African Journal of Microbiology Research Vol. 5(24), pp. 4097-4104, 30 October, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.123
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Screening of contaminated soils for biodegradable plastic producing bacteria and profiling of their resistance markers

Nighat Naheed*, Nazia Jamil and Shahida Hasnain

Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore-54590, Pakistan.

Accepted 12 October, 2011

Polyhydroxyalkanoates (PHA) are the biodegradable polyesters produced by bacteria to overcome the environmental stress. Contaminated environments have a large number of bacteria which can accumulate PHA as their energy reserves. Molasses, oil/ghee and sewerage contaminated soils were selected for the screening of biodegradable plastic- producing bacterial strains. The selected contaminated environments showed great diversity of bacterial flora, *Pseudomonas* (39.39%) being the most common. The 54 bacterial isolates were further screened by Sudan Black B staining after 48 h of incubation on polyhydroxyalkanoates (PHA) - detection media with glucose as a carbon source and Nile Blue A viable colony staining method. The PHA granules could not be detected in 6 bacterial isolates (AS1, AS2, AP2, AP1, LS13 and VM1). In soil samples contaminated with molasses (sugar industry waste), 95% and in samples contaminated with oil/ghee industry waste all bacterial isolates showed PHA accumulation ability. The accumulated PHA was extracted by direct addition of sodium dodesyl sulphate (SDS). The bacterial isolates SM5, SM11, PG5, UOS2, LS10, AP4 and LS3 accumulated more than 50% PHA of their wet cell mass. No correlation was observed between bacterial growth and the PHA production. The bacterial strains showed multiple resistant markers for selected antibiotics (penicillin and streptomycin) and heavy metals (copper and cadmium). A high level of resistance was observed for penicillin (100 - 4500 µg/ml) and copper (1.5 - 17 mM/ml) in PHA- producing strains.

Key words: Biodegradable plastic, polyhydroxyalkanoates, molasses, oil/ghee, contaminated soil.

INTRODUCTION

Biodegradable plastics are a group of biopolymers synthesized by many bacteria and archaea. Among the various biodegradable polymer materials, polyhydroxy-alkanoates (PHA) provide a good alternative to petrochemical plastics because they are biodegradable as well as biocompatible and ecofriendly. Non petroleum-based biological polyesters are considered to be one of the most important next-generation polymers in the future in light of limiting natural resources. The properties of PHA are also similar to those of polyethylene (PE) and polypropylene (PP) (Kim and Lenz, 2001; Rehm, 2003).

Many micro-organisms can accumulate PHA as intracellular energy and carbon storage inclusions when the carbon substrate is in excess to other nutrients such as nitrogen, sulfur, phosphorus or oxygen (Madison and Huisman, 1999; Reddy et al., 2003). There are almost 250 organisms known to produce PHA, but only a few species can produce PHA at a high concentration e.g. *Alcaligenes latus* (Yamane et al., 1996), *Pseudomonas oleovorans* (Brandl et al., 1988), *Cupriavidus necator* (formerly *Ralstonia eutropha)* (Kim et al., 1994).

PHAs can be classified into different types according to the number of repeating units in the polymers. The polymers containing monomers of C3 to C5 hydroxyl fatty acids are referred to as short-chain-length PHA (scl-PHA) e.g. polyhydroxybutyrate (PHB) and hydroxyvalerate (PHV).

Similarly, the polymers composed of C6 to C16 hydroxyl fatty acids or aliphatic carbon sources are termed as medium- chain -length PHA (mcl-PHA) (Kim

*Corresponding author. E-mail: nighat_shabbir@yahoo.co.nz.

and Lenz, 2001; Sudesh et al., 2000)

The environments get contaminated with heavy metals and antibiotics from natural sources or directly or indirectly from human activities such as rapid industrialization, urbanization, and anthropogenic sources (Ware et al., 2006). The general use of antibiotics has been increased in many activities leaded by man, as agriculture, hospitals, animal husbandry, industry and prophylaxis. Production of PHA for industrial scale is affected by the heavy contamination from environment in the fermentor. Different scientists prefer to exploit the strains which are resistant to some antibiotics for controlling contamination (*de* Lima et al., 1999).

This study was carried out in three phases. The sugar, ghee industry and sewerage waste contaminated soil samples were screened for potential PHA producing bacterial strains in terms of diversity, estimation of PHA production and characterizing them for resistance markers.

MATERIALS AND METHODS

Sampling

The soil samples were collected aseptically from sugar, ghee/oil industry and sewerage waste disposal sites. For air samples, PDA (Polyhydroxyalkanoates detection agar) media plates were exposed to contaminated environments for 30 min. and are incubated at 37°C for 24 h (Cappuccino and Sherman, 2004).

Selection and characterization

The serially diluted soil samples were spread on the PDA media plates, after 24 h of incubation at 37°C the isolated colonies were selected and further purified by streak plate technique (Cappuccino and Sherman, 2004). The selected and purified colonies were coded according to the sample collection area. The colonies were then preserved as glycerol stocks and were analyzed for Gram reaction and performed a series of biochemical tests e.g. Oxidase, Catalase, Urease, TSI, Lactose Fermentation Test, Citrate, Motility, Indole and Sugar Fermentation (glucose, sucrose, lactose and maltose). The strains were characterized by the help of Bergey's Manual of determinative bacteriology.

Growth conditions

The selected colonies were grown on PDA- medium, consisted of (g/l) $(NH_4)_2SO_4$ 0.2, KH_2PO_4 13.3, $MgSO_4$ 1.3, Citric Acid 1.7. Trace element solution 10 ml/l, (g/l, $FeSO_4.7H_2O$ 10, $ZnSO_4.7H_2O$ 2.25, $CuSO_4.5H_2O$ 1, $MnSO_4.5H_2O$ 0.5, $CaCl_2.2H_2O$ 2.0, $Na_2B_4O_7.10H_2O$ 0.23, $(NH_4)_6Mo_7O_{24}$ 0.1, 35%HCl 10 ml); Bacto agar, 15 g/l. The pH of the medium was adjusted to 6.8 - 7.0. Glucose was added separately in the final concentration of 2% (Lee and Choi, 1999). The colonies were screened out qualitatively with Sudan black B (Lee and Choi, 1999) and Nile Blue A (Spiekermann et al., 1999) staining procedure. The potential PHA producing strains were subjected to shake flask culture for PHA estimation. The inoculums were prepared in 5 ml of PHA detection broth. The initial O.D. of all cultures was maintained at 0.05 nm with model U2020 IRMECO UV-VIS spectrophotometer (Germany). Cell growth was monitored by measuring the absorbance at 600 nm.

Extraction of biodegradable plastic

The recovery of the biodegradable plastic was done by direct addition of SDS (Kim et al., 2003). SDS treatment was carried out in 30 ml flasks containing 10 ml culture broth. The ratio of SDS to the wet cell mass (w/w) was 0.5:1 and reaction time was 1 h at 37°C and 150 rpm. After the reaction the heat treatment was conducted at 121°C for 15 min followed by centrifugation at 13000 x g for 10 min. The harvested solid sample including PHA was washed with distilled water. The recovered PHA was dried overnight at room temperature. The PHA content was defined as the percent ratio of PHA to wet cell concentration. The experiments were performed in three replicates. Pearson correlation coefficient was calculated with the formula:

$$r = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2\sum(y-\bar{y})^2}}$$

Where x and y are sample means.

Antibiotic and heavy metal resistance

Plate dilution technique was used to determine the levels of resistance for the selected antibiotics (penicillin and streptomycin) and heavy metals (copper and cadmium). $CuSO_4$ and $CdCl_2$ salts were used as the source of heavy metals in the media. The stock solutions were prepared in autoclaved distilled water (Sambrook and Russell, 2001).

RESULTS

Microbial diversity

In microbial diversity terms, 12 different genera (*Pseudomonas, Citrobacter, Enterobacter, Listeria Klebsiella, Escherichia, Lactobacillus, Alcaligenes, Streptococcus, Planococcus, Flavobacterium* and *Bacillus*) were isolated from three different groups of samples. Four of them (*Pseudomonas, Bacillus, Lactobacillus* and *Alcaligenes*) were common in all sampling sites. *Citrobacter, Listeria* and *Streptococcus* were found in the soil samples contaminated with sugar industry waste. The ghee Industry waste contaminated soil sample had *Enterobacter, Klebsiella* and *Escherichia* other than the four groups mentioned above. The bacterial strains which were gram negative rods, typically positive for Oxidase, Catalase and Citrate while negative for Indole and motile were grouped in genus *Pseudomonas* comprising 36.36% of total isolates. The bacterial strain UOS1 and PG1 were gram negative rods with raised, mucoid colonies, motile, positive for Citrate, ferment sucrose, generated gas from glucose and negative for Indole and H_2S production were considered to belong to genus *Enterobacter*. The 25 % bacterial isolates from ghee industry waste contaminated soil were identified as genus *Klebsiella.* These were found to be gram negative rods, non-motile and Oxidase negative. Among the gram positive bacterial strains *Bacillus, Lactobacillus* and genu *Listeria* were represented. SM2, UM4, VM1 and PG8 were spore

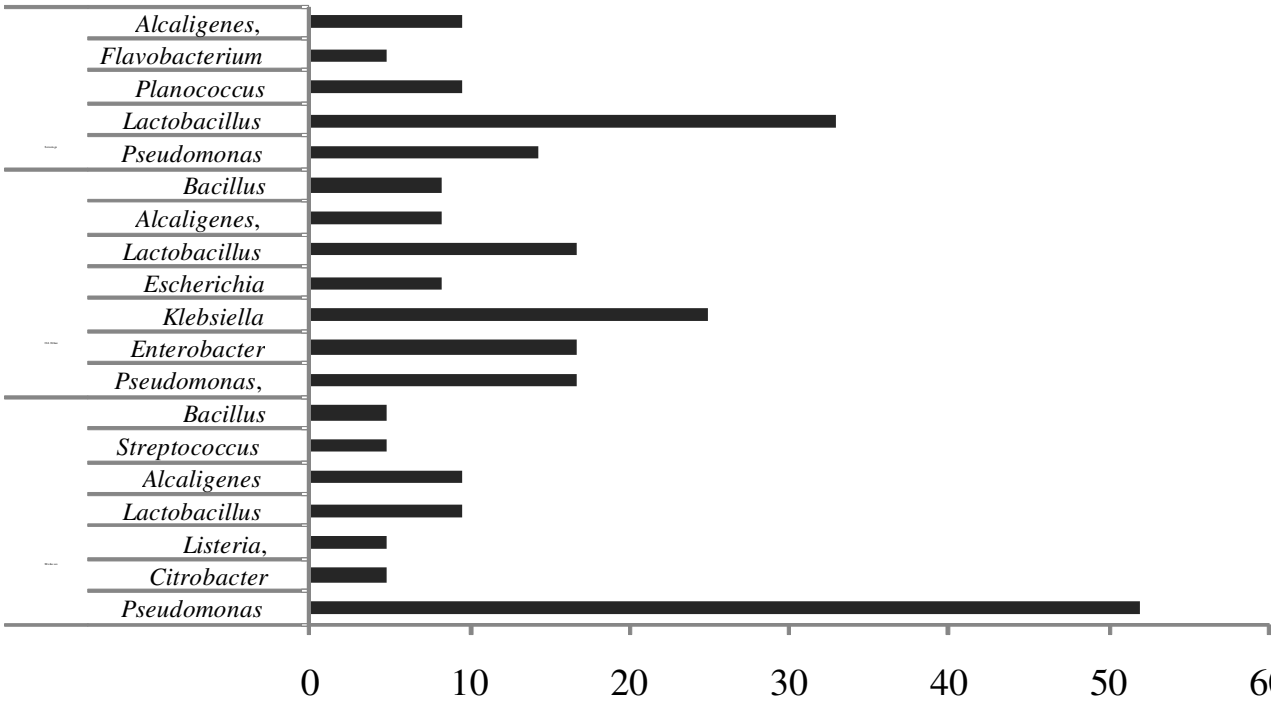

Figure 1. Percentage of isolated genera represented in different contaminated environments.

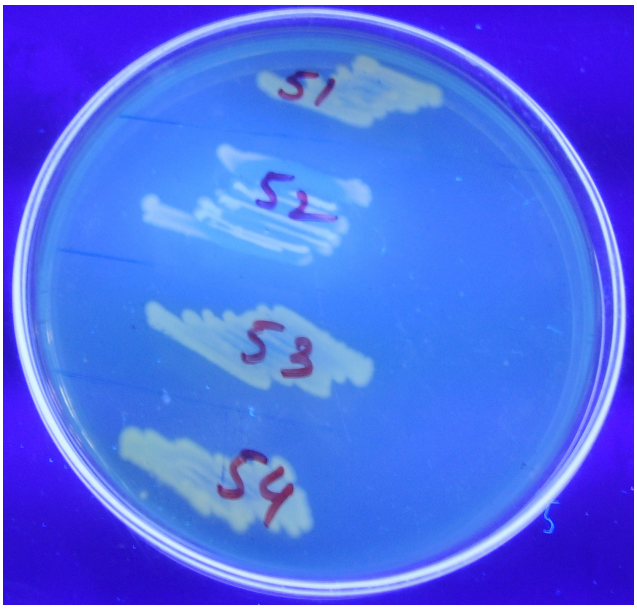

Figure 2. Nile Blue A staining.

formers and were positive for Catalase test. SM9, VM2, PG5, PG7 were non-spore formers, Catalase negative and positive for lactose fermentation. Out of 54 strains 21 were isolated from molasses, 12 from oil/ghee and 21 from sewerage contaminated environment. In molasses contaminated soil 52.38% of isolated bacterial flora belonged to genus *Pseudomonas,* while from oil/ghee contaminated samples *Pseudomonas* was only 16.66%. The data indicates that in both samples *Pseudomonas* (39.39%) *was* the most common genus. The *Bacillus* and *Lactobacillus* were the next more abundant genera representing 12.12% each of the total isolates (Figure 1).

PHA production

The 54 purified bacterial strains were subjected to Sudan Black B staining. The results showed that 48 strains were positive and in 6 bacterial isolates (VM1, LS13, AP1, AP2, AS1 and AS2) the PHA granules could not be detected. In the presence of Nile Blue A (5 µg/ml of PDA-media) most of the strains showed florescence when exposed to ultra violet light (312 nm) after appropriate cultivation period (Figure 2). The 48 potential PHA producing strains were grown in PHA detection media with 2% glucose as a carbon source. The percentage content of PHA was calculated after 24 h of incubation at 37°C and 150 rpm. Maximum absorbance (7.152) was observed in strain SM10 at 600 nm with wet cell weight as 70.66 g/l but percent PHA content was only 18.13. Strain SM5 showed 69.9% PHA accumulation with only 20.33 g/l wet cell mass. The bacterial strains suspected to be *Pseudomonas* showed PHA production in the range 59.52 - 18.13%. The bacterial strains SM4, SM5, and SM11 from molasses contaminated soil sample and UM3, UM3, PG3, PG5, PG8 and UOS2 from oil/ghee and LS1,

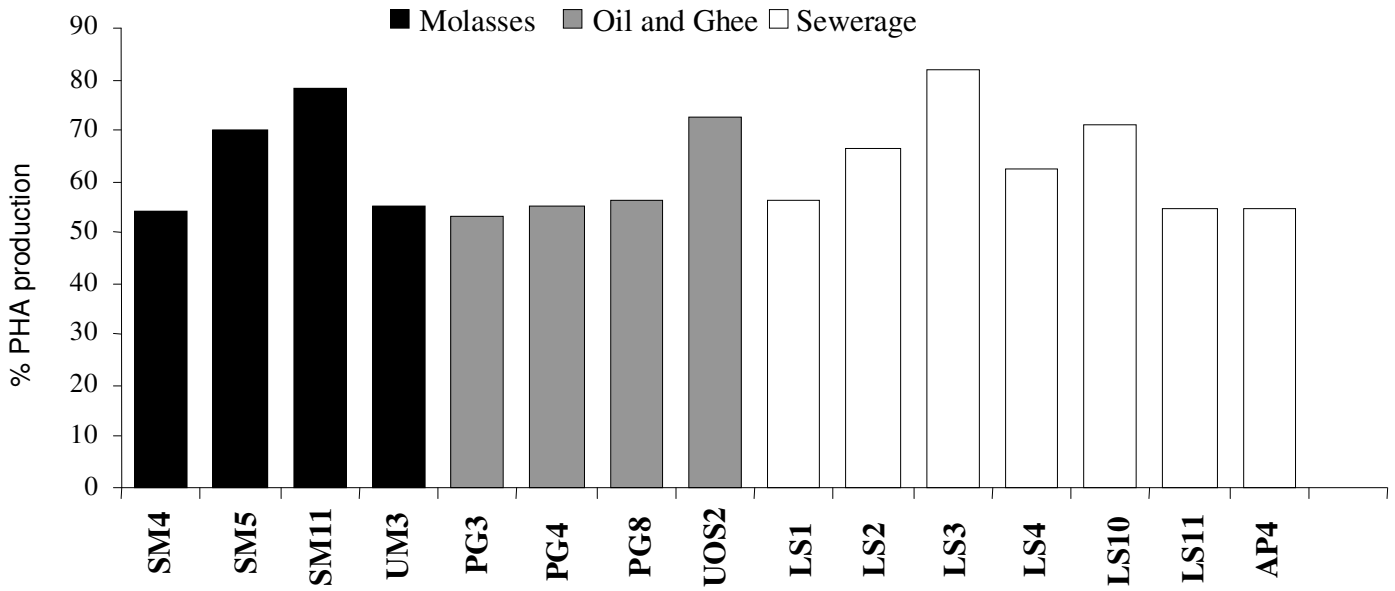

Figure 3. Comparison of %PHA production by the selected bacterial strains isolated from different contaminated environments.

LS2, LS3, LS4, LS10, LS11 and AP4 FROM sewerage contaminated soil sample could accumulate more than 50% PHA of their wet cell mass (Figure 3 and Table 1). The Pearson correlation coefficient was less than zero (-0.524 and -0.179) in gram negative and gram positive bacterial strains for biomass and percent PHA production thus no linear correlation could be established between these two variables.

Antibiotic and heavy metal resistance

All the purified strains were tested for their antibiotic and heavy metal resistance. In strains isolated from molasses contaminated environment penicillin resistance is more prevalent as compared to streptomycin resistance. The level of resistance ranged from 500 to 4500 µg/ml for penicillin and 50 to 1800 µg/ml for streptomycin. The bacterial strain UM9 showed highest level of penicillin resistance, 4500 µg/ml but was sensitive for streptomycin at 25 µg/ml level. On the other hand bacterial strain UM4 showed highest level of resistance at 3000 µg/ml and was also resistant for streptomycin at 1800 µg/ml (Table 1). In oil/ghee contaminated environment, resistance range for penicillin was 100 to 4000 µg/ml and for streptomycin was 600 to 1700 µg/ml. The bacterial strain PG8 showed highest level of penicillin resistance (4000 µg/ml) and for streptomycin was 1600 µg/ml. Similarly, PG4 showed resistance for penicillin at 2500 µg/ml and for streptomycin at 1700 µg/ml level. In third selected environment the penicillin sensitivity was more prevalent phenomena but some of the strains showed high level of resistance (4500 µg/ml) as in LS1, AS2 and AS3 (Table 1).

The heavy metals selected were copper and cadmium. The 0.5 mM concentration was selected as the minimum level for both heavy metals. In molasses contaminated environmental isolates, the resistance range was 1.5 to 17 mM for copper and 1.5 to 4.5 mM for cadmium. In oil/ghee contaminated environment it was 1.5 to 12 mM and 1.5 to 5 mM for copper and cadmium respectively (Table 1). The bacterial strains SM10 and UM4 showed maximum level of resistance to copper and cadmium respectively in molasses contaminated environment. Similarly in oil/ghee contaminated environment high level of resistance (1.2 and 5 mM) was observed in strains PG2 and PG4 respectively for both heavy metals applied. It was observed that in strains isolated from sewerage sites, the copper resistance (> 6.5 mM) was more common (Table 1).

DISCUSSION

The contaminated soils have a large diversity of PHA producing bacterial strains and diversity measurements are reflection of the dynamic status of an ecosystem (*de* Lima et al., 1999). The sugar, ghee industry and sewerage waste disposal sites were selected for sampling to conduct the present study. The selection of environment plays an important role in the isolation of PHA- producing strains as it must have conditions i.e. nitrogen limitation and carbon source either any carbohydrate or long chain fatty acids (Chen et al., 2001). A total of 54 bacterial strains were isolated 97% of which were PHA producers as anticipated from the staining results of Sudan black B and Nile Blue A. The 61% of the total isolates were from sugar industry waste

Table 1. Biopolymer production and characterization of bacterial isolates from contaminated environments.

S/N	Strain Code	% PHA	Antibiotic Resistance (µg/ml)		Metal resistance mM		S/n	Strain Code	% PHA	Antibiotic Resistance (µg/ml)		Metal resistance mM	
			Pen	Sm	Cu	Cd				Pen	Sm	Cu	Cd
1	SM1	45.64	4000	1600	4.5	2.5	28	PG7	36.35	3500	1600	4.5	3.5
2	SM2	20.51	3500	1600	4.5	1.5	29	PG8	56.19	4000	1600	S	1.5
3	SM3	29.41	3500	300	1.5	S	30	UOS1	36.16	400	S	1.5	S
4	SM4	54.2	4000	50	10.5	1.5	31	UOS2	72.54	3500	1600	S	1.5
5	SM5	69.9	3000	1600	10.5	3.5	32	UOS4	32.39	3000	S	1..5	S
6	SM6	48.53	4000	50	9.5	1.5	33	UOS6	21.76	3500	600	12	4.5
7	SM7	48.71	3000	1100	10.5	1.5	34	LS1	56.01	4500	S	6.5	1.5
8	SM8	38.09	3000	S	3.5	2.5	35	LS2	66.61	S	S	6.5	S
9	SM9	13.11	2300	1700	13.5	3.5	36	LS3	81.81	S	S	5.5	S
10	SM10	18.13	2500	S	17	1.5	37	LS4	62.5	S	S	5.5	S
11	SM11	78.27	3500	S	8	1.5	38	LS5	35.71	1200	600	6.5	2
12	VM1	ND	3000	S	4.5	S	39	LS6	35.29	S	50	6.5	2
13	VM2	37.73	4500	S	4	1.5	40	LS7	33.33	S	S	5.5	2.5
14	VM3	39.13	3000	500	11	1.5	41	LS8	47.74	3500	600	5.5	2
15	VM4	42.85	3500	500	5.5	1.5	42	LS9	32.93	S	600	5.5	S
16	VM5	37.09	500	500	5	1.5	43	LS10	71.16	S	S	5.5	1.5
17	UM1	23.93	3000	500	6.5	3.5	44	LS11	54.54	S	600	5.5	S
18	UM3	55.43	2200	600	6.5	1.5	45	LS12	44.44	S	S	5.5	S
19	UM4	43.03	3000	1800	16	4.5	46	LS13	ND	S	S	5.5	1.5
20	UM6	47.64	3000	S	13	3.5	47	LS14	11.11	S	50	5.5	1.5
21	UM9	41.81	4500	S	S	S	48	AP1	ND	50	50	5.5	S
22	PG1	43.75	2200	S	9	1.5	49	AP2	ND	S	600	6	S
23	PG2	41.22	3000	1600	12	1.5	50	AP3	14.26	S	600	4.5	S
24	PG3	53.3	2000	1600	11	4.5	51	AP4	54.54	25	S	5.5	S
25	PG4	55.32	2500	1700	15	5	52	AS1	ND	4500	800	5.5	S
26	PG5	50.49	100	S	4.5	2.5	53	AS2	ND	S	S	5.5	S
27	PG6	37.35	3000	1100	S	1.5	54	AS3	9.91	4500	S	5.5	S

Pen: penicillin; Sm: streptomycin; Cu: copper; Cd: cadmium.

contaminated environment. The PHA production was estimated by using 2% glucose as a sole carbon source. It was observed that 20% strains showed more than 50% PHA production of their biomass. The strains SM4, SM5, SM11 and UM3 produced 54.2, 69.9, 59.52 and 55.66% PHA respectively. The range of production was observed from 59.52 to 18.13%. The findings are supported by Chen et al. (2001) who reported that 40% of the isolated bacteria from molasses contaminated environment were capable of growing rapidly and synthesizing PHA at the same

time.

The 37% of the PHA producing strains were isolated from oil/ghee contaminated soil from ghee industry waste disposal sites. For the purpose of comparison the estimation of PHA production was done under the same growth conditions. The 41% of the strains showed more than 50% PHA production of their biomass. It indicates that although the strains were isolated from fatty acid rich environment but they were also capable to grow and accumulate PHA in the presence of sugars as well. The strains PG3, PG4, PG5, PG8 and UOS2 exhibited 53.3, 55.32, 50.49, 56.19 and 72.54% PHA production (Table 1). As Silva et al. (2004) described the high cell density cultures are indispensable in a PHA production process since they allow high productivity however this part of the study also demonstrated that there was no linear corre-Clation between the biomass and the percent PHA production. The value of Pearson correlation coefficient was less than zero as it should fall between -1 and +1 so the value greater than zero means a linear correlation between the two variables. A number of studies have reported the isolation and characterization of PHA - producing bacteria from natural habitats including marine water, sewage sludge and contaminated soils (Jamil et al., 2007; Razzaq et al., 2010; Arshad et al., 2007; and Rehman et al., 2007).

The PHA-synthesizing bacteria are present in various locations (Lopes et al., 2009) but there is no systematic study concerning PHA accumulation by microorganism (Gallert et al., 2005). On analysis for biochemical reactions, the 9 maximum PHA producing bacterial strains were identified as *Pseudomonas* (SM5, SM11, UM3 and UOS2), *Klebsiella* (PG3 and PG4), *Alcaligenes* (SM4) and *Bacillus* (PG8). Several studies had examined PHA production using these bacteria such as *Alcaligenes* (Rehm, 2003), *Pseudomonas sp*. (Ashby et al., 2002) *Escherichia and Klebsiella* (Zhang et al., 1994) and *Bacillus* (Singh et al., 2009). The *Pseudomonas* and *Bacillus* are among the most common genera of soil bacteria and *Escherichia* is encountered where sewerage contamination occurs (Kim et al., 1994).

Gram-positive bacteria have another potential advantage in terms of raw materials for PHA production. So PG5 and PG8 strains can be exploited for larger scale PHA production as the gram-positive genera are capable of naturally synthesizing commercially important copolymer (poly 3-hydroxybutyrate-co-3-hydroxyvalerate) [P (3HB-co-3HV)] from abundant and inexpensive carbon sources such as glucose. In contrast, gram-negative bacteria need expensive structurally related substrates such as propionicacid, valericacid, or other fatty acids with an odd number of carbon atoms to produce 3HV unit. There relatively high expenditure involved is a major hindrance in PHA copolymer production. Hence, gram-positive producers could considerably reduce the production cost (Mizuno et al., 2010).

Micro-organisms frequently contain extra-chromosomal elements that determine resistance to toxic compounds such as antibiotics and heavy metals (Gallert et al., 2005). The penicillin and streptomycin are most common antibiotics and are extensively used in many applications from household to industry. They are used for controlling contaminations in fermentations at industrial levels. The selected sampling sites are supposed to have a high level of exposure by these antibiotics. So in order to survive in these environments the organisms must develop mechanisms of resistance for tolerating high concentration of these antibiotics (Oliveira et al., 2000; Gallert et al., 2005). The data confirmed that all the isolated bacterial strains from both samples exhibited high level of resistance for penicillin which is more commonly used antibiotic as compared to streptomycin which inhibited the growth of 35% of the isolated bacteria. In molasses and oil/ghee contaminated environments, all the strains which are able to produce PHA are resistant to penicillin (500 to 4500 µg/ml and 1200 to 4500 µg/ml). The industry is also a major source of copper and cadmium contamination in the environment (Ali et al., 1996). The copper (58%) and cadmium resistance (55%) is equally prevalent factor in PHA producing strains of the selected contaminated environment (Figure 4).

Another important aspect of the study is that the maximum PHA producing bacterial strains showed multiple resistant markers for selected antibiotics and heavy metals applied. SM4 and PG8 had tolerated 4000 µg/ml of penicillin in the media and 50 and 1600 µg/ml of streptomycin respectively. Similarly SM4and SM5 (10.5 mM each) SM6, PG3 and PG4 (9.5, 11and 15 mM respectively) were notably resistant for copper.

Conclusions

This study indicates that contaminated environments are source of a large number of potential biodegradable plastic producing bacterial strains and as these are isolated from contaminated sites most of them showed multiple resistant markers for some of commonly used antibiotics and heavy metals. Although the strains are isolated from different environments in which the growth conditions and carbon sources are different but they are capable to grow and accumulate PHA equally well in the presence of sugars.

AKNOWLEDGEMENT

This research work was supported by International Foundation of Science (Sweden).

REFERENCES

Ali K, Javid MA, Javid M (1996). Pollution and Industrial Waste.6th National Congress Soil Sci, Lahore, 122: 131.

Arshad MU, Jamil N, Naheed N, Hasnain S (2007). Analysis of bacterial production of biopolymers. Afr. J. Biotechnol., 6 (9): 1115-1121.

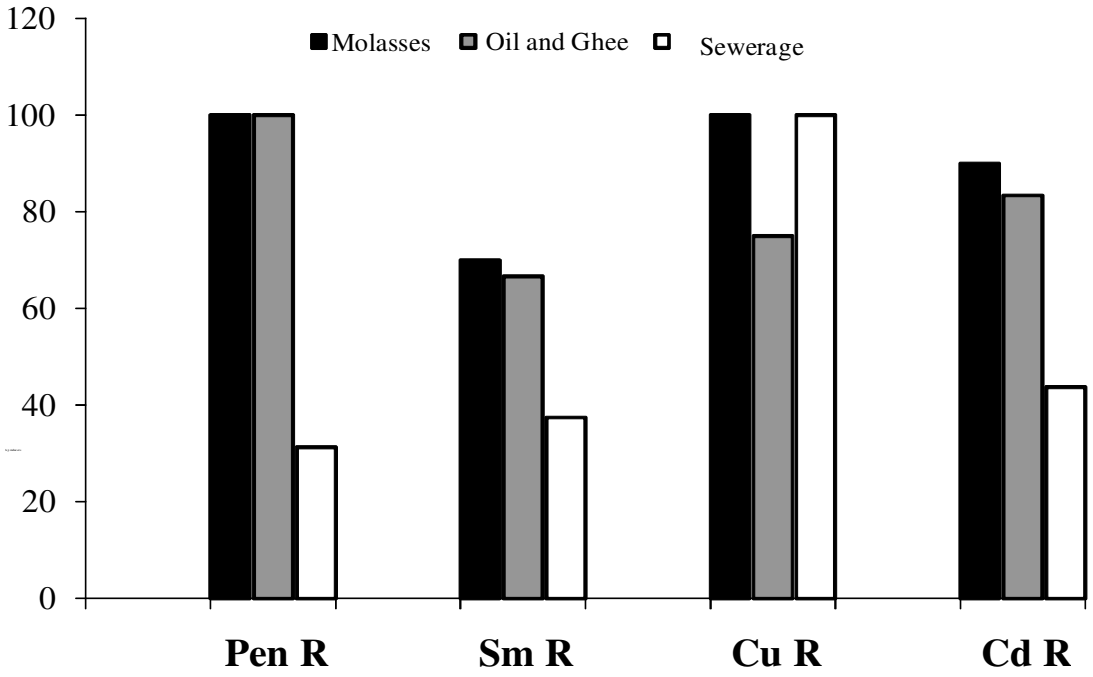

Figure 4. Prevalence of antibiotic and heavy metal resistance in PHA producing bacterial strains isolated from different contaminated environments.

Ashby RD, Solaiman DKY, Foglia TA (2002). The synthesis of short strains from contaminated and non-contaminated sites for the and-medium-chain length poly (hydroxyalkanoate) mixtures from glucose or alkanoic acid-grown *Pseudomonas oleovorans*. J. Ind. Microbiol. Biotechnol., 28:147–15.

Brandl H, Knee EJ, Fuller RC, Gross RA, Lenz RW (1988). Ability of the phototrophic bacterium *Rhodospirillum rubrum* to produce various poly β-hydroxyalkanoates: Potential Sources for Biodegradable Polyesters. Int. J. Biol. Macromol., 11: 49–55.

Cappuccino JG, Sherman N (2004). Microbiology A Laboratory Manual. Pearson Education: pp 7-15.

Chen G, Wu Q, Chen J (2001). Biosynthesis of Polyhydroxyalkanoates. Tsinghua Sci. Technol., 63: 193-199.

de Lima TS, Grisi BM, Bonato MCM (1999). Bacteria Isolated from a Sugarcane Agroecosystem: Their Potential Production of Polyhydroxyalkanoates and Resistance to Antibiotics. Revista de Microbiologia. 30: 214-224.

Gallert C, Fund K, Winter J (2005). Antibiotic resistance of bacteria in raw and biologically treated sewage and in groundwater below leaking sewers. Appl. Microbiol. Biotechnol., 69: 106–112.

Jamil N, Ahmed N, Edwards DH (2007). Characterization of biopolymer produced by *Pseudomonas* sp. CMG607w of marine origin. J. Gen. App. Microbiol., 53: 105-109.

Kim BS, Lee SC, Lee SY, Chang HN, Chang YK, Woo SI (1994). Production of Poly3-Hydroxybutyric Acid by Fed-Batch Culture of *Alcaligenes eutrophus* with Glucose Concentration Control. Biotechnol. Bioeng., 43: 892–898.

Kim M, Cho KS, Ryu HW, Lee EG, Chang YK (2003). Recovery of poly 3-hydroxybutyrate from high cell density culture of *Ralstonia eutropha* by direct addition of sodium dodecyl sulfate. Biotechnol. Lett., 25:55-598.

Kim YB, Lenz RW (2001). Polyesters from Microorganisms. Adv. Biochem. Eng. Biotechnol., 71: 51-79.

Lee SY, Choi J (1999). Polyhydroxyalkanoates: Biodegradable Polymer. In Arnold L, Demain J, Davis E. (2nd eds). Manual of industrial microbiology and biotechnology, Am. Soc. Microbiol., Washington, pp 616 - 627.

Lopes MSG, Rocha RCS, Zanotto SP, Gomez JGC, *da* Silva LF (2009). Screening of bacteria to produce polyhydroxyalkanoates from xylose. World J. Microbiol. Biotechnol., 25: 1751–1756.

Madison LL, Huisman GW (1999). Metabolic Engineering of Poly3-Hydroxyalkanoates: from DNA to Plastic. Microbiol. Mol. Biol. Rev., 63: 21–53.

Mizuno K, Ohta A, Hyakutake M, Ichinomiya Y, Tsuge T (2010). Isolation of polyhydroxyalkanoates-producing bacteria from a polluted soil and characterization of the isolated strain *Bacillus cereus* YB-4. Polym. Degrad. Stab. doi:10.1016/j.polymdegradstab.2010.01.033

Oliveira RC, Jose GC, Gomez B, Torres B, Netto LC, de Silva BLF (2000). A suitable procedure to choose antimicrobials as controlling agents in fermentations performed by bacteria. Braz. J. Microbiol., 31: 87-89.

Razzaq A, Jamil N, Naheed N, Hasnain S (2010). Bacteria from contaminated urban and hilly areas as a source of polyhydroxyalkanoates production. Afr. J. Biotechnol., 9 (13): 1919-1925.

Reddy CS, Ghai R, Rashmi VC, Kalia (2003). Polyhydroxyalkanoates: An Overview. Bioresour. Technol., 87:137-146.

Rehm BHA (2003). Polyester Synthases: Natural Catalysts for Plastics. Biochem. J., 376: 15-33.

Rehman S, Jamil N, Hasnain S (2007). Screening of different contaminated environments for polyhydroxyalkanoates producing bacterial strain. Biologia, Sect. Cell. Mol. Biol., 62(6): 650-656.

Sambrook J, Russell DW (2001). Molecular Cloning, (3rd eds) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Silva LF, Taciro MK, Ramos ME, Carter JM, Pradella JGC, Gomez, JGC. (2004). Poly-3-hydroxybutyrate (P3HB) production by bacteria from xylose,glucose and sugarcane bagasse hydrolysate. J. Ind. Microbiol. Biotechnol., 31: 245–254.

Singh M, Patel SKS, Kalia VC (2009). *Bacillus subtilis* as potential producer for polyhydroxyalkanoates. Microb. Cell Factory, 8: 38.

Spiekermann PHA, Bernd RK, Rehm D, Baumeister, Steinbüchel A (1999). A sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds. Arch. Microbiol., 171: 73–80.

Sudesh SH, Abe, Doi Y (2000). Synthesis, Structure and Properties of

Polyhydroxyalkanoates: Biological Polyesters. Prog. Polymer Sci., 25: 1503-1555.
Ware GW, David M, Whitacre DM, Albert LA, De Voogt P, Gerba CP, Hutzinger O, James B, Knaak FL, Mayer DP, Morgan D L, Park, Tjeerdema, Raymond SH, Yang FA, Gunther KK, Krishnani SA (2006). Heavy Metals Remediation of Water Using Plants and Lignocellulosic Agrowastes. Rev. Environ. Contam. Toxicol., 188: 59 - 84.
Yamane T, Chen X, Fueda S (1996). Growth-Associated Production of Poly 3-Hydroxyvalerate from *N*-Pentanol by a Methylotrophic Bacterium, *Paracoccus denitrificans.* Appl. Environ. Microbiol., 62: 380–384.
Zhang H, Obias V, Gonyer K, Dennis D (1994). Production of polyhydroxyalkanoates in sucrose-utilizing recombinant *Escherichia coli* and *Klebsiella* strains. Appl. Environ. Microbiol., 60: 1198 – 120.