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# Studies on degradation efficiency of polycaprolactone by a naturally-occurring bacterium

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**Abstract**

We report a strain of *Bacillus*, isolated from the rhizosphere of the mangrove *Sesuvium portulacastrum*, that degrades polycaprolactone (PCL) on timescales that are a factor of three shorter than hitherto reported, with complete degradation in only 20 days. The bacterium has been identified as *Bacillus pumilus* by means of 16S rRNA gene sequencing and FAME analysis; it secretes proteases and lipases and its 'de-polymerase' activity is evident by the zone of clearing in emulsified PCL. It is an aerobic chemoheterotroph capable of utilizing a variety of carbohydrates. Although not a true psychrophile, is a mesophile, growing optimally over a temperature range 30-45° C and pH range 5-12.5. It is a halophile tolerating NaCl concentrations up to 10% w/v, and is unique in degrading and utilizing PCL and its monomer, ε-caprolactone (CL), as a sole carbon source. Degradation of PCL was monitored using Fourier Transform Infrared (FTIR) spectroscopy, light microscopy and scanning electron microscopy (SEM). This degradation was found to be enhanced by salts (NaCl, KCl, $MgSO_4$, $Na_2HPO_4$) and at medium pH values in excess of 7. Under the same growth conditions, another standard *Bacillus pumilus* strain showed somewhat reduced PCL-degradation.

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## Introduction

Biodegradable polymers– as a potential, partial solution to the problems of plastic accumulation and degradation, were developed during the last decade. Biodegradable plastics are degraded under environmental conditions or in municipal/industrial waste treatment facilities, with aerobic composting and anaerobic bio-gasification of waste currently in use. Aerobic biodegradation behavior of plastics is well studied and several polymer-degrading microorganisms have been isolated and identified.Sanchez (2000) have isolated an *Aspergillus* sp. that degrades poly 3-hydroxybutyrate at high temperatures; Kathiresan (2003) have isolated bacteria as well as fungi from the soil which degrade polyethylene; Hayase (2004) isolated a strain of *Bacillus pumilus* which degraded many polymers including blends of polycaprolactone (PCL) and starch. Of the variety of bio-plastics available, PCL and its blends are one among the ever-growing list of most commonly used biodegradable polymers, essentially because of their water resistance, chemical stability and fast degradability. Previous studies in aerobic as well as anaerobic landfill model reactors have shown PCL biodegradation within 120 d with slow degradation during the first 80 d followed by a faster rate (Ishigaki *et al*., 2004). Studies by Khatiwala *et al.* (2008) showed complete degradation of PCL only when the films were treated with UV irradiation (in 68 d) but almost negligible loss of weight when biodegradation was carried out directly.

Polymer degradation assays with mixed cultures are practice-oriented and mimic naturally occurring conditions but, are not suitable to elucidate the biochemical and

mechanistic details of degradation. (Kathiresan *et al*., 2003). It is therefore imperative that an appropriate methodology be developed to enhance and determine biodegradation of PCL under defined conditions so as to suit diverse scientific and technological pursuits, and to identify an organism that degrades the polymer and/or utilizes it as a carbon source. In the present study, we have focused attention on an exhaustive screening for the isolation of PCL-degraders from environmental samples (soil, sand, sewage and mangrove rhizosphere). We have chosen to investigate mangroves, which are known to exist under diverse conditions and are hotspots for microbial diversity.

## Materials and Methods

**Media :** Media components used were from HiMedia Laboratories, India. Minimal medium utilized to check the growth of isolates with CL or PCL as the sole carbon source was $KH_2PO_4$, 1 g $l^{-1}$; $K_2HPO_4$, 1 g $l^{-1}$; $NH_4NO_3$, 1 g $l^{-1}$; $CaCl_2$, 0·02 g $l^{-1}$; $FeCl_3$, 0·05 g $l^{-1}$; $MgSO_4$, 0·2 g $l^{-1}$; agar, 15 g $l^{-1}$. Medium for the halo assay contained peptone, 5·0 g $l^{-1}$; NaCl, 2·4g $l^{-1}$; $MgSO_4 \cdot 7H_2O$, 7·0 g $l^{-1}$; $MgCl_2 \cdot 6H_2O$, 5.3 g $l^{-1}$; KCl, 0·7 g $l^{-1}$; $CaCl_2$, 0·1 g $l^{-1}$. Medium for checking contribution of salt to PCL degradation contained; peptone, 5·0 g $l^{-1}$ with either of the following salts: NaCl, 2·4g $l^{-1}$; $MgSO_4 \cdot 7H_2O$, 7·0 g $l^{-1}$; $MgCl_2 \cdot 6H_2O$, 5·3 g $l^{-1}$; KCl, 0·7 g $l^{-1}$ or $CaCl_2$, 0·1 g $l^{-1}$.

**Isolation of bacterial strains:** Samples of sand, soil, sewage and mangrove rhizosphere were collected in sterile plastic containers from beach, garden, sewage treatment plant and mangroves respectively. Mangrove sediment samples were collected from the rhizosphere of four mangroves species – *Avicennia officinalis, Exoecaria agallocha, Sesuvium portulacastrum and Sonneratia alba*. For selective enrichment of PCL degraders, commercially available films of PCLS (1 cm X 4 cm) were buried in the samples, at a depth of three cm from the surface, for 20-30 d, with intermittent observations. For sewage samples, the film was suspended in sewage with constant shaking at 50 rpm. The supernatants from soil/mangrove samples were subjected to serial dilutions and plated on nutrient medium. Bacterial colonies were picked and maintained on nutrient agar (NA) for further studies. Polymer degradation by individual isolates was tested by inoculating each of them in sterile soil containing films of PCLS; the films observed for degradation patterns. Subsequently, this was confirmed by spot inoculation by halo assay to compare the zones of clearing on plates.

**Identification of bacteria :** Morphological characteristics and biochemical tests were performed according to *Bergey's Manual of Systematic Bacteriology* (Williams and Sharpe, 1989). Incubations were performed at 30°C unless mentioned otherwise. Positive and negative controls were maintained as and when necessary. CBS-i1 was tested for the utilization of a variety of polysaccharides, monosaccharides and sugar alcohols using HiMedia Hicarbo kit. The bacterium was further characterized by its FAME fingerprints, separated by gas chromatography and identified by Sherlock Microbial Identification System Database (Siegel *et al.,* 2000). For 16S rRNA gene sequencing, genomic DNA was extracted and the gene amplified using forward (5'-TAGAGTTTGATCGTGGCT-3') and reverse (5'-TTGGTTACCTTGTTACGA-3') primers.

**Growth characterization:** Experiments for characterization of the bacterium were performed in triplicates. To ascertain the effect of temperature on growth, CBS-i1 was inoculated in medium containing peptone, 5·0 g $l^{-1}$ (pH 7) pre-adjusted to the required temperatures (5-55° C). To determine the effect of pH, CBS-i1 was inoculated in medium containing peptone, 5·0 g $l^{-1}$ of varying pH (4-13) and grown at 40°C. Growth was assessed by monitoring the optical density up to 96 hr. Since CBS-i1 was obtained from a mangrove it was desirable to check the effect of NaCl on growth; hence, it was inoculated in medium containing peptone 5·0 g $l^{-1}$ (pH 7) with NaCl (5-200 g $l^{-1}$) and grown at 40°C. Growth was assessed by monitoring the optical density and measuring the viable cell counts on NA plates for up to 72 h.

The susceptibility of the bacterium to antibiotics was tested as per standard protocols. Discs for the following antibiotics were used; amikacin, ampicillin, chloramphenicol ciproflaxin, clindamycin, erythromycin, gentamycin, kanamycin, nalidixic acid, streptomycin, tetracycline and vancomycin (30 μg).

**PCL degradation studies :** For further degradation studies, CBS-i1 was inoculated (4 x $10^6$ cells) in beakers containing sterile soil with films of PCL or PCLS buried at a depth of three centimetre. Moisture content of soil was maintained at 35 % and incubated at 40° C. At various time points (4-12 d) films were removed and utilized for film characterization experiments. PCLS film degradation patterns were observed microscopically and on a Fei Quanta 200 ESEM system. Polymer degradation was characterized by spotting CBS-i1 on plates containing emulsified PCL (Capromer PD4-05, $M_w$-550; provided by BASF) in peptone 5·0 g $l^{-1}$ (pH 5, 7 and 9), incubated at 15-50° C for 24-72 h and degradation assessed by observing zones of clearing. To test the ability of CBS-i1 to grow using CL or PCL as the sole carbon source, the isolate was grown on solid minimal media supplemented with either 0·1-1·0 g $l^{-1}$ ε-caprolactone (Sigma) or 0·1-1·0 g $l^{-1}$ emulsified PCL ($M_w$-550 Da) in minimal medium at 40° C. To check the effect of salt on rate of degradation of polymer and/or enzyme induction, CBS-i1 was spot inoculated on plates containing emulsified PCL ($M_w$-550 Da). Infrared spectra

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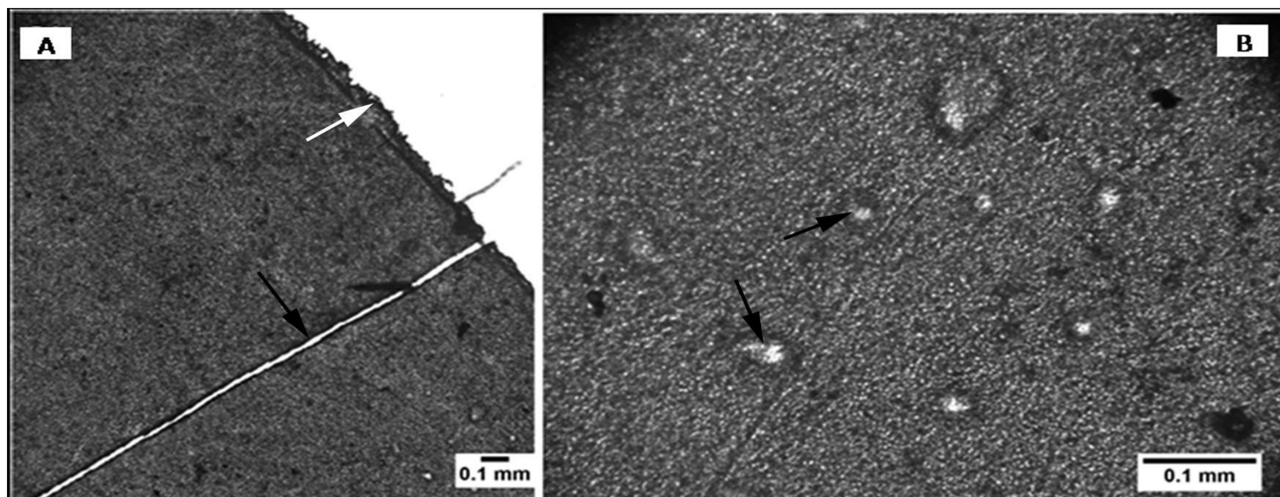


**Fig. 1 :** Biodegradation of PCLS films by *Bacillus pumilus* (CBSi1). PCLS blend films, post-degradation, were viewed under a compound microscope and showed the presence of linear tears - black arrow, and serrated edges - white arrow (A at 40X) and circular pits and holes - black arrows (B at 63X)

of samples were obtained using Varian FTIR in ATR mode. 25 scans with a resolution of 2 $cm^{-1}$ were carried out from 400 $cm^{-1}$ to 4000 $cm^{-1}$.

## Results and Discussion

**Identification of bacteria :** Thirteen polymer-degrading bacterial species from mangroves, 12 from soil and 3 from sand were isolated. Since no degradation was observed in sewage, the sample was not analyzed further. Comparative studies of degradation showed that CBS-i1, the isolate from the mangrove *S. portulacastrum* was most efficient. It also was the most efficient at utilizing PCL and its monomeric form, CL, as a sole carbon source. In case of other mangrove sediments, PCLS degradation was seen within 45-50 d (except *S. portulacastrum* where degradation was observed in 20 d) *vis-a-vis* the samples in sand and soil (60 d). In earlier reports, rates of degradation have enhanced only after UV irradiation for 4 h (Kathiresan *et al.*, 2003). UV is known to aid in the polymer breakdown by inducing the formation of free radicals (Bei *et al*., 1999). Films of PCLS degraded by CBS-i1 showed circular and linear cuts near the edges within the first 8-12 d followed by enhancement of degradation on the same lines, with simultaneous appearance of new cuts (Fig. 1). 16S rRNA gene sequence (Fig. 2) and FAME analysis, together established CBS-i1 as being *B. pumilus* GC sub group B with a similarity index of 0.83.

**Growth characterization :** Growth and biochemical characteristics revealed CBS-i1 to be an aerobic, sporulating gram positive, motile rod. While it utilized several carbohydrates such as cellobiose, D-arabinose, L-arabinose, dextrose, esculin, fructose, galactose, glucoseamine, glycerol, mannitol, mannose, α-methyl-D-glucoside ribose, salicin, sucrose, trehalose and xylose; it did not utilize adonitol, citrate, dulcitol, inositol, insulin, lactose, malonate, maltose, α-methyl-D-mannoside, ortho-nitrophenyl-β galactoside, raffinose, rhamnose, sodium gluconate, sorbitol, xylitol. CBS-i1 tested positive for catalase, oxidase, protease and gelatinase assays and negative for urease, amylase and nitratase production (Table 1). Many bacteria, including *Bacillus* sp., have been identified to grow in close contact with various mangrove plants (Abhaykumar and Dube, 1991; Kathiresan and Bingham, 2001; Ravikumar *et al*., 2005). These are known to survive osmotic stress and thus could have varied applications. Since CBS-i1 was obtained from a mangrove, it was desirable to check the effect of NaCl on growth. CBS-i1 showed growth in media with NaCl concentrations ranging from 0-90 g $l^{-1}$ and survived concentrations up to 200 g $l^{-1}$. The bacterium was susceptible to several antibiotics (using antibiotic discs from HiMedia - 30µg of amikacin, ampicillin, chloramphenicol ciproflaxin,

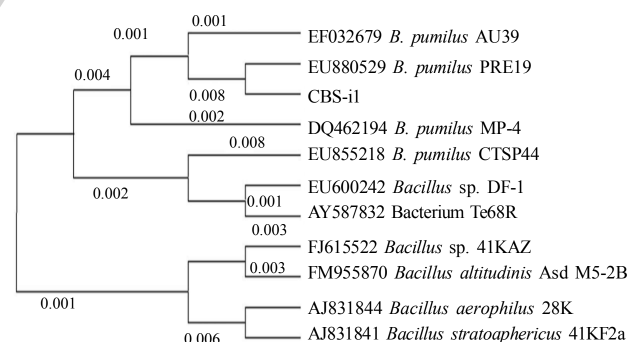


**Fig. 2 :** Phylogenetic tree generated using Neighbor Joining Method, Mega 3.1. Based on this method, the novel 16S rRNA gene sequence, with the GenBank assignment number GQ220330.1 is closest to EU880529 B. pumilus PRE19

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**Table 1 :** Morphological and growth properties of *Bacillus pumilus* CBSi1. All experiments for characterization of the bacterium were performed in triplicates. The characteristics of the isolate (CBSi1) match that of *Bacillus pumilus* (as mentioned in the Bergey's manual of Systematic Bacteriology, William and Sharpe, 1989)

| Property | Result |
|---|---|
| Gram Staining | Gram +ve |
| Shape | Rod |
| Motility | Motile |
| Spore formation | + |
| Colony Characteristics | |
| Size | 2-3mm |
| Shape | Circular |
| Colour | White / Cream |
| Margin | Serrate |
| Elevation | Raised |
| Opacity | Opaque |
| Consistency | Butyrous |
| Carbon Source | |
| Adonitol | - |
| Cellobiose | + |
| Citrate | - |
| D-Arabinose | + |
| Dextrose | + |
| Dulcitol | - |
| Esculin | + |
| Fructose | + |
| Galactose | + |
| Glucoseamine | + |
| Glycerol | + |
| Inositol | - |
| Inulin | - |
| Lactose | - |
| L-Arabinose | + |
| Malonate | - |
| Maltose | - |
| Mannitol | + |
| Mannose | + |
| Melezitose | - |
| Melibiose | - |
| ONPG | - |
| Raffinose | - |
| Rhamnose | - |
| Ribose | + |
| Salicin | + |
| Sod.Gluconate | - |
| Sorbitol | - |
| Sorbose | - |
| Sucrose | + |
| Trehalose | + |
| Xylitol | - |
| Xylose | + |
| á-Methyl-D-glucoside | - |
| á-Methyl-D-mannoside | - |
| Miscellaneous | |
| Catalase | + |
| Nitratase | - |
| Oxidase | + |
| Urease | - |

clindamycin, erythromycin, gentamycin, kanamycin, nalidixic acid, streptomycin, tetracycline and vancomycin per disc).

Bacteria from the genus *Bacillus* are well known degraders of various compounds and their potential for enzyme production is being tapped for various industrial applications (Shukor *et al*., 2008, Vetter *et al*., 1995). *Bacillus pumilus*, a well characterized member of the genus *Bacillus*, is listed in the Bergey's Manual of Systemic Bacteriology as a saprophyte residing in soil. Reports on its existence in diverse niches such as intestinal tract, processed foods, oceans, high altitudes, soils, etc. have been cited (Matarante *et al*., 2004; Barbosa *et al*., 2005). While, it has been documented to degrade various biodegradable polymers (Hayase *et al*., 2004) this is the first evidence of a *B. pumilus* from the rhizosphere of *S. portulacastrum* that not only degrades, but utilizes PCL and its monomeric ring form CL as a sole carbon source.

**PCL degradation studies :** Scanning electron microscope images of the degraded PCLS film showed gross changes in the texture of the film; these being clearly absent in the control film (Fig. 3). As assessed by formation of zones around colonies, the degradation of PCL ($M_w$-550) was seen in 48-72 h (Fig. 4B); thus confirming the production of PCL hydrolyzing enzyme/s. Under same growth conditions, a

**Table 2:** IR spectroscopy of the PCL film. Infrared spectra of samples were obtained using Varian FTIR in ATR mode. Twenty-five scans with a resolution of 2 $cm^{-1}$ were carried out from 400 $cm^{-1}$ to 4000 $cm^{-1}$.The results are expressed in ratios of absorbance at peaks corresponding to characteristic band ratios (as depicted in Table 3). Note the increase in the Crystallinity and Carbonyl index post-PCL degradation

| Duration (days) | Crystallinity index from the ratio of abs peaks at 1293 and 1162 $cm^{-1}$ | Carbonyl index from the ratio of abs peaks at 1720 and 1467 $cm^{-1}$ |
|---|---|---|
| 0 | 0.4577 | 4.8387 |
| 7 | 0.5802 | 5.3149 |
| 14 | 0.6260 | 5.3621 |

**Table 3 :** IR spectra bands of PCL. Characteristic peaks of the IR spectra bands of PCL assigned for resonance frequencies involved in PCL degradation. (Elzein *et al.,* 2004 and Khatiwala *et al.,* 2008)

| Band position ($cm^{-1}$) | Vibrator |
|---|---|
| 2944 | Asymmetric $CH_2$ stretching |
| 2865 | Symmetric $CH_2$ stretching |
| 1720 | Carbonyl stretching |
| 1467 | $CH_2$ Scissoring |
| 1293 | C-O and C-C stretching in the crystalline phase |
| 1239 | Asymmetric COC stretching |
| 1187 | OC-O stretching |
| 1162 | C-O and C-C stretching in the amorphous phase |

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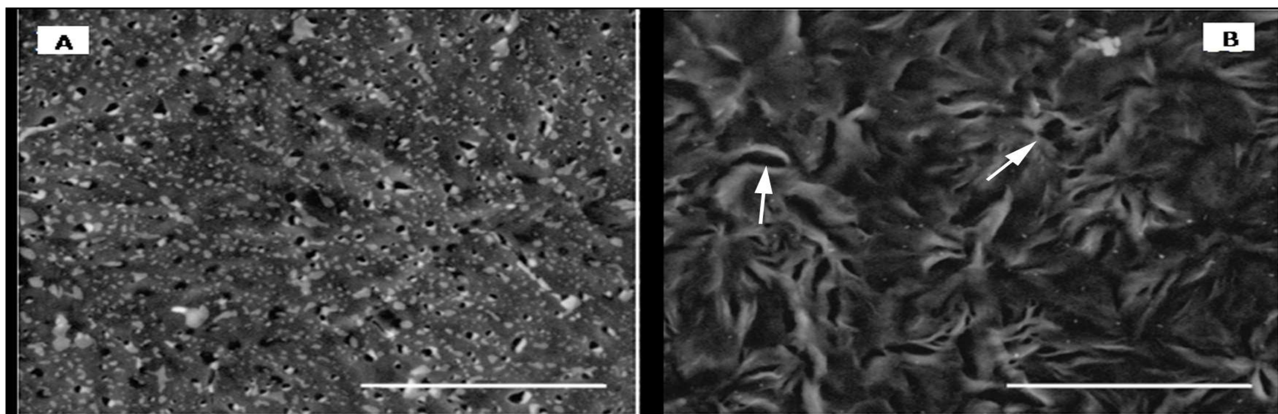


**Fig. 3 :** Biodegradation of PCLS films by *Bacillus pumilus* (CBSi1) as seen in a scanning electron microscope. A representative film of PCL after degradation in the presence of CBSi1 for 7 days (B) shows changes on the surface (white arrows). Control film (A), Scale bar – 20 µm

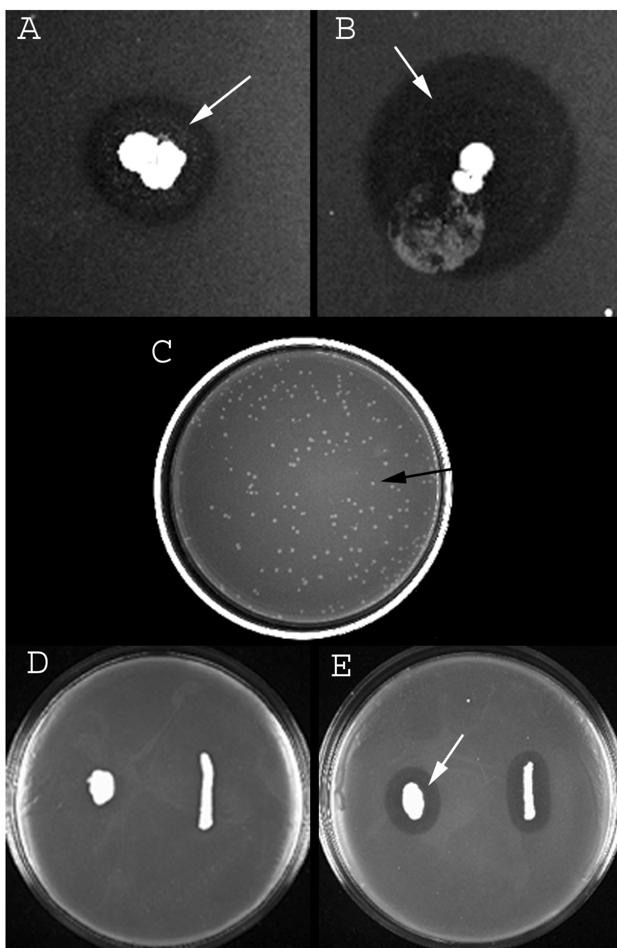


**Fig. 4 :** PCL degradation and CL utilization. Plates containing PCL ($M_w$-550 Da, emulsified in 0.5 % peptone-agar) were spotted and/or streaked with CBS-i1 or Control strain. After 48 hr, zones of clearing around colonies of CBSi1 were larger (B) as compared to control *B. pumilus* NCIM strain 2327 (A); see white arrows for comparison. Growth of CBSi1 on plates was observed within 3 d on which CL was supplied as the sole carbon source (C). Addition of 2 % NaCl to the medium with emulsified PCL enhanced the zone of clearing. As seen in (D), these zones were small and diffused in agar devoid of NaCl and large and clear in agar with 2 % NaCl (E).

standard *Bacillus pumilus* strain from NCIM showed almost 3-fold lesser zone of clearing as compared to CBSi1 isolate (Fig. 4A). Growth of isolate, CBSi1 was observed within 3 d on media in which CL was supplied as the sole carbon source (Fig. 4C). Degradation of PCL was seen at varying NaCl concentrations (Fig. 4D-E and 5A), different salts (Fig. 5C) and at a wide range of pH (from 5-9, Fig. 5B). Enhanced zone formation seen due to the addition of Na, K and Mg salts in the plate assays could be due to increased enzyme production and/or enhanced enzyme activity. Induction of hydrolyzing enzymes and increased activity in the presence of salts is known (Akel *et al*., 2009). Further study of the enzyme would help understand the mechanism of salt-dependent increased degradation.

PCL hydrolysis has been shown in the presence of several enzymes such as proteases, lipases and cutinases. CBS-i1 secreted proteases that exhibited hydrolysis of proteins such as gelatin and casein (Fig. 6). Since, CBS-i1 tested negative for amylase production, we concluded that the degradation of PCLS occurred through the selective degradation of PCL. Zymographs have shown that a ‘depolymerase’ is indeed secreted and also associated with the cell pellet (Fig. 6E, star and arrows). Polymer degradation by the depolymerase may occur by the breakage of crystalline and/or amorphous parts. FTIR was used to analyze the preferential breakdown as well as to study the bonds being broken. Infrared spectrum bands at 1293 and 1162 $cm^{-1}$ are assigned to the backbone C-C and C-O stretching (Table 3) in the crystalline and amorphous PCL respectively (Coleman and Zarian, 1979). Incubation of the PCL film in an inoculated medium (7 and 14 d) increased the crystallinity index (ratio of the absorbance peak at 1293 and 1162, Table 2); thus indicating the preferential breakdown of the amorphous parts of the polymer (Elzein *et al.*, 2004; Khatiwala *et al.*, 2008). A ratio between the absorbance peaks of carbonyl at 1720 to that of $CH_2$ at



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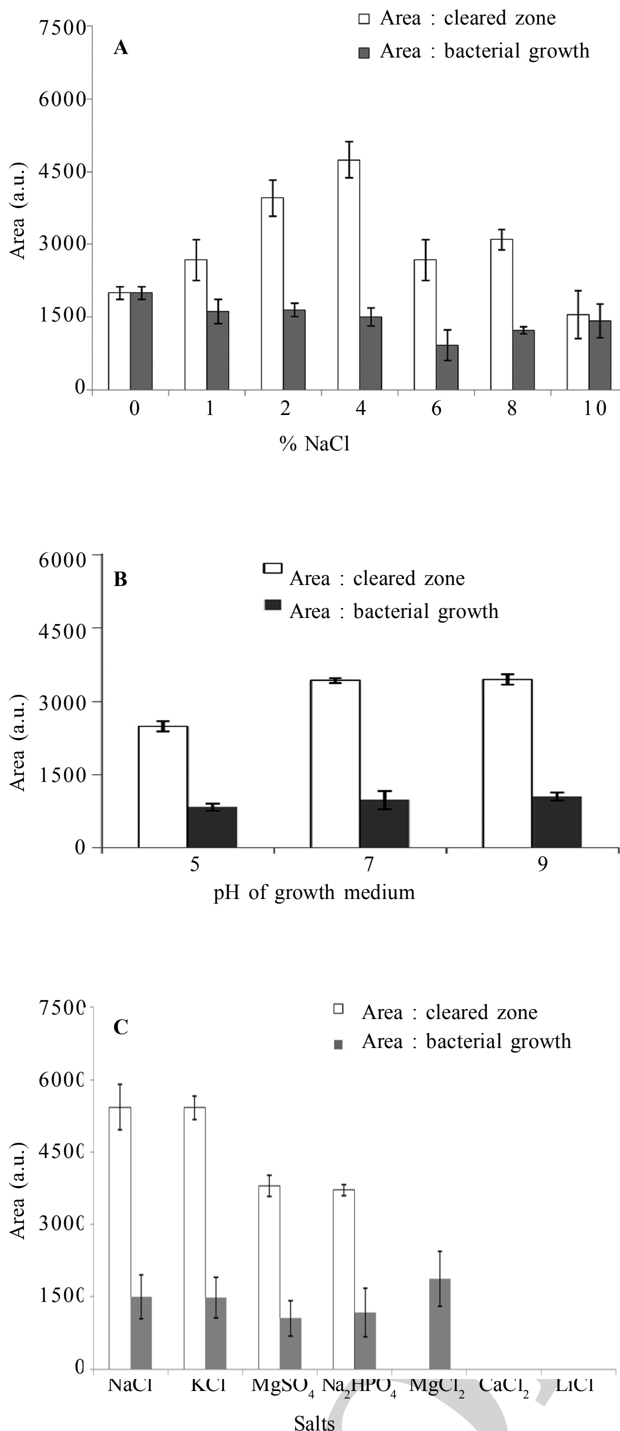


**Fig. 5 :** Quantification of zones of clearing on emusified PCL plates. Areas of the colony size and zones of clearing (represented by gray and white bars respectively) were estimated using ImageJ and plotted as arbitrary units. Fig (A) shows the size of the zones of clearing was largest when 4 % NaCl was added to the medium. At neutral and alkaline pH the zones of clearing were larger than that at pH 7 even though the colony size was similar (B). Enhanced zone formation was seen due to the addition of Na, K and Mg salts in the medium (C)

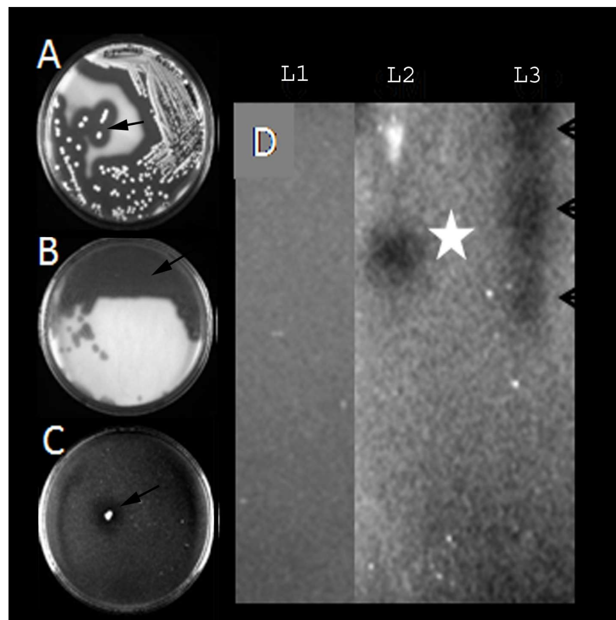


**Fig. 6 :** Secretion of the 'depolymerase' in the medium. *Bacillus pumilus* (CBS-i1) when grown on media containing gelatinase, casein and tributyrin secretes a gelatinase (A), caseinase (B), lipase (C) respectively as seen by the zones of clearing (marked by black arrows). The depolymerase which breaks down PCL has been identified using PCL-zymograph (white star and arrows). Concentrated crude supernatant was loaded in lane 2 (L2) and cell pellet in lane 3 (L3). Concentrated crude supernatant from isolate CBSi1 unprimed by PCL was loaded on lane 1(L1)

1467 $cm^{-1}$ was found to increase (Table 2) indicating the preferential breakage of $CH_2$ bonds over that of the carbonyl bonds.

The natural ubiquity and phylogenetic diversity of bacteria belonging to the genus *Bacillus* has led us to the isolation of a PCL-degrading halophilic and psychrophilic mesophile. The biodegradation of the polymer is seen within 20 d without any prior physical or chemical treatment of the polymer; fastest ever reported thus far. The isolate resides in the rhizosphere of *S. portulacastrum*, a niche unknown to harbor such PCL-degrading strain of *Bacillus*. The 3-fold improvement in the time of PCL degradation along with the characteristic geometric degradation pattern of the films, necessitates further experiments so as to identify the gene/s, and subsequently the enzyme(s) involved. This identification would help generate hereupon a modified bacterium whose depolymerase secretion is increased by partitioning the enzyme(s) associated with the pellet and/or by increasing the efficiency of degradation.

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## References

Abhaykumar, V.K. and H.C. Dube: Epiphytic bacteria of mangrove plants *Avicennia marina* and *Sesuvium portulacastrum*. *Indian J. Mar. Sci.*, **20**, 275-276 (1991).

Akel, H., F. Al-Quadan and T.K. Yousef: Characterization of a purified thermostable protease from hyperthermophilic *Bacillus* strain HUTBS71. *Euro. J. Scie. Res.*, **31**, 280-288 (2009).

Barbosa, T.M., C.R. Serra, R.M. La Ragione, M.J. Woodward and A.O. Henriques: Screening for *Bacillus* isolates in the broiler gastrointestinal tract. *Appl. Environ. Microbiol.*, **71**, 968-978 (2005).

Bei, J., W. He, X. Hu and S. Wang: Photodegradation behavior and mechanism of block copoly(caprolactoone-ethylene glycol). *Polym. Degrad. Stabil.*, **67**, 375-380 (2000).

Claus, D. and R.C.W. Berkeley: The Genus *Bacillus* Cohn. In: Bergey's Manual of Systematic Bacteriology (Eds.: P.H.A. Sneath, N. S. Mair, M. E. Sharpe and J. G. Holt). The Williams & Wilkins Co., Baltimore, **2**, 1105-1139 (1986).

Coleman, M.M. and J. Zarian: Fourier-transform infrared studies of polymer blends. II. Poly (α-caprolactone)-poly(vinylchloride) system. *J. Polym. Sci. Polym. Phy. Ed.*, **17**, 837-850 (1979).

Elzein, T., M. Nasser-Eddine, C. Delaite, S. Bistac and P. Dumas: FTIR study of polycaprolactone chain organization at interfaces. *J. Colloid Interface Sci.*, **273**, 381-387 (2004).

Hayase, N., H. Yano, E. Kudoh, C. Tsutsumi, K. Ushio, Y. Miyahara, S. Tanaka and K. Nakagawa: Isolation and characterization of poly (butylene succinate-co-butylene adipate)-degrading microorganism. *J. Biosci. Bioeng.*, **97**, 131-133 (2004).

Ishigaki, T., W. Sugano, A. Nakanishi, M. Tateda, M. Ike and M. Fujita: The degradability of biodegradable plastics in aerobic and anaerobic waste landfill model reactors. *Chemosphere*, **54**, 225-233 (2004).

Kathiresan, K. and B.L. Bingham: Biology of mangroves and mangrove ecosystems. *Adv. Mar. Biol.*, **40**, 81-251(2001).

Kathiresan, K.: Polythene and plastics-degrading microbes from the mangrove soil. *Rev. Biol. Trop.*, **51**, 629-634 (2003).

Khatiwala, V.K., N. Shekhar, S. Aggarwal and U.K. Mandal: Biodegradation of poly (α-caprolactone) (PCL) film by *Alcaligenes fecalis*. *J. Polym. Environ.*, **16**, 61-67 (2008).

Matarante, A., F. Baruzzi, P.S. Cocconcelli and M. Morea: Genotyping and toxigenic potential of *Bacillus subtilis* and *Bacillus pumilus* strains occurring in industrial and artisanal cured sausages. *Appl. Microbiol. Biotechnol.*, **70**, 5168-5176 (2004).

Ravikumar, S., G.P. Williams, S. Shanthy, N.A. Gracelin, S. Babu and P.S. Parimala: Effect of heavy metals (Hg and Zn) on the growth and phosphate solubilising activityin halophilic phosphobacteria isolated from Manakudi mangrove. *J. Environ. Biol.*, **28**, 109-114 (2007).

Sanchez, J., A. Tsuchii and Y. Tokiwa: Degradation of polycaprolactone at 50° C by a thermotolerant *Aspergillus sp. Biotechnol. Lett.*, **22**, 849-853 (2000).

Siegel, J.P., A.R. Smith and R.J. Novak: Cellular fatty acid analysis of isolates of *Bacillus thuringiensis* Serovarkurstaki, strain HD-1. *Biol. Control*, **17**, 82-91(2000).

Shukor, M.Y., N. Gusmanizar, N.A. Azmi, M. Hamid, J. Ramli, N.A. Shamaan and M.A. Syed: Isolation and characterization of an acrylamide degrading *Bacillus cereus*. *J. Environ. Biol.*, **30**, 57-64 (2009).

Vetter, R., D. Wilke, B. Moeller, M. Mueller, I. Muecke, M. Takenberg, and G. Konieczny-janda: Alkaline proteases from *Bacillus pumilus*. *United States Patent*, **5478742** (1995).

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