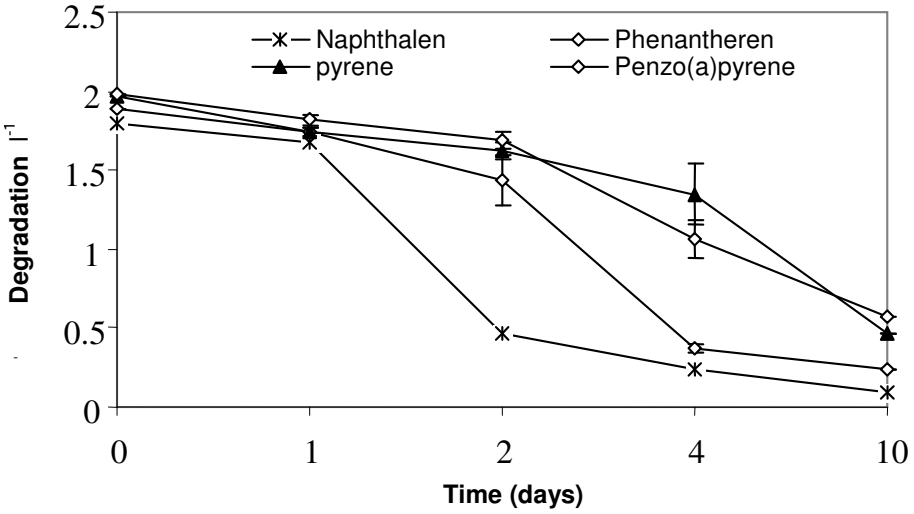

Figure 4. Degradation of PAHs by *Candida* viswanathii Strain AH70 in mixture solutions. The initial concentration of each PAH was: naphthalene, 1.79mg l^{-1}; phenanthrene, 1.89 mg l^{-1}; pyrene, 1.96 mg l^{-1}; benzo(a)pyrene, 1.98 mg l^{-1} Each point represents mean ± S.E. of triple assays.

identification and typing of the *Candida* species (Suezawa et al., 2006).

Degradation of PAHs by strain AH70

The initial concentrations, residual and degradation ratios of all of the LMW-PAHs and HMW-PAHs by the yeast strain AH70 are described in (Table 1 and Figure 4). The data indicate that all of the PAHs mixture was degraded significantly by the yeast. The removal efficiency for the LMW-PAHs (naphthalene and phenanthrene) ranged from 89.76 – 77.21% and that for the HMW-PAHs (pyrene and benzo (a) pyrene) ranged from 60.77 – 55.53% after degradation for 10 days.

It is interesting that the HMW-PAHs including pyrene and benzo(a)pyrene, were removed with a relatively high efficiency (60.77 – 55.53%). Previous studies demonstrated that the HMW-PAHs could not be utilized as carbon sources for yeasts such as *S. cerevisia, D. hansenii, C. lipolytica, C. albicans, C. guilliermondii, C. maltosa* and *P. anomala* (Cerniglia and Crow, 1981; Hofmann 1986; McGillivray and Sharis, 1993; Zinjarde and Pant, 2002; Pan et al., 2004). The efficient removal of these compounds should be removed through co-metabolism by the yeast using the LMW-PAHs as the carbon sources. Hesham et al. (2006) found that the 5 ring benzo(a)pyrene decomposed by *P. anomala* only through co-metabolism. Members of the genera *Candida*, *Pichia*, *Rhodotorula* and *Sporidiobolus* are considered potential degraders for hazardous organic pollutants, such as aliphatic and polycyclic aromatic hydrocarbons (McGillivray and Sharis, 1993; Zinjarde and Pant, 2002; Pan et al., 2004; Hesham et al., 2006).

Conclusion

By selecting phenanthrene or other aromatic hydrocarbon compounds rather than naphthalene in enrichment methods as the sole carbon source, more powerful strains that could grow on HMW-PAHs was obtained. The strain AH70 exhibited faster growth than others and could utilize a mixture of LMW and HMW-PAHs. By sequencing of D1/D2 of large subunit 26S rRNA gene, the strain AH70 was identified to be *C. viswanathii.*

ACKNOWLEDGEMENT

The authors would like to thank Faculty of Agriculture, Assiut University, Egypt for supporting his study.

REFERENCES

Abliz P, Fukushima K, Takizawa K, Nishimura K (2004). Identification of pathogenic dematiaceous fungi and related taxa based on large subunit ribosomal DNA D1/D2 domain sequence analysis. FEMS Immun. Med. Microbiol. 40: 41-49.

Aitken MD, Stringfellow TW, Nagel DR, Kazunga C, Chen SH (1998). Characteristics of phenanthrene-degrading bacteria isolated from soils contaminated with polycyclic aromatic hydrocarbons. Can. J. Microbiol. 44: 743-752.

Andres S, Andrzej M, Christian J, Aloys H (1999). Degradation of fluorene, anthracene, phenanthrene, fluoranthene, and pyrene lacks connection to the production of extracellular enzymes by *Pleurotus ostreatus* and *Bjerkandera adusta*. Int. Biodeterior. Biodegrad. 43: 93-100.

Anonymous (1984). U.S. Environmental Protection Agency, (EPA)., Creosote-special review. Position document 213.U.S. Environmental Protection Agency, Washington, DC.

Barnett JA, Payne RW, Yarrow D (2000). How yeasts are classified. In: Barnett JA, Payne RW, Yarrow D (ed), Yeasts: Characteristics and rd Identification, 3 ed. Cambridge University Press, Cambridge, pp. 15-22

Boekhout T, Kurtzman CP, Donnell K, Smith MT (1994). Phylogeny of the yeast genera Hanseniasora (Anamorph Kloeckera) Dekkera (Anamorph Brettanomyces) and Eeniella as inferred from partial 26S ribosomal DNA nucleotide sequences. Int. J. Syst. Bacteriol. 44: 781-786.

Cerniglia CE, Crow SA (1981). Metabolism of aromatic hydrocarbons by yeasts. Arch. Microbiol. 129: 9-13.

Cerniglia CE (1992). Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation, 3: 351-368

Frutos RL, Fernandez–Espinar MT, Querol A (2004). Identification of species of the genus Candida by analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Ant. van. Leeuwenhoek, 85: 175-185.

Goldman R, Enewold L, Pellizzari E, Beach BJ, Bowman DE, Krishnan SS, Shields GP (2001). Smoking increase carcinogenic polycyclic aromatic hydrocarbons in human lung tissue. Cancer Res. 61: 6367-6371.

Guffogg SP, Thomas-Hall S, Holloway P, Watson K (2004). A novel psychrotolerant member of the hymenomycetous yeasts from Antarctica: *Cryptococcus watticus* sp. nov. Int. J. Syst. Evol. Microbiol. 54: 275-277.

Harju S, Fedosyuk H, Peterson KR (2004). Rapid isolation of yeast genomic DNA: Bust n' Grab. BMC Biotechnol. 4: 8.

Hesham A, Wang Z, Zhang Y, Zhang J, Lv W, Yang M (2006). Isolation and identification of a yeast strain capable of degrading four and five ring aromatic hydrocarbons. Ann. Microbiol. 56: 109-112.

Hofmann KH (1986). Oxidation of naphthalene by Saccharomyces cerevisiae and Candida utilis. J. Basic Microbiol. 26: 109-111.

Hong SG, ChunJ, Oh HW, Bae KS (2001). Metschnikowia koreensis sp. nov. a novel yeast species isolated from flowers in Korea. Int. J. Syst. Evol. Microbiol. 51: 1927-1931.

Jacques RJS, Okeke BC, Bento FM, Teixeira AS, PeralbaMCR, Camargo FAO (2008). Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. Bioresour. Technol. 99: 2637-2643.

Juhasz AL, Naidu R (2000). Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int. Biodeterior. Biodegrad. 45: 57-88.

Khan S, Cao Q, Lin AJ, Zhu YG (2008). Concentrations and bioaccessibility of polycyclic aromatic hydrocarbons in wastewater-irrigated soil using in vitro gastrointestinal test. Environ. Sci. Pollut. Res. 15: 344-353.

Klaasen CD (2001). Casarett and Doull's Toxicology: The Basic Science of Poisons. New York: McGraw-Hill.

Kurtzman CP, Robnett CJ (1997). Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large subunit (26S) ribosomal DNA gene. J. Clin. Microbiol. 35: 1216-1223.

Kurtzman CP, Robnett CJ (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Ant. van. Leeuweenhoek 73: 331-371.

Kurtzman CP (2001). Six new anamorphic ascomycetous yeasts near Candida tanzawaensis. FEMS Yeast Res. 1: 177-185

Leblond JD, Schultz TW, Sayler GS (2001). Observations on the preferential biodegradation of selected components of polyaromatic hydrocarbon mixtures. Chemosphere, 42: 333-343.

Mastrangelo G, Fadda E, Marzia V (1996). Polycyclic aromatic hydrocarbons and cancer in man. Environ. Health Perspect. 104: 1166-1170.

McGillivray AR, Sharis MP (1993). Biotransformation of polycyclic aromatic hydrocarbons by yeasts isolation from coastal sediments. Appl. Environ. Microbiol. 59: 1613-1618.

McNally DL, Mihelcic JR, Lueking DR (1999). Biodegradation of mixtures of polycyclic aromatic hydrocarbons under aerobic and nitrate-reducing conditions. Chemosphere, 38: 1313-1321.

Pan F, Yang Q, Zhang Y, Zhang S, Yang M (2004). Biodegradation of polycyclic aromatic hydrocarbons by Pichia anomala. Biotechnol. Lett. 26: 803-806.

Phaff HJ, Starmer WT, Kurtzman CP (1999). Pichia lachancei sp.nov. associated with several Hawaiian plant species. Int. J. Syst. Bacteriol. 49: 1295-1299.

Pothuluri JV, Cerniglia CE (1994). Microbial metabolism of polycyclic aromatic hydrocarbons. In Chaudry GR, ed. Biological Degradation and Bioremediation Toxic Chemicals. London: Chapman and Hall, pp. 92-124.

Romero MC, Cazau MC, Giorgieri S, Arambarri AM (1998). Phenanthrene degradation by microorganisms isolated from a contaminated stream. Environ. Pollut. 101: 355-359.

Santos EC, Jacques RJS, Bento FM, Peralba MCR, Selbach PA, Sá ELS, Camargo FAO (2008). Anthracene biodegradation and surface activity by an iron-stimulated Pseudomonas sp. Bioresour. Technol. 99: 2644-2649.

Scorzetti G, Fell JW, Fonseca A, Statzell-Tallman A (2002). Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS Yeast Res. 1497: 1-23.

Starmer WT, Phaff HJ, Ganter PF, Lachance MA (2001). Candida orba sp. nov. a new cactus-specific yeast species from Queensland, Australia. Int. J. Syst. Bacteriol. 51: 699-705.

Suezawa Y, kimura I, Inoue M, Gohda N, Suzuki M (2006). Identification and typing of miso and soy sauce fermentation yeasts, Candida etchellsii and Candida versatilis based on sequence analyses of the D1D2 domain of the 26S ribosomal RNA gene, and the region of internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2. Biosci. Biotechnol. Biochem.70: 348-354.

Wesselink J, Iglesia B, James SA, Dicks JL, Roberts IN, Rayward-Smith VJ (2002). Determining a unique defining sequence for yeast species using hashing techniques. Bioinform. 18: 1004-1010.

Yang OX, Yang M, Pritsch K, Yediler A, Hagn A, Schloter M, Kettrup A (2003). Decolorization of synthetic dyes and production of manganese-dependent peroxidase by new fungal isolates. Biotechnol. Lett. 25: 709-713.

Yarrow D (1998). Methods for the isolation, maintenance, classification and identification of yeasts. In: Kurtzman C. P. and Fell J. W. (eds), The Yeasts, A th Taxonomic Study, 4 edn. Elsevier Science BV, Amsterdam, The Netherlands, pp. 77-100.

Zinjarde SS, Pant AA (2002). Hydrocarbon degraders from tropical marine environments. Mar. Pollut. Bull. 44: 188-121.