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ORIGINAL RESEARCH ARTICLE

Genetic Analysis of Plastic Degrading Bacterial Strains

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ABSTRACT

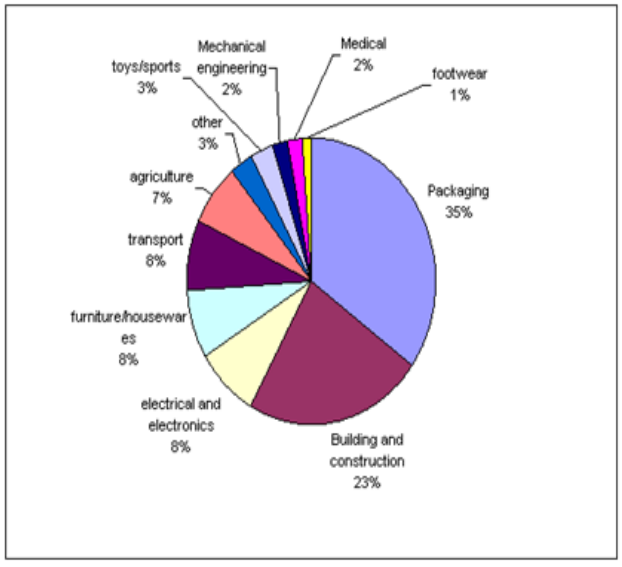
Though plastics have become indigenous, now days, they are very hazardous and should be disposed off, properly. Land filling, incineration and recycling are the most common methods employed for the disposal of plastics and all methods have their own environmental and health hazards. So, biodegradation will be the right choice for the proper disposal of plastic wastes. Soil samples from the compost yard have the rich consortia of biodegrading microbes. These samples were inoculated into mineral salt medium with plastic as the sole carbon source for the isolation of the plastic degrading strains. Characterization of bacterial strains was done based on molecular characterization. To ensure that plastic degradation is plasmid mediated, the plasmid was isolated and purified by the alkaline lysis method from these strains and after plasmid curing they were found to lose this property. Transformed strains showed the ability to degrade plastics.

Key words: Land filling, incineration, recycling, biodegradation, molecular characterization.

INTRODUCTION

Plastics are widely employed in the variety of uses. Out of which, packaging represents the largest single sector of plastics almost in all the nations. The sector accounts 35% of plastics consumption which is the highest, when compared to other usages (APME, 1999). Worldwide Plastics Industry witnessed a steady growth in the year 2007.

USES OF PLASTICS



Asia has been world's largest plastics consumer for several years, accounting for about 30% of the global consumption excluding Japan, which has share of about 6.5%. Next to Asia is North America with 26% share, then Western Europe with 23% share in the global market.

Due to the indigenous use of plastics, the plastic waste stream emerges from domestic, industrial and municipal refuse (Jayasekara *etal.*, 2005). The plastic waste is disposed off through land filling, incineration, recycling and degradation (Aamer Ali Shah, 2007). Out of these, first three methods have a number of demerits and hazards.

Microorganisms such as bacteria fungi and actinomycetes are involved in the degradation of both natural and synthetic plastics (Gu *et al*, 2000a).

A wide variety of actinomycetes like Streptomyces strains and fungi like *Aspergillus* sp and *Penicillium* sp are reported active against polyethylene(PE) (Zheng *et.al*, 2005).

Curvalaria sp, *Fusarium* sp, *Aureobasidium* sp, *Cladosporium* sp, *Pseudomonas* sp and *Comamonas* sp are found to be effective against polyurethane (Zheng *et.al*, 2005).

Pseudonocardiaceae sp, *Micromonosporaceae* sp, Thermonosporaceae sp (Tokiwa and Jarerat, 2003) *Bacillus* sp, TT96, *Aspergillus* sp and

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Streptomyces sp (Sanchez *et.al.* 2000) are effective against PHA.

MATERIALS AND METHODS

PLASTIC FILMS

High density polyethylene (HDPE) and Low density polyethylene (LDPE) which are widely used to manufacture carry bags, milk and oil pouches were used for the study (Gosh *et.al.*, 2004).

ENRICHMENT OF PE DEGRADING MICROORGANISMS

Soil samples were collected from a plastic dumpsite inside Madras Christian college campus, Chennai. A total 1g of the soil sample was suspended in 10ml of sterile Milli-Q water and vortexed for 15 minutes. Nearly 100 µl of suspension was used as inoculums. Erlenmeyer flasks containing 100 ml of mineral salt medium, strips of untreated polyethylene, 0.01 %(w/v) glucose and 1 ml of inoculums were used for maintaining the first pre-culture. Thee later subcultures did not contain glucose but only the polymer as the sole carbon source. After three successive sub-culture, in which microorganisms were grown on Polyethylene (PE) and without glucose, pure cultures were isolated on Nutrient agar plates (Himedia Limited, Mumbai, India) for bacterial isolation (Artham.T& Doble.M, 2008).

I. Identification of PE degrading bacterial strains

The molecular characterization of the bacterial isolates was done based on 16s rDNA sequencing. Amplification of 16s rDNA fragment of the strains were performed with 125–1, 562 ng of bacterial DNA as template using 0.4 µM of 8f (AGAGTTTGATCCTGGCTCAG) and 0.4 µM of 1492r (GGTTACCTTGTTACGACTT) eubacterial universal primers [Mohana,s., et al 2005, Eden, P.A., *et al*, 1991, Weisburg, W.G., *et al*,1991] in a reaction mixture which contained 1.5 mM MgCl2, 0.3 mM each of dNTPs, and 2.5 U of Taq polymerase (InvitroGene), in a What man Biometra® thermo cycler. The polymerase chain reaction (PCR) program included an initial denaturation of 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1.5 min, and a final extension at 72°C for 15 min. The amplified product was resolved on 1% (w/v) agarose gel. PCR product elution was carried out with Wizard SV Gel and PCR Cleanup System (Promega). The nucleotide sequence was determined manually using the dideoxynucleotide chain termination method [Thermo Sequenase kit (Amersham)] or automatically using BigDye® Terminator v3.1 Cycle Sequencing Kit in the ABI PRISM 3100-Avant Genetic Analyzer with universal and reverse primers. DNA sequence analyses were performed using the Blast and Bioedit programs.(Center for Cellular & Molecular Biology, Hyderabad) . The isolates were identified based on the similarity scores and the sequences were submitted to NCBI Gen Bank to get accession number (Soni.R , *et al*., 2008).

II. Screening and Curing of plasmid from bacterial strains

The bacterial strains used in the study were isolated by the selective enrichment method and maintained in the laboratory (Mashetty *et al*., 1996). The bacterial capable of degrading PE were initially grown on a mineral salts medium amended with 0.2% (v/v) PE before autoclaving. The flasks were inoculated at 37°c on an orbital shaker at 160rmp for 4-6 days. The PE – grown cells at mid-exponential phase were pelleted by centrifugation and washed with GTE (Glucose-Tris-EDTA) buffer. The pellet was re-suspended in GTE buffer and the plasmid was isolated by the alkali lysis method (Sambrook *et al*., 1989). The aqueous phase was washed with phenol-chloroform, and the plasmid DNA was precipitated by adding two volumes of ethanol at room temperature. The precipitated DNA was recovered by centrifugation and subjected to electrophoresis after re-dissolving the DNA pellet in TE (10 MM Tris, 1 MM EDTA pH 8.0) on 0.8% agarose gel.

Two methods were tried to cure the plasmid from Bacillus and Pseudomonas sp to check whether the cured organism retain the ability to utilize PE as the sole source of carbon. In the first method, spontaneous loss of the above phenotype was testing by growing the organism on LB agar by up to five or six subculture on agar plates containing 0.2% PE. In the second method, the bacterium was grown in LB broth in the presence of ethidium bromide (Rani *et al*., 1996).

TRANSFORMATION OF DEGRADATIVE PLASMID

The PE degradative plasmids were transformed in to *E.coli* DH5 α strain by using the procedure of Hanahan (Hanahan *et al*., 1983).

RESULTS

Identification of plastic degrading bacterial strains:

Plastic degrading bacterial strains were identified as *Pseudomonas mediterranea* strain M2, Accession number (Gen bank) - JQ904748 &

Bacillus megaterium strain B1, Accession number (Gen bank) - JQ904750.

The partial 16s Ribosomal RNA gene of Pseudomonas mediterranea M2 strain is

Origin

```
   1 cgggatccag agtttgatcc tggctcagaa cgaacgctgg cggcaggcct aacacatgca
  61 agtcgagcgg tagagaggtg cttgcacctc ttgagagcgg cggacgggtg agtaatgcct
 121 aggaatctgc ctggtagtgg gggataacgc tcggaaacgg acgctaatac cgcatacgtc
 181 ctacgggaga aagcagggga ccttcgggcc ttgcgctatc agatgagcct aggtcggatt
 241 agctagttgg tgaggtaatg gctcaccaag gcgacgatcc gtaactggtc tgagaggatg
 301 atcagtcaca ctggaactga gacacggtcc agactcctac gggaggcagc agtggggaat
 361 attggacaat gggcgaaagc ctgatccagc catgccgcgt gtgtgaagaa ggtcttcgga
 421 ttgtaaagca ctttaagttg ggaggaaggg ccattaccta atacgtgatg gttttgacgt
 481 taccgacaga ataagcaccg gctaactctg tgccagcagc cgcggtaata cagagggtgc
 541 aagcgttaat cggaattact gggcgtaaag cgcgcgtagg tggttcgtta agttggatgt
 601 gaaagccccg ggctcaacct gggaactgca ttcaaaactg tcgagctaga gtatggtaga
 661 gggtggtgga atttcctgtg tagcggtgaa atgcgtagat ataggaagga acaccagtgg
 721 cgaaggcgac cacctggact gatactgaca ctgaggtgcg aaagcgtggg gagcaaacag
 781 gattagatac cctggtagtc cacgccgtaa acgatgtcaa ctagccgttg ggagccttga
 841 gctcttagtg gcgcagctaa cgcattaagt tgaccgcctg gggagtacgg ccgcaaggtt
 901 aaaactcaaa tgaattgacg ggggcccgca caagcggtgg agcatgtggt ttaattcgaa
 961 gcaacgcgaa gaaccttacc aggccttgac atccaatgaa ctttccagag atggattggt
1021 gccttcggga acattgagac aggtgctgca tggctgtcgt cagctcgtgt cgtgagatgt
1081 tgggttaagt cccgtaacga gcgcaaccct tgtccttagt taccagcacg ttatggtggg
1141 cactctaagg agactgccgg tgacaaaccg gaggaaggtg gggatgacgt caagtcatca
1201 tggcccttac ggcctgggct acgcacgtgc tacaatggtc ggtacagagg gttgccaagc
1261 cgcgaggtgg agctaatccc ataaaaccga tcgtagtccg gatcgcagtc tgcaactcga
1321 ctgcgtgaag tcggaatcgc tagtaatcgc gaatcagaat gtcgcggtga atacgttccc
1381 gggccttgta cacaccgccc gtcacaccat gggagtgggt tgcaccagaa gtagctagtc
1441 taaccttcgg ggggacggtt accacggtgt gattcatgac tggggtgaag tcgtaacaag
1501 gtagccgtag gatcccg
```

And the *Bacillus magaterium* strain B1 plasmid PE sequence is

Origin

```
   1 gatcattttg ccgattatgt ttcaatcaga aagttgtgtt cgatttcgga cagatatttt
  61 gaatatttat attcaactgt taactaaaac tgtctgcaaa ttaatcaatg tctctagtaa
 121 aattccgatt ttatacccac tcctattttc atcaatagga catgatataa taaaaaagaa
 181 cggagattga ggcctccgtt ctcttggttg tacttgttgc aatagtacgt ttatttggtt
 241 acgtatatta tagcaagtac tccaacaaaa aatcaatcgt tggaggaatt tagaaatgaa
 301 tggaattatc aaaacaataa aaacaacgca atatgctcaa atacataacg cgccattaca
 361 aacggacctg gaggacttgc ggtctatcgg tctattaagt catattatga gcttaggtga
 421 aggttggacg attaggaaga cccagttaca aaacaaattt tctcgtagaa atgtagacgc
 481 tgcttggaag gaattagcta ttaaacaata cgctgtcggt tttagcgcat atgttgacgg
 541 aaaaaaagat tatttttacg ccgtatcaga tattccgatt tcgcaagcta atttcgaatt
 601 gcttgtcatt gaacaaataa atctcttaca atcgcaaggt aaaaacgtca tgacgttaag
 661 cgtgatacaa cattcgccac ttgaaatcac tgaaaaaata tctgatgtac aaaatgtaca
 721 tcagatacaa aataagcaaa tttcttctga tgtacgttct gtacaacaca gtgtgtacaa
 781 cacagaacgt acacctataa ataaaaaaga aacaaatgaa aaattaacaa atgaaaaaat
 841 tagtagtagt ccacgtgatt ttattgatga taaattacga gaaaaatata ataatgtacc
 901 tttcgacgaa gtgaaaagtg aaatgcttaa cgatactgta atcgttgata cgaataagca
 961 atataaatca ctattagaat accgtcttaa acattggaaa ccgaagcaaa caaaaaggaa
1021 acgtgtagta cgtaatgcac gtaaagaaat ggttccaaag tggctacaaa caaatgatga
1081 aaatgcagta gcagaagatc ttccagtgaa taatgatttt gaagctgaaa aggaaaaaat
1141 gaaggcggag cttatgcaat tagatgcaga attaaaagca ggtaatcgtt aataaatatt
1201 gaattacacc tacaaaagcg agagcgaacg aggtgaagaa cgtgtcagaa aacgaacgcg
1261 catattggaa tcatgaagta gctgaacaac ttgacatagg tacaagcaca ttaagaaaat
1321 ggtgcttaga attggaagag aacgagtacg tgttctctaa aggcgaacaa gaaagccgag
1381 ctttttaaa acgtgatatt gatgttttaa tgaacatgaa aaatgaaata cgaaataaga
1441 aaaaatcact caaagatgcg gctaaaatcg cgttagaaaa ggcaagaacg ggggtcgttc
1501 tcgtagaaca agaacaggaa caagcacccc ccgttcccgt cgaacaagca cagccgatgt
1561 tcaccttgga ggacatgcgg aacattgtac gtgaagagct acaggaacaa gctaaagcac
1621 gtgagaacga acgagataaa gcactaatgg gtgtcatgcg tgaattacag gatgtaaaaa
1681 aaatgatagc tgcttctcaa gaggtaagta aaaaaaaatg gtgggagttt tggaaaacct
1741 aatttttta gaaatcttga gtgcaatata gtttaataca tcttgagcaa cttcaatgga
1801 gtattatcaa caacatgtaa taggttattt cataatacag agaagaagtt ttattaaaaa
1861 atgatacctt caaggtgtat ctttttttgt agcatggtat atagcatata atattttccc
1921 tttatgtctg ttatataggc aattacaaag ttttcttaaa gataaggtca agtgggatgc
```

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1981 aattttcgag attgttattg atagatacta taaattacgt aagtaattat gtattatcaa
2041 agctgctttt tttctcctgt tttgtttgta tgatgaatgg gggagacggc ggcaaggatg
2101 aaggtataag aaaagccctc aagatttgtt gagggctttt cttacatgtt tggttgatta
2161 tagctactgt ggtaaagagc ttgctttttt caatgtattc ctgaacaagg ttgaacaaat
2221 agctttggga ataaataagt ggttggaaaa gttgtgggtt atttaaaaaa tagataaata
2281 acagggagac gagaaatgat cctttaaaaa cgatttatta aattcatctt ttcttaatgt
2341 ttgtaaggga gaataagtca aaaagacctg tagttgtgtg actacagatc ttgtctctta
2401 ataactgtgg gagtagattg gattcgctcc tacaattacc tcgttccccc gttcagtctt
2461 ttttaagaaa gcttgacccc tttcttctgg actgatatta agagcgtcaa acagcttgtc
2521 atacgtgtcc attgtcacgt tgctgttccc gccctctaca cgagagattg ttttttgtgt
2581 tgtattagcc attaaagcaa ggtcatcttg tgatatgcct ttgctcatac gcttctctag
2641 gattagttgc ccgaagatag aggctgtact gccaaataaa gatttgtgca actcaggatt
2701 tttagccttc accatttccc tacctaaatt ccgtaggttt gccatgaaaa atcatctcct
2761 tatagcttat tggtagaggt catcaaaata tttacggtaa tcatcgaaat gtgcccttac
2821 ttgtttaaac acctcgtacg tttcctgcat gttttcatct gttgggtcat cgttatctcc
2881 ttctttccct tctttttcga aaggattcac taaacaatag aaaagttggt tagtatcagg
2941 atgatattta gggaagaagg tagcacgaaa atgccaattt aatcgctcga cgcttaatcg
3001 taactcataa attggttctg cacgtaatgc cttaatagcc tttggacgta aagtgtactc
3061 tactccgtca attgggtctg ttgttaattc aaaccctacg aagaaatgat ttccctctgc
3121 gtctctacct ctaggtggta attgagcttt gggaacagtc gctagtgcat ccattgcttt
3181 agtgataaac ccgattttga ttaaggaatc tttggcgggg tcttcaaata acatagacat
3241 gtaatccgtc atctgatcta cacctttaa atcgggtaat gagataacct ctgctactat
3301 atttaccata catcatatca tataatacct aaattgtgaa aaaatttaag gcgtatatga
3361 cgtttccccc ctttgatgta aaattaacgc cttttacagc gatatataaa ttttaatata
3421 aaaaattaaa aaaaggggaa aaacttcctt tttaattaag tgagataaaa agattaaaat
3481 ggtgtttttt tatgcaggta tttcaagcgc gttattcaca gaaacgttgt taaatcaaca
3541 atgtataaaa agatgcataa tcaagaaaac tcttctttct gcgagccctt gcctcccaag
3601 gtttcttttt tgtggtgggt tccggaaaga gtgtttatgt aaactagctt tgtatatcgt
3661 atacataccc aaatgtttct tatctagtaa aagaatggtg atataattat aaatagatct
3721 acacagggag taagaatatg atgaatccaa aagaaaagca aagttccaaa tttagcgttg
3781 tgtttaagtc ctacgaacaa tttttgaaga agattttaaa tatgttcttt actagatgat
3841 atttcataca aaaaaggctt ttaagaaata taacacttaa aagctttttt tggtgcctat
3901 aagctgtttt atgttaacta gaaagaaata tataaacata tacatagcac tttttattga
3961 ttaaaaagct gagtacatta gttctcagct ttctttatct ttatcgttat tttcaattag
4021 cgtaaacaaa tcgtccaacg ttacatcaaa atacatcatc aatttagcta gtaaactggc
4081 tggtattcgt ctgttattca tatcacggtt gttcgccaaa tcgtttattg tatcgcgtct
4141 ttcattgatt tcttcagata atttccttac agaaatgtct tgctcttcta aatatttttt
4201 tagatgacat tgcaatttta cgttctccaa aattgacctc cttctcaaaa aaattcaata
4261 ttttattgac ctttcattat gtaagatgct atatttatgt taacaatttg acacgtttaa
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4381 gaaaaagctg aaaaattgtt agtggggatc gaaaatgttt taggcatggc taatgaactt
4441 tttgacgagg tagcacgttt gaaacaagtc gaggaggagt gtaaaatcct aaaagaaaaa
4501 ttgtttttga cccaatttac acgctcggaa aaagaagtgt ttgaactcgc acttgacagt
4561 ctttcatctt caaaaatggc tgaatggcta ttcaaagaac ggagcacaat ttcacaacaa
4621 cgtaaggata tttgcaaaaa attaggcgtt aaaaatatca aagaagcagt acaaaaattt
4681 caaaaccttc accaagaacc gcaacaagaa atccaccaag aatcaccaca aaaactcgta
4741 aacatttcat aaaaaatgaa aaaagcgtat cgacagcagg tgcccacgag gggaatcagc
4801 cacctaaata gttgaaaata tggaatattt ttaggggttt gaaggacat tgcaaacgtt
4861 ttcagtgtac ccactggaaa aaatcgtggt atcacggtta agctaggctc tgcctagcct
4921 agcacagtat gtgatagcaa aaacccctta tgctctttcg aagaaataac ggattaattt
4981 ccagttacta tttttttga aaaaaggagt tggttttttt ggatattatt attcgcaata
5041 tcggcaaaga atacgtcaaa gcaattgatg aaaaagcgaa ggcattgaac atgtctcgca
5101 atgaattttt gaacagatat ttaatcggat acgcacatca tcgggaagca cttgggaggg
5161 aagaccgact ggaaaagttt cttaatgaaa cacaacagca aatgttagct ttcacaatgc
5221 taatggatat gatgacagat actttacgag aattaagtgg attggacgat gatgaatcgt
5281 aatcatatga cggttttttt atttaagggt cttctgaaac acgaggcaat acagcaaacg
5341 tacgaaaatc gcctgtattt gatttttgat gtgtcggaag ataaataatt gccttatcat
5401 taaaaacgtc atataaggca atatggaa
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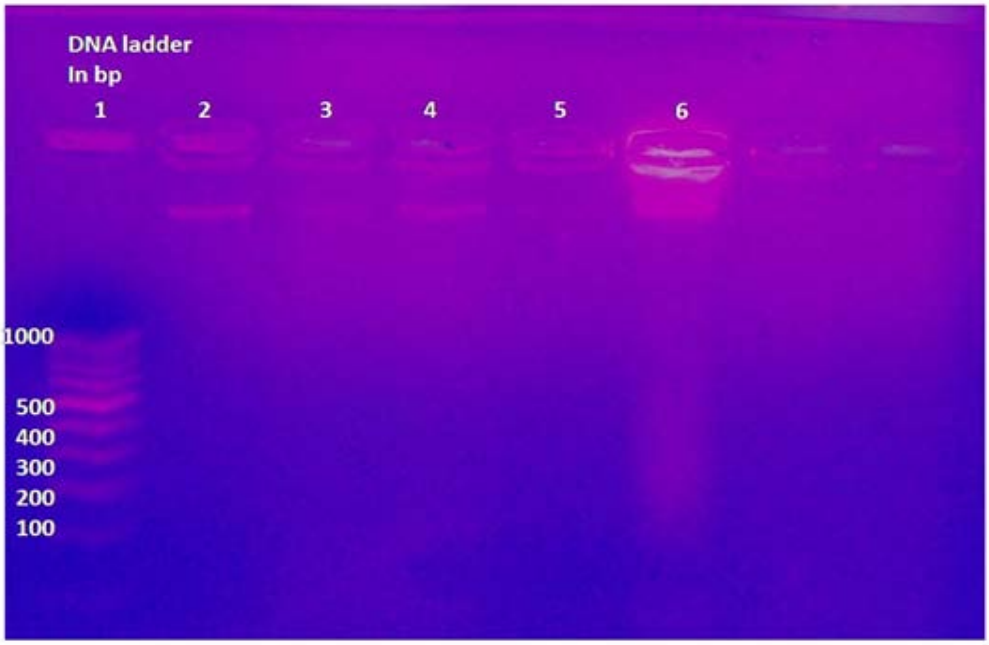
Plasmid isolation:

Degradative plasmids, which are responsible in degrading HDPE & LDPE were isolated from both the strains taken from the mineral salt broth (**Figure A**).

Figure A: Isolation of degrading plasmids



Lane : 1 - Marker DNA
Lane: 2,3 - Plasmids from Bacillus megaterium.
Lane: 4,5,6 - Plasmids from Pseudomonas mediterranea.

Plasmid curing:

Plasmids were not isolated from both the strains after curing & these strains lost the ability to degrade LDPE & HDPE.

Transformation of plasmid:

Transformed E.coli DH5α strains showed the ability to degrade polyethylene.

DISCUSSION

The 16s rDNA sequences were matched for local-alignment through NCBI-BLAST. Based on the scores and identity percentages, the isolates identified.(Soni.R *et al*., 2009).Similarly in the present study the isolates identified as Pseudomonas mediterranea and Bacillus megaterium by molecular characterization. The plasmids were isolated from the culture capable of degrading polyethylene. A similar observation was reported in Moraxella sp, where 60-70 kb plasmids encoding phlalate 4,5–dioxygenase and 4,5-dihydro-4,5-dihydroxyphthalate dehydrogenase are involved in o-phthalate catabolism (Rani,M. et al,1996). Plasmid cured cells lost their ability to grow on Dimethylphthalate (DMP) (Javed Hussain Niazi, et al, 2001) and in this present study,200kb plasmids capable of degrading PE were isolated from the strains and upon plasmid curing they lost that ability. Transfer of plasmids to recipient conferred the characteristics of the donor cells (Griffith, 1928) upon transformation, the recipient cells gained the ability to degrade PE.

CONCLUSION

Plastic degrading bacterial strains were isolated from compost soil and characterization of these isolates was done by molecular characterization.

To demonstrate that biodegradation of plastics is plasmid mediated, plasmids were isolated from these strains when plasmid curing was done they lost the PE degrading activity.

Transformed *E.coli* DH5α strain showed the ability to degrade PE.

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