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Full Length Research Paper

Isolation and characterization of nitrogen fixing bacteria from raw coir pith

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Coir fibre is the hard fibre extracted from the coconut husk and coir pith is a lignocellulosic byproduct released during the extraction of coir fibre. The pith is not degraded under normal environmental conditions and accumulates in the fibre extraction units occupying sprawling space in the units. The inherent properties of coir pith make it useful as a plant nutrient. Nitrogen fixing bacteria could be isolated from the coir pith and four strains were amplified with *nifH* gene *viz*. NF-4 (*Lysinibacillus* sp.), NF-7 (*Ochrobactrum* sp.), NF-12 (*Paenibacillus* sp.) and an uncultured bacterial clone which shows only 50% similarity to NF-18 (*Clostridium* sp). The present study targeted the isolation and characterization of natural flora of nitrogen fixing organisms in the coir pith.

Key words: Coir pith, *nif*H gene, nitrogen fixation, organic manure.

INTRODUCTION

Coir pith or coir dust is a major byproduct of coir fiber extraction industries (Reghuvaran and Ravindranath, 2010). Normally, the pith is dumped as an agricultural waste and accumulates in the form of heaps of coarse and fine dust. Coir pith thus produced decomposes very slowly in the soil as its pentosan-lignin ratio is below 0.5 (Ghosh et al., 2007), and because of the chemical and structural complexity of its lignin-cellulose complex (Ramalingam et al., 2005). Large amounts of coir pith (approximately 7.5 million tons annually in India) accumulate nearby coir processing units, causing severe disposal problems, fire hazards and ground water contamination due to the release of phenolic compounds (Namasivayam et al., 2001). Coir pith contains 87% of organic matter, 6.28% organic carbon, 0.73% nitrogen (Reghuvaran and Ravindranath, 2010) and 13% of ash content (Thampan, 1987). Lignin is generally synthesized by polymerization of coniferyl, sinapyl and p-coumaryl alcohol to produce large molecules of indefinite size in which aromatic monomers are linked by a variety of chemical bonds. The structural feature has important implications for effective bio-degradation by microorganisms (Crawford and Crawford, 1976; McCarthy et al., 1984). It is estimated that 15,840 million coconuts are produced annually in India. In agro-industrial wastes; lignin is a main contributor of the total carbon producing polycyclic aromatic hydrocarbon components such as benzopyrine, catechol, hydroquinone, phenanthrene and naphthalene when degraded by heat (Kjallstrand et al., 1998). Coir pith is low in nitrogen content, C: N ratio mounting to 112:1 (Nagarajan et al., 1985). Microbial degradation of this waste is generally considered to be safe, effective and an environmental friendly process and certain mushrooms have showed good potentials for degrading coir pith (Vijaya et al., 2008).

Nitrogen fixation can be considered as one of the most interesting microbial activity as it makes the recycling of nitrogen on earth possible and gives a fundamental contribution to nitrogen homeostasis in the biosphere (Aquilantia et al., 2004). It is the reduction of N_2 (atmospheric nitrogen) to NH_3 (ammonia). Free living prokaryotes with the ability to fix atmospheric dinitrogen (diazotrophs) are ubiquitous in soil. In natural eco-systems, biological nitrogen fixation is the most important source of nitrogen. The capacity for nitrogen fixation is

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widespread among bacteria. The estimated contribution of free living N-fixing prokaryotes to the nitrogen input of soil ranges from 0 to 60 kg/ha/year (Burgmann et al., 2003). In recent years, many studies have addressed the importance and contribution of biological nitrogen fixation in ecologically unique terrestrial and aquatic habitats by focusing on the diversity of *nif*H sequences (Zehr et al., 2003). Such studies have provided a rapidly expanding database of *nif*H sequences and revealed a wide diversity of uncultured diazotrophs (Tan et al., 2003).

Plant-associated nitrogen fixing bacteria have been considered as one of the possible alternatives for inorganic nitrogen fertilizer for promoting plant growth and yield (Ladha and Reddy, 2000). A variety of nitrogen fixing bacteria like *Acetobacter, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Derxia, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas* and *zoogloea* have been isolated from the rhizosphere of various crops (Barraquio et al., 2000). A significant reduction in the use of nitrogen-fertilizer could be achieved if biological nitrogen fixation is made available to crop plants (Dawe, 2000). Nitrogenous fertilizers are one of the most widely used chemical fertilizers, as deficiency of nitrogen in the soil often limits crop yields. Consumption of nitrogen fertilizer in Asia has increased from 1.5 to 47 million tones (mt) during the last 35 years (Dawe, 2000). Only less than 50% of the added nitrogen is available to the plants. The enzymatic reduction of nitrogen to ammonia replenishes the loss of nitrogen from soil-plant ecosystems and is achieved through biological nitrogen fixation. Diazotrophs in the soil are the main source of nitrogen input in primary production eco-systems (Cleveland, 1999).

Nitrogen fixers in the environment are diverse. Bacteria of the genus *Azospirillum* are well-known examples of so-called associative nitrogen fixers, which are widespread in the soils of tropical, subtropical and temperate regions. These bacteria develop close relationships with the roots of various wild and agricultural plants (Tyler et al., 1979; Steenhoubt and Vanderleyden, 2000). The studies of these microorganisms carried out over the last few decades have primarily been aimed at gaining insight into the molecular nature of plant-microbial interactions in order to develop efficient modern genetic and agricultural biotechnologies (Burdman et al., 2001; Fedonenko et al., 2001). Associative nitrogen fixing bacteria such as *Azospirillum brasilense, Herbaspirillum seropedicae* and *Acetobacter diazotrophicus* may benefit their host plants as nitrogen biofertilizers and plant growth promoters. The latter two organisms were the first nitrogen-fixing bacteria suggested to be endophytes (Baldani et al., 1997; James and Oliveres, 1997). *A. lipoferum* and *A. brasilense* were for long the only known members of the genus *Azospirillum (*Tarrand et al., 1978).

A large number of *nif*H primers have been designed to study the diversity of diazotrophs (Poly et al, 2001; Rosch et al., 2002; Widmer et al., 1999; Shaffer et al., 2000). All nitrogen fixers carry a *nif*H gene that encodes the Fe protein of the nitrogenase. In this study, the structure of the *nif*H gene pool was investigated by RFLP analysis of the *nif*H gene, which has been amplified from DNA directly extracted from soil samples (Poly et al., 2001) and other techniques, such as PCR cloning (Zehr et al., 1995, 1998). The *nif*H genes are very diverse, some of them are characteristic of an ecological niche (Chelius and Lepo, 1999; Shaffer et al., 2000), which shows the habitats of soil nitrogen fixing bacteria and the structure of *nif*H gene pools relationships.

MATERIALS AND METHODS

Sampling location

Samples of coir pith were collected from the accumulated heap in the Alappuzha district of Kerala in India. The samples were randomly collected in sterile plastic bags and stored at 4°C in the laboratory for the further experiments.

Isolation of nitrogen fixing bacteria

General plating techniques were followed for screening and isolation. Individual colonies were picked, purified and assayed as pure cultures for nitrogenase activity using N-deficient medium. Pure cultures of nitrogen fixing isolates were readily obtained by repeated sub-culturing and confirmed through Gram staining technique.

Extraction and analysis of DNA

Genomic DNA was obtained by using standard bacterial procedure (Sambrook et al., 1989).

The DNA stock samples were quantified by UV spectro-photometer at 260 and 280 nm using the convention that one absorbance unit at 260 nm wavelength equals 50 µg DNA per ml. The absorbance in the UV range of 260 and 280 nm were studied for determination of DNA concentration and purity. Purity of DNA was confirmed on the basis of optical density ratio at 260:280 nm. The quality of DNA was further confirmed using agarose gel electrophoresis (Naniatis et al., 1982). 16S rDNA fragment was amplified by PCR from the bacterial genomic DNA using 16s rDNA universal primers (10 to 30 F: 5′ –GAG TTT GAT CCT GGC TCA G-3′ and 530 R: 5′-G(AT)A TTA CCG CGG CGG CTG-3′).

PCR amplification of the *nif*H gene fragment

One hundred nanogram of DNA were used as template in PCR. Selected primers *Nif*H for-5' TAYGGNAARGGNGGHATYGGYATC and *Nif*H rev -5' ATRTTRTTNGCNGCRTAVABBGCCATCAT were used to amplify (Poly et al., 2001). PCR was carried out in a final reaction volume of 25 µL in 200 µL capacity thin wall PCR tubes. The PCR tubes with all the components were transferred to thermal cycler.

The thermo cycling conditions consisted of an initial denaturation step at 94°C for 3 min, 30 amplification cycles of 45 s at 94°C, 30 s at 55°C, 60 s at 72°C and a final extension step at 72°C for 5 min with Gene Amp PCR system (Perkin-Elmer Co., Norwalk, Conn.).

Analysis of DNA amplification by AGE

Commercially available 100 bp ladder was used as standard molecular weight DNA. Analysis of the PCR products was carried out by electrophoresis. Electrophoresis was done by 5 µL of PCR product with 4 µL bromophenol blue (loading dye) in agarose gels (1.5%). The voltage of 100 V and current of 45 A for a period of 1 h 20 min till the bromophenol blue travelled 6 cm from the wells was applied. We viewed the gels on UV transilluminator and photographed the gel for documentation.

Purification and DNA sequencing of samples

Amplified PCR product was purified using column purification as per manufacturer's guidelines (Thermo Scientific, Fermentas Molecular Biology Tools). The isolated DNA having ratio between 1.8 to 2.0 can be considered to be of good purity and further used for sequencing reaction.

Sequencing of purified 16SrDNA gene segment

The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on ABI3730xl genetic analyzer (Applied Biosystems, USA).

16S rRNA sequence analysis

Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequences at the 3′ and 5′ ends (considering peak and quality values for each base) using the sequence analysis tools. The edited sequences (16S rDNA) were then used for similarity searches using Basic Local Alignment Search Tool (BLAST) programme in the NCBI GenBank (www.ncbi.nlm.nih.gov) DNA database for identifying the bacterial strains. The phylogenetic tree was constructed by the methods implemented in the TREECONW software package.

Sequence deposition

The 16s rRNA, nifH gene fragments of the strain NF4, NF7, NF12 and NF18 have been deposited in the GenBank under the accession numbers JN230510, JN230511, JN230512 and JN230513, respectively.

RESULTS AND DISCUSSION

Coir pith is a rigid fluffy material and in the present study, an attempt was made to isolate microorganisms in the coir pith samples drawn from accumulated heaps of coir pith on coir fiber extraction units stored for the past 3 years without any type of treatment (Figure 6). Four nitrogen fixing bacteria composed of different physiological and biochemical characters were isolated and sequenced. Out of the 19 isolated colonies, four strains were amplified with *nifH* gene *viz*. NF-4 (*Lysinibacillus sp.*), NF-7 (*Ochrobactrum sp*), NF-12 (*Paenibacillus sp*) and an uncultured bacterial clone was isolated which shows only 50% similarity to NF-18 (*Clostridium sp.*). By repeated plating on to nitrogen deficient agar media, pure cultures of the four bacterial colonies were obtained. High molecular weight DNA was observed in agarose gel evaluation.16s rDNA fragment was amplified by PCR from genomic DNA using 16S rDNA universal primer. Column purification yielded contaminant free PCR product.

A large number of *nifH* primers were designed to study the diversity of diazotrophs (Poly et al., 2001; Rosch et al., 2002; Widmer et al., 1999; Shaffer et al., 2000). Here, the design of the appropriate primers was done with utmost priority for the novel primers, otherwise the use of highly generated primers in combination with low stringency amplification conditions could result in biased conclusions. In the present study, predesigned degenerated primers were used for the coir pith based micro flora analysis viz. *Nif*H for-5' TAYGGNAARGGN-GGHATYGGYATC and NifH rev -5' ATRTTRTTN-GCNGCRTAVABBGCCATCAT (Edwards et al., 1989). The *nif*H primers were designed from the available *nif*H sequences of different organisms from NCBI GenBank (www.ncbi.nlm.nih.gov). After the amplification of microbial DNA from coir pith with *nif* H primers (Figure 1), the edited sequences (16s rDNA) were then subjected to the similarity searches using BLAST programme (Table 1). The BLAST results show that the four cultures have greater similarity with *Lysinibacillus* sp., *Ochrobactrum* sp., *Paenibacillus* sp. and *Clostridium* sp. The results have been furnished in Figures 2 to 5.

Coir pith is very slow in microbial decomposition due to the presence of lignin and accumulates in coir fiber extraction units. The microflora inhabiting coir pith is therefore limited, as the lignocellulose complex resists biodegradation. Herein, an effort is made here to isolate the microorganisms in coir pith which possess the nitrogen fixing ability. The DNA isolated from the natural microflora in coir pith showed similarities with *Lysinibacillus* sp., *Ochrobactrum* sp., *Paenibacillus* sp. and *Clostridium* sp.

These bacteria are important nitrogen fixing species and most of them have other applications too. It is observed that activities that have been found to be associated with *P. polymyxa* treatment on plants in field experiments include nitrogen fixation, soil phosphorous solubilization, production of antibiotics, auxins, cytokinins, chitinase and hydrolytic enzymes, as well as the promotion of increased soil porosity (Timmusk and Wagner, 1999; Timmusk et al., 1999).

All these activities might be of importance for plant growth promotion. Timmusk et al. (2009) reported *P. polymyxa* B2, B5 and B6 antagonistic mechanisms against the well characterized model of oomycetic pathogens *Phytophthora palmivora* and *Pythium aphanidermatum. P. polymyxa* (previously *Bacillus polymyxa*; Ash et al., 1994) is a common soil bacterium belonging to plant growth promoting rhizobacteria

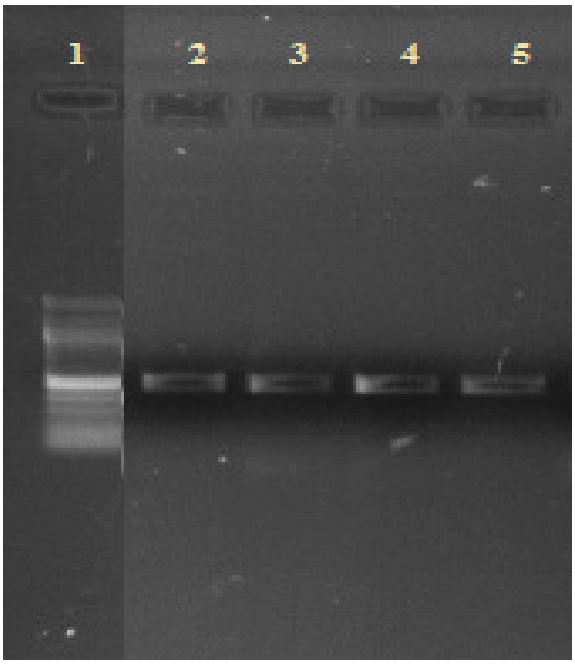
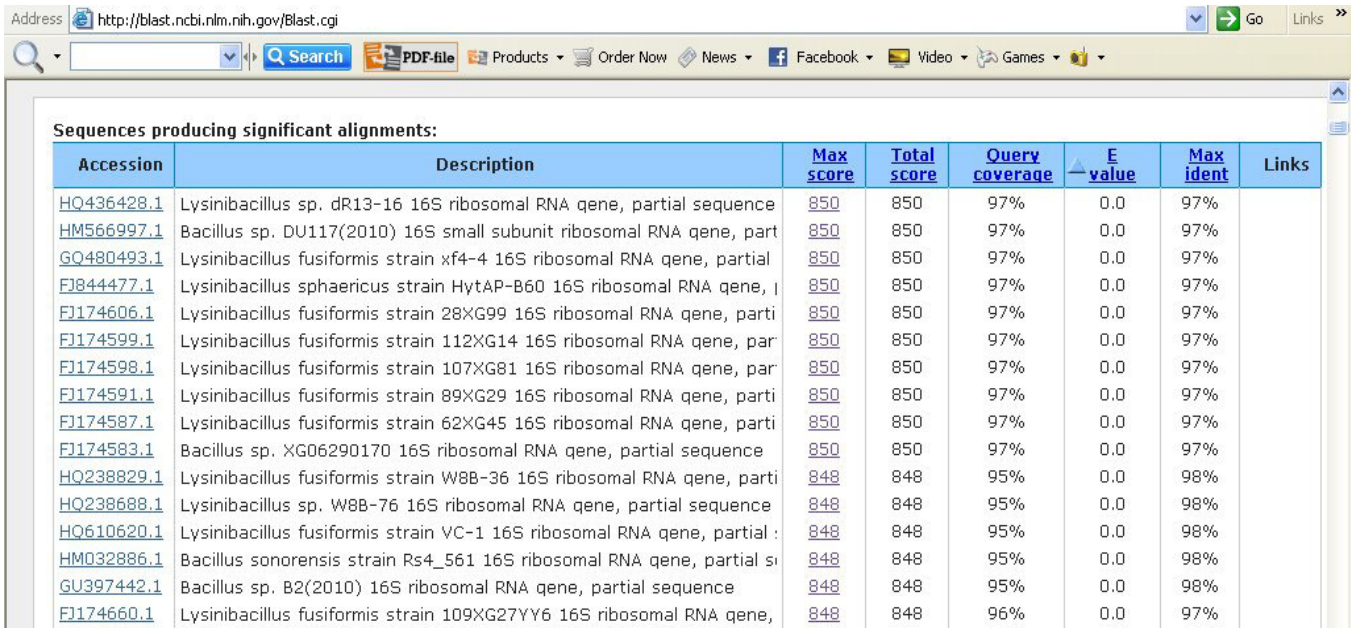


Figure 1. Amplification of coir pith DNA with *nifH* primers. Lane 1, 100 bp molecular weight marker, lane 2, NF-4; lane 3, NF-7; lane 4, NF-12 and lane 5, NF-18.

Table 1. Blast results of top two genes showed maximum similarity.

Organisms	Accession No	Description	Maximum identity (%)
Lysinibacillus sp.	FJ 174660.1	*Lysinibacillus fusiformis* strain 109XG27YY6 16S ribosomal RNA gene, Partial sequence.	97
	HM 032886.1	*Bacillus sonorensis* strain 16S ribosomal RNA gene, Partial sequence.	98
Ochrobactrum sp.	HM 629806.1	*Ochrobactrum sp*. BE3. 16S ribosomal RNA gene, Partial sequence.	96
	EU 301689.1	*Ochrobactrum tritici*. 16S ribosomal RNA gene, Partial sequence.	96
Paenibacillus sp.	EU 912456.1	*Paenibacillus sp.* BL18-3-2. 16S ribosomal RNA gene, Partial sequence.	98
	FJ 468006.1	*Paenibacillus polymyxa* strain. MS 0102 16S ribosomal RNA gene, Partial sequence.	98
Clostridium sordellii sp.	HP 259293.1	*Clostridium sordellii* strain MA2 16S ribosomal RNA gene, Partial sequence.	85
	HQ 259292.1	*Clostridium sordellii* MA1 16S ribosomal RNA gene, Partial sequence.	85

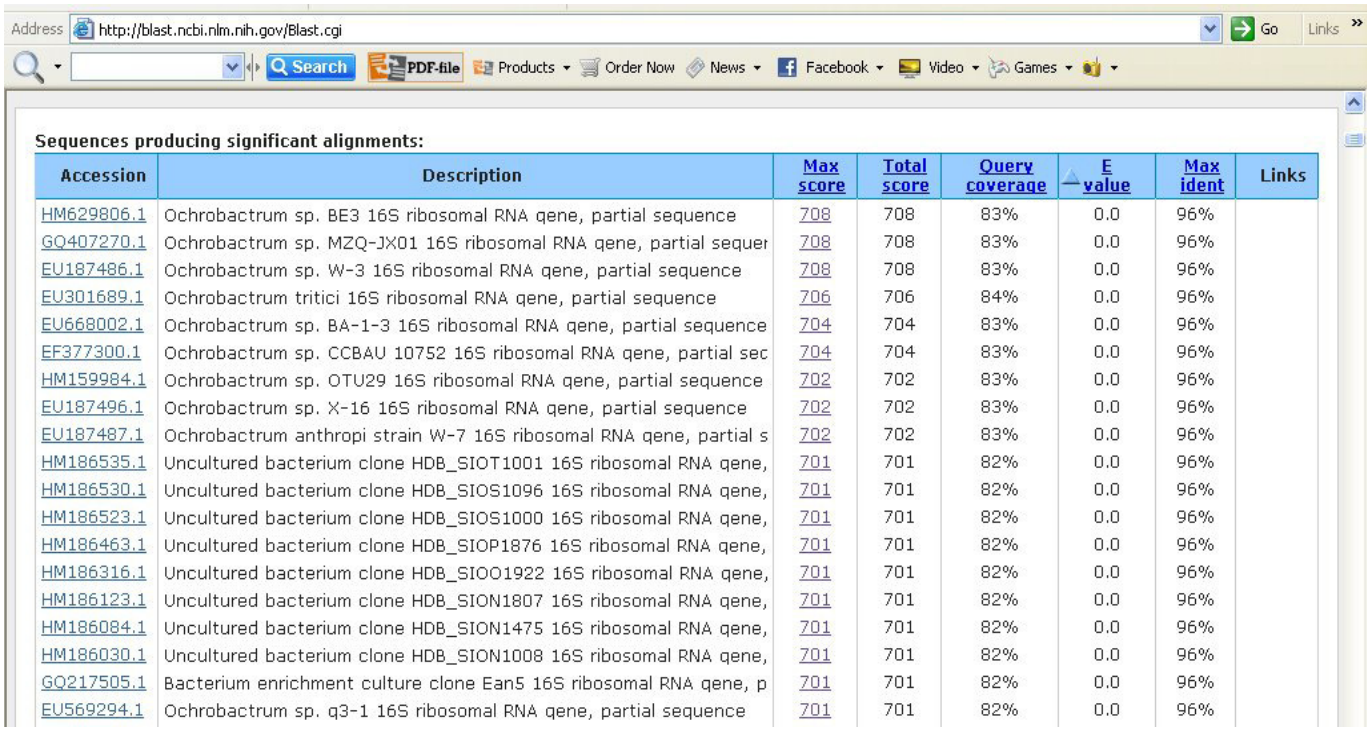
(PGPR) also present in the coir pith sample. The activities associated with *P. polymyxa* include nitrogen fixation (Heulin et al., 1994), soil phosphorous solubilization (Jisha and Alagawadi, 1996), as well as promotion of increased soil porosity (Gouzou et al., 1993; Timmusk and Wagner, 1999; Timmusk et al., 1999). Besides, it produces antimicrobial substances active against fungi and bacteria (Rosado and Seldin, 1993; Picard et al., 1995; Kajimura and Kaneda, 1996). *P. polymyxa* also has been used for the control of plant



Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
HQ436428.1	Lysinibacillus sp. dR13-16 16S ribosomal RNA gene, partial sequence	850	850	97%	0.0	97%	
HM566997.1	Bacillus sp. DU117(2010) 16S small subunit ribosomal RNA gene, part	850	850	97%	0.0	97%	
GQ480493.1	Lysinibacillus fusiformis strain xf4-4 16S ribosomal RNA gene, partial	850	850	97%	0.0	97%	
FJ844477.1	Lysinibacillus sphaericus strain HytAP-B60 16S ribosomal RNA gene,	850	850	97%	0.0	97%	
FJ174606.1	Lysinibacillus fusiformis strain 28XG99 16S ribosomal RNA gene, parti	850	850	97%	0.0	97%	
FJ174599.1	Lysinibacillus fusiformis strain 112XG14 16S ribosomal RNA gene, par	850	850	97%	0.0	97%	
FJ174598.1	Lysinibacillus fusiformis strain 107XG81 16S ribosomal RNA gene, par	850	850	97%	0.0	97%	
FJ174591.1	Lysinibacillus fusiformis strain 89XG29 16S ribosomal RNA gene, parti	850	850	97%	0.0	97%	
FJ174587.1	Lysinibacillus fusiformis strain 62XG45 16S ribosomal RNA gene, parti	850	850	97%	0.0	97%	
FJ174583.1	Bacillus sp. XG06290170 16S ribosomal RNA gene, partial sequence	850	850	97%	0.0	97%	
HQ238829.1	Lysinibacillus fusiformis strain W8B-36 16S ribosomal RNA gene, parti	848	848	95%	0.0	98%	
HQ238688.1	Lysinibacillus sp. W8B-76 16S ribosomal RNA gene, partial sequence	848	848	95%	0.0	98%	
HQ610620.1	Lysinibacillus fusiformis strain VC-1 16S ribosomal RNA gene, partial	848	848	95%	0.0	98%	
HM032886.1	Bacillus sonorensis strain Rs4_561 16S ribosomal RNA gene, partial s	848	848	95%	0.0	98%	
GU397442.1	Bacillus sp. B2(2010) 16S ribosomal RNA gene, partial sequence	848	848	95%	0.0	98%	
FJ174660.1	Lysinibacillus fusiformis strain 109XG27YY6 16S ribosomal RNA gene,	848	848	96%	0.0	97%	

Figure 2. Blast result of Culture 4. Based on the 16s rDNA analysis, the culture 4 showed 97% similarity with *Lysinibacillus* sp. (accession no: HQ436428.1).



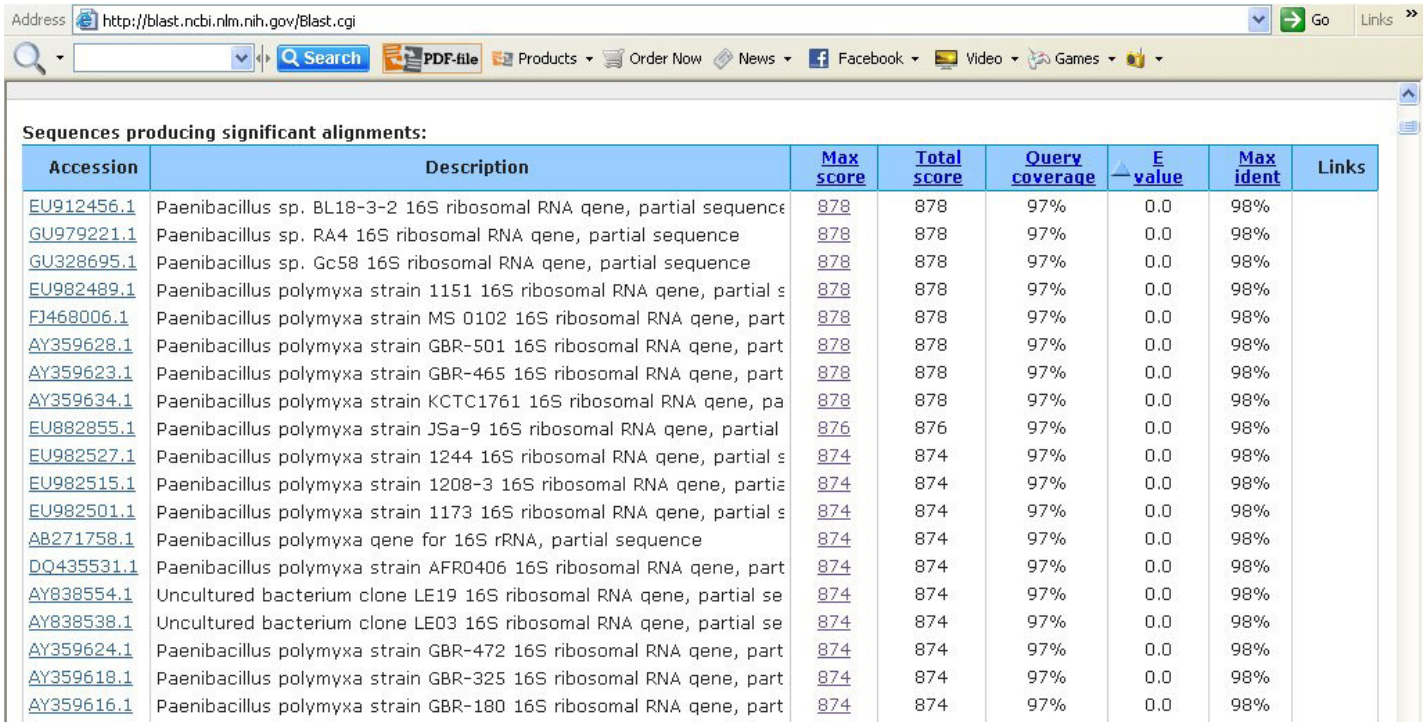
Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
HM629806.1	Ochrobactrum sp. BE3 16S ribosomal RNA gene, partial sequence	708	708	83%	0.0	96%	
GQ407270.1	Ochrobactrum sp. MZQ-JX01 16S ribosomal RNA gene, partial sequer	708	708	83%	0.0	96%	
EU187486.1	Ochrobactrum sp. W-3 16S ribosomal RNA gene, partial sequence	708	708	83%	0.0	96%	
EU301689.1	Ochrobactrum tritici 16S ribosomal RNA gene, partial sequence	706	706	84%	0.0	96%	
EU668002.1	Ochrobactrum sp. BA-1-3 16S ribosomal RNA gene, partial sequence	704	704	83%	0.0	96%	
EF377300.1	Ochrobactrum sp. CCBAU 10752 16S ribosomal RNA gene, partial sec	704	704	83%	0.0	96%	
HM159984.1	Ochrobactrum sp. OTU29 16S ribosomal RNA gene, partial sequence	702	702	83%	0.0	96%	
EU187496.1	Ochrobactrum sp. X-16 16S ribosomal RNA gene, partial sequence	702	702	83%	0.0	96%	
EU187487.1	Ochrobactrum anthropi strain W-7 16S ribosomal RNA gene, partial s	702	702	83%	0.0	96%	
HM186535.1	Uncultured bacterium clone HDB_SIOT1001 16S ribosomal RNA gene,	701	701	82%	0.0	96%	
HM186530.1	Uncultured bacterium clone HDB_SIOS1096 16S ribosomal RNA gene,	701	701	82%	0.0	96%	
HM186523.1	Uncultured bacterium clone HDB_SIOS1000 16S ribosomal RNA gene,	701	701	82%	0.0	96%	
HM186463.1	Uncultured bacterium clone HDB_SIOP1876 16S ribosomal RNA gene,	701	701	82%	0.0	96%	
HM186316.1	Uncultured bacterium clone HDB_SIOO1922 16S ribosomal RNA gene,	701	701	82%	0.0	96%	
HM186123.1	Uncultured bacterium clone HDB_SION1807 16S ribosomal RNA gene,	701	701	82%	0.0	96%	
HM186084.1	Uncultured bacterium clone HDB_SION1475 16S ribosomal RNA gene,	701	701	82%	0.0	96%	
HM186030.1	Uncultured bacterium clone HDB_SION1008 16S ribosomal RNA gene,	701	701	82%	0.0	96%	
GQ217505.1	Bacterium enrichment culture clone Ean5 16S ribosomal RNA gene, p	701	701	82%	0.0	96%	
EU569294.1	Ochrobactrum sp. q3-1 16S ribosomal RNA gene, partial sequence	701	701	82%	0.0	96%	

Figure 3. Blast result of Culture 7. Based on the 16s rDNA analysis, the culture 7 showed 96% similarity with *Ochrobactrum* sp. (accession no: HM629806.1).

disease (Mavingui and Heulin, 1994; Kim, 1995; Shishido et al., 1996; Dijksterhuis et al., 1999; Kharbanda et al., 1999). It displayed potent antimicrobial properties against both Gram negative and Gram positive pathogenic bacteria. The antimicrobials produced by this strain were isolated from the fermentation broth and subsequently analyzed by liquid chromatography-mass spectrometry.

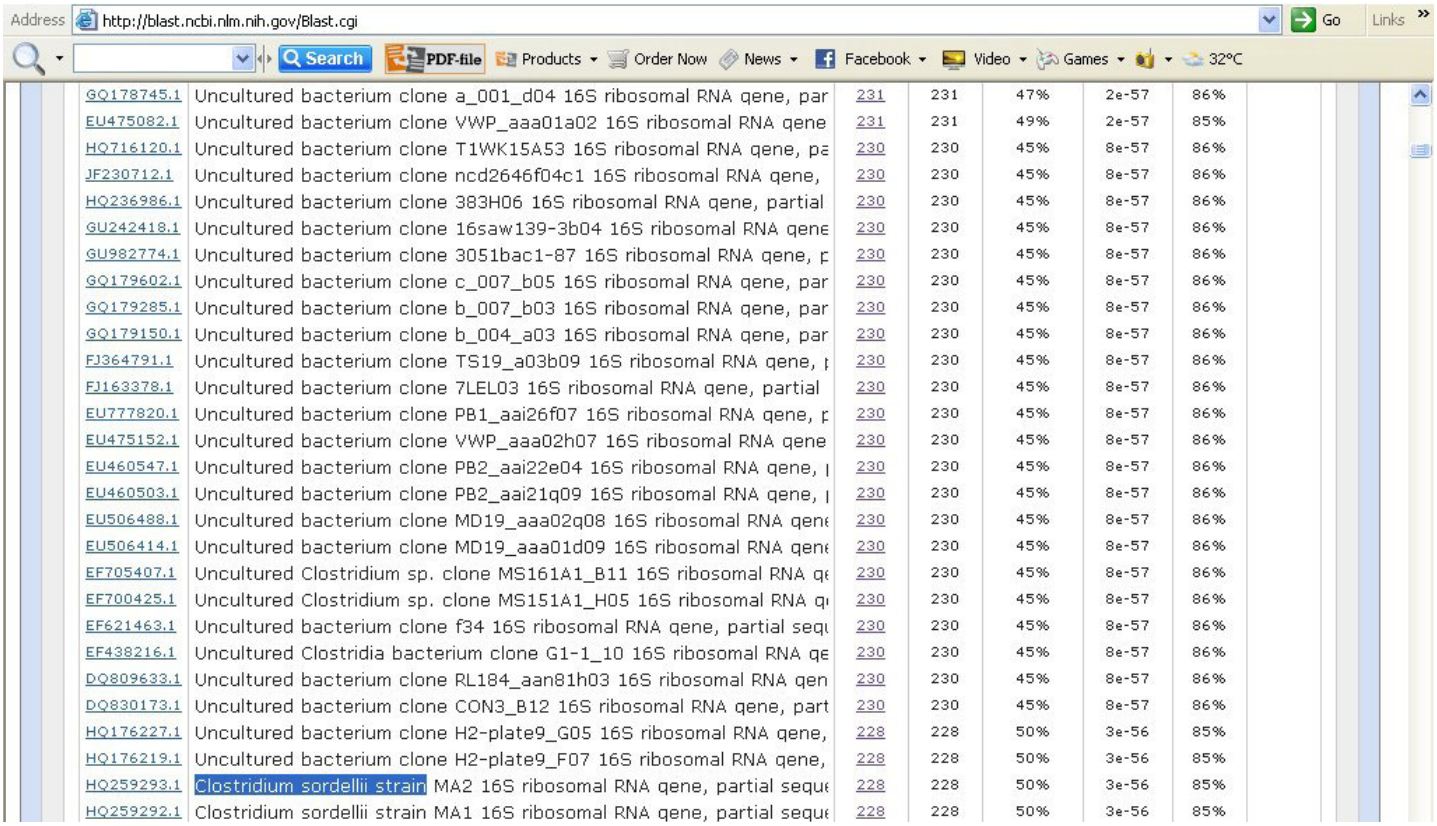
Another important bacteria isolated from the coir pith



Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
EU912456.1	Paenibacillus sp. BL18-3-2 16S ribosomal RNA gene, partial sequence	878	878	97%	0.0	98%	
GU979221.1	Paenibacillus sp. RA4 16S ribosomal RNA gene, partial sequence	878	878	97%	0.0	98%	
GU328695.1	Paenibacillus sp. Gc58 16S ribosomal RNA gene, partial sequence	878	878	97%	0.0	98%	
EU982489.1	Paenibacillus polymyxa strain 1151 16S ribosomal RNA gene, partial s	878	878	97%	0.0	98%	
FJ468006.1	Paenibacillus polymyxa strain MS 0102 16S ribosomal RNA gene, part	878	878	97%	0.0	98%	
AY359628.1	Paenibacillus polymyxa strain GBR-501 16S ribosomal RNA gene, part	878	878	97%	0.0	98%	
AY359623.1	Paenibacillus polymyxa strain GBR-465 16S ribosomal RNA gene, part	878	878	97%	0.0	98%	
AY359634.1	Paenibacillus polymyxa strain KCTC1761 16S ribosomal RNA gene, pa	878	878	97%	0.0	98%	
EU882855.1	Paenibacillus polymyxa strain JSa-9 16S ribosomal RNA gene, partial	876	876	97%	0.0	98%	
EU982527.1	Paenibacillus polymyxa strain 1244 16S ribosomal RNA gene, partial s	874	874	97%	0.0	98%	
EU982515.1	Paenibacillus polymyxa strain 1208-3 16S ribosomal RNA gene, partia	874	874	97%	0.0	98%	
EU982501.1	Paenibacillus polymyxa strain 1173 16S ribosomal RNA gene, partial s	874	874	97%	0.0	98%	
AB271758.1	Paenibacillus polymyxa gene for 16S rRNA, partial sequence	874	874	97%	0.0	98%	
DQ435531.1	Paenibacillus polymyxa strain AFR0406 16S ribosomal RNA gene, part	874	874	97%	0.0	98%	
AY838554.1	Uncultured bacterium clone LE19 16S ribosomal RNA gene, partial se	874	874	97%	0.0	98%	
AY838538.1	Uncultured bacterium clone LE03 16S ribosomal RNA gene, partial se	874	874	97%	0.0	98%	
AY359624.1	Paenibacillus polymyxa strain GBR-472 16S ribosomal RNA gene, part	874	874	97%	0.0	98%	
AY359618.1	Paenibacillus polymyxa strain GBR-325 16S ribosomal RNA gene, part	874	874	97%	0.0	98%	
AY359616.1	Paenibacillus polymyxa strain GBR-180 16S ribosomal RNA gene, part	874	874	97%	0.0	98%	

Figure 4. Blast result of Culture 12. Based on the 16s rDNA analysis, the culture 12 showed 98% similarity with *Paenibacillus sp.* (accession no: EU912456.1).



GQ178745.1	Uncultured bacterium clone a_001_d04 16S ribosomal RNA gene, par	231	231	47%	2e-57	86%
EU475082.1	Uncultured bacterium clone VWP_aaa01a02 16S ribosomal RNA gene	231	231	49%	2e-57	85%
HQ716120.1	Uncultured bacterium clone T1WK15A53 16S ribosomal RNA gene, pa	230	230	45%	8e-57	86%
JF230712.1	Uncultured bacterium clone ncd2646f04c1 16S ribosomal RNA gene,	230	230	45%	8e-57	86%
HQ236986.1	Uncultured bacterium clone 383H06 16S ribosomal RNA gene, partial	230	230	45%	8e-57	86%
GU242418.1	Uncultured bacterium clone 16saw139-3b04 16S ribosomal RNA gene	230	230	45%	8e-57	86%
GU982774.1	Uncultured bacterium clone 3051bac1-87 16S ribosomal RNA gene, p	230	230	45%	8e-57	86%
GQ179602.1	Uncultured bacterium clone c_007_b05 16S ribosomal RNA gene, par	230	230	45%	8e-57	86%
GQ179285.1	Uncultured bacterium clone b_007_b03 16S ribosomal RNA gene, par	230	230	45%	8e-57	86%
GQ179150.1	Uncultured bacterium clone b_004_a03 16S ribosomal RNA gene, par	230	230	45%	8e-57	86%
FJ364791.1	Uncultured bacterium clone TS19_a03b09 16S ribosomal RNA gene, p	230	230	45%	8e-57	86%
FJ163378.1	Uncultured bacterium clone 7LEL03 16S ribosomal RNA gene, partial	230	230	45%	8e-57	86%
EU777820.1	Uncultured bacterium clone PB1_aai26f07 16S ribosomal RNA gene, p	230	230	45%	8e-57	86%
EU475152.1	Uncultured bacterium clone VWP_aaa02h07 16S ribosomal RNA gene	230	230	45%	8e-57	86%
EU460547.1	Uncultured bacterium clone PB2_aai22e04 16S ribosomal RNA gene, p	230	230	45%	8e-57	86%
EU460503.1	Uncultured bacterium clone PB2_aai21q09 16S ribosomal RNA gene, p	230	230	45%	8e-57	86%
EU506488.1	Uncultured bacterium clone MD19_aaa02q08 16S ribosomal RNA gene	230	230	45%	8e-57	86%
EU506414.1	Uncultured bacterium clone MD19_aaa01d09 16S ribosomal RNA gene	230	230	45%	8e-57	86%
EF705407.1	Uncultured Clostridium sp. clone MS161A1_B11 16S ribosomal RNA ge	230	230	45%	8e-57	86%
EF700425.1	Uncultured Clostridium sp. clone MS151A1_H05 16S ribosomal RNA ge	230	230	45%	8e-57	86%
EF621463.1	Uncultured bacterium clone f34 16S ribosomal RNA gene, partial seq	230	230	45%	8e-57	86%
EF438216.1	Uncultured Clostridia bacterium clone G1-1_10 16S ribosomal RNA ge	230	230	45%	8e-57	86%
DQ809633.1	Uncultured bacterium clone RL184_aan81h03 16S ribosomal RNA gen	230	230	45%	8e-57	86%
DQ830173.1	Uncultured bacterium clone CON3_B12 16S ribosomal RNA gene, part	230	230	45%	8e-57	86%
HQ176227.1	Uncultured bacterium clone H2-plate9_G05 16S ribosomal RNA gene,	228	228	50%	3e-56	85%
HQ176219.1	Uncultured bacterium clone H2-plate9_F07 16S ribosomal RNA gene,	228	228	50%	3e-56	85%
HQ259293.1	Clostridium sordellii strain MA2 16S ribosomal RNA gene, partial sequ	228	228	50%	3e-56	85%
HQ259292.1	Clostridium sordellii strain MA1 16S ribosomal RNA gene, partial sequ	228	228	50%	3e-56	85%

Figure 5. Blast result of Culture 18. Based on the 16s rDNA analysis, the culture 18 showed 85% similarity with *Clostridium sordellii*. (Accession No: HQ259293.1)

Figure 6. Coir pith heaps accumulating in fiber extraction units.

sample was *Ochrobactrum* sp., which has several properties of importance. The genus *Ochrobactrum* was described first by Homes et al. (1988) and belongs to the α-2 subclass of the Proteobacteria (De Ley, 1992). *Ochrobactrum tritici* was identified as Bacterial strain 5bv11, isolated from a chromium-contaminated waste water treatment plant, which is resistant to a broad range of antibiotics and metals viz. Cr (VI), Ni (II), Co (II), Cd (II) and Zn (II) (Branco et al*.,* 2004). Holmes et al. (1988) proposed *Ochrobactrum anthropi* as a sole and type species of *Ochrobactrum*, but they observed heterogeneities in geno- or phenotypic characters within the tested *O. anthropi* collection. *O. anthropi* strains have been isolated from samples originating from different continents. *Ochrobactrum sp*. contains root associated bacteria that enter bivalent interactions with plant and human hosts. Several members of these genera show plant growth promoting as well as excellent antagonistic properties against plant pathogens and were therefore utilized for the development of biopesticides (Weller, 1988; Whipps, 2001).

Most available *O. anthropi* isolates are from human clinical specimens (Lebuhn et al., 2000). *O. anthropi* LMG 5140 has also been isolated from arsenical cattle dipping fluid (Holmes et al., 1988). Moreover, there are some reports on the presence of *O. anthropi* in soil, on wheat roots and in internal root tissues of different plants (Anguillera et al., 1993; McInroy and Kloepper, 1994; Sato and Jiang, 1996).

More also, NF-4(*Lysinibacillus* sp.) could be isolated from the coir pith samples. It is described by Ahmed et al. (2007) as spore forming, Gram-positive, motile, rod shaped and boron-tolerant. A large number of Bacillus strains, including *B. fusiformis* capable of degrading different hydrocarbons, have been isolated from oil contaminated soils (Bento et al*.,* 2003). It is also considered as a growth promoting agent. In addition to all the useful organisms, some pathogenic microorganisms could also be observed with 50% possibility.

Conclusion

The results of the study indicated that coir pith is a source of nitrogen fixing bacteria and other useful microorganisms. Coir pith, which was considered as a problematic waste, has been found to harbor useful microorganisms with potential use as plant nutrient. Coir pith can be used as good organic manure after supplementing with efficient nitrogen fixing bacteria. It can therefore be concluded that the coir pith has great potential for use as a source of many useful microorganisms including nitrogen fixing bacteria.

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