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Molecular and biochemical characterization of solid waste biodegrading microbes isolated from the municipal waste dump site of Karachi City

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Abstract
The current study aimed to determine the productive microorganisms for waste biodegradation. Ten samples were collected from different areas of Karachi waste dump sites. The identification and characterization of isolates were done by morphological and biochemical tests. The isolated microbes were qualitatively screened for the action of making industrially key bio-enzymes. The potential enzyme-producing microbes were subjected to a waste degradation test for 90 days. Changes in odor, color and weight loss of decomposing garbage were noted. The highest degradation ability in term of weight loss was shown by *Aspergillus* (SSI_{14}) and *Bacillus* (SSI_6). Four metals (zinc sulphate, cadmium chloride, copper sulphate and iron sulphate) were chosen for metals tolerance test and minimum inhibitory concentration (MIC) of the isolated microbial strains. The current study reported that Zn was found to be more lethal in comparison to Cu and Fe. Molecular identification of the isolated microbes was done by polymerase chain reaction. The microbial strains were characterized by the polymerase chain reaction (PCR) followed by confirmation of the product by agarose gel electrophoresis. The findings can be served as baseline data to develop microbial apparatus for biodegradation and management of solid waste.

Keywords: Aspergillus, Biochemical test, biodegradation, metals, polymerase chain reaction.

Caracterização molecular e bioquímica de micróbios biodegradadores de resíduos sólidos isolados da lixeira municipal da cidade de Karachi

Resumo
O presente estudo visou determinar os microrganismos produtivos para a biodegradação de resíduos. Foram recolhidas dez amostras de diferentes áreas de depósitos de resíduos de Karachi. A identificação e caracterização dos isolados foram feitas através de testes morfológicos e bioquímicos. Os micróbios isolados foram selecionados qualitativamente para a ação de fazer bio-enzimas industriais chave. Os potenciais micróbios produtores de enzimas foram submetidos a um teste de degradação de resíduos durante 90 dias. Registaram-se alterações no odor, na cor e na perda de peso do lixo em decomposição. A maior capacidade de degradação em termos de perda de peso foi demonstrada por Aspergillus (SSI14) e Bacillus (SSI6). Foram escolhidos quatro metais (sulfato de zinco, cloreto de cádmio, sulfato de cobre e sulfato de ferro) para o ensaio de tolerância dos metais e para a concentração mínima inibitória (MIC) das estirpes microbianas isoladas. O estudo atual relatou que Zn foi considerado mais letal em comparação com e Fe. A identificação molecular dos micróbios isolados foi feita por reação em cadeia de polimerase. As estirpes microbianas foram caracterizadas pela reação em cadeia de polimerase (PCR) seguida de confirmação do produto por eletroforese de gel de agarose. As descobertas podem ser servidas como dados de base para desenvolver aparelhos microbianas para a biodegradação e gestão de resíduos sólidos.

Palavras-chave: Aspergillus, Teste Bioquímico, biodegradação, metais, reação em cadeia de polimerase.

Introduction

Municipal solid waste is released through the various family units, businesses, and institutions' activities. Such waste is segregated into various categories including waste or trash that is thrown but can be utilized for different other useful purposes, whereas garbage is considered a useless material due to its organic nature. However, waste material is considered inorganic some examples of waste are paper, domestic waste, yard squander, fabric, plastic, metals, and glass (Drackner, 2005; Townsend et al., 2015). However, based on the composition dry waste is more adjustable and adaptable in comparison to wet waste. The release of waste from construction sites is one of the problems faced by Urban people. Natural solids are available in the very high amount

of waste released from cultivation, foodstuff and marketplace (Cecchi et al., 2011). Waste materials are released due to expenditure, leisure, manufacture, and physical activities such waste materials are unwanted and proscribed as favorable in many ways. Such waste materials are created because of regular activities over some time (Hamed & Hameed, 2017).

The global city's strong waste creation arrived at 1.3 billion tons every year (365 days) in 2010 & it is predictable on the way to enhance to 2.2 billion tons each year (365 days) by 2025 (Hoornweg & Bhada-Tata, 2012). Dumps places are referred to as the zone locales where material waste from many sources and processes is deposited (Odeyemi, 2012). The natural waste found in municipal solid waste produce smell, mud, contamination, or unattractive chaos during decomposition. This problem can be solved by the application of microbial flora as supplements. The utilization of microbial flora on waste makes it less toxic by the release of various secondary metabolites of microbial flora. Such microbial flora also plays an essential role in converting complex waste into simpler forms. Various studies reported that there is a likelihood to get a vast variety of microbial flora after the screening of waste dump places with worthwhile applications (Siddiqqui, 2017). It is encouraged to utilize enzymatic methods in comparison to the synthetic chemicals, as it requires more research to be done and explore microbial diversity of biological molecules. There are various microbial flora that can be built with a diversity of bio-molecules similar to cellulose under proper settings (Siddiqui et al., 2000).

The microbial population in the soil means a massive problem on earth. These microorganisms carry a wide spectrum which includes bacteria, archaea, yeast, fungi, algae, and protozoa (Braga et al., 2016). Both scholarly and modern researchers understood that soil microorganisms are a potential cause to get exceptional naturally energetic metabolites. Microbes have been found to produce many important chemicals including ethanol, acetone, biomolecules, fragrance, and antimicrobials (Begum et al., 2017). Therefore, the current study aimed to identify the productive microorganisms for municipal waste biodegradation.

Materials and Methods

Collection of waste materials

Microbes can grow up in a broad array of humidity ranks. the samples were collected from various sites in Karachi including, Gulistan-e-Joahar, Garden east, Garden west, Pahar Ganj north Nazimabad, Iqra university north campus sector 7, Golimar Chowrangi, Kachra Kundi with Saifee college boundary wall North Nazimabad, near Dr. Ziauddin hospital North Nizamabad campus, Saddar Cantt Station and People's Chowrangi Karachi, Pakistan dump site.

Physicochemical properties of solid waste

Moisture Content (%) and pH Of Waste (Soil) Samples were carried out by applying the formula given by AWPA (**American Wood Protection Association**) (Emmanuel et al., 2017), whereas the pH was optimized via pH strips.

Microbial isolation from solid waste

Microbial strains were isolated via serial dilution technique followed by the inoculum streaking on Nutrient agar plates and incubated at 37 ^{0}C for 24 hours. Colonies were counted under the colony counter and cfu/mL was calculated. Purified culture colonies were preserved in 2% glycerol vials at -20 ˚C.

Biochemical parameters

Isolated microbes were identified by gram staining for morphological characteristics, and biochemical characteristics, isolates were streaked on Nutrient agar, Cetrimide agar, Mannitol salt agar, Sabouraud dextrose agar, MacConkey agar incubated at 37 ˚C for 24 hours.

Extracellular enzymes production

Isolated microbes were screened to produce industrially essential enzymes. The isolated microbes were streaked on starch agar, carboxymethylcellulose agar, skim milk agar, and egg yolk agar made based on their composition. The streaked plates were incubated at 37◦C. Then the plates were flooded with indicator solution and a clear zone around the growth was obtained which revealed enzyme production (Emimol et al., 2012).

Selection of potential microbes for waste degradation

The garbage was autoclaved and inoculated with 5mL microbial suspension. Control treatments were also performed. The initial waste sample was weighed and kept at room temperature. The waste biodegradation was calculated by the weight loss method and from that very information, the waste biodegradation was noted (Gautam et al., 2012).

Metal tolerance assay

Heavy metals tolerance test was performed by agar well diffusion method (Agarwal & Singh, 2012). Heavy metals (cadmium chloride, zinc sulphate, copper sulphate and iron sulphate) were used for the isolated microbes. Different concentrations of 10 mM, 50 mM, 100 mM, and 500 mM of each metal were poured into the wells of microbial swab lawned plates followed by incubation at 37◦C for 48 hours. The minimum Inhibitory Concentration (MIC) of heavy metals test was performed by gradually falling the concentration of heavy metals (Gautam et al., 2017; Sabdono et al., 2012). The protocols have been optimized as per the laboratory requirements.

Molecular identification

The deoxyribonucleic acid (DNA) was extracted according to the colony/ broth method with some modifications (Tong, 2011). Assessment of DNA purity was done by Nanodrop (2000 Spectrophotometer, Thermo Scientific). The extracted DNA concentration in ng/µl and Ration of absorbance at 260nm/280nm were noted. Molecular characterization of extracellular enzymes

producing microbes from municipal solid waste dump sites of various areas of Karachi Pakistan was done by the amplification of targeted regions by Polymerase Chain Reaction (PCR).

The PCR reaction mixture containing DNA template (3.0µl), Forward primer (2.0 µl), Reverse primer (2.0 µl), Master mix (13.0 µl), Triton 25% 100X (3.0 µl), and Nuclease free water (7.0µl). The PCR cycle for fungal and bacterial strains was mentioned in Table 1.

Table 1. PCR cycle parameters for microbial and fungal strains.

Step	Temperature and time for fungal	Temperature and time for Bacterial
Initial denaturation	94ºC for3 minutes	94ºC for 1minutes
Denaturation	94ºC for 45 seconds	90ºC for 30 seconds
Annealing	55ºC for 45 seconds	54ºC for 30 seconds
Extension	72ºC for 2 minutes	72ºC for 1minute
Final extension	72ºC for 7 minutes	72ºC for 8 minutes
Cycles	34	35

The 16s rRNA gene fragment of isolated microbes was carried out through a polymerase chain reaction in a thermal cycler (BioRAD). The forward universal primers (27F and ITS-1F) and the reverse primers (1492R and ITS-86R) were used for microbial DNA amplification. The amplified product was run on 1% agarose gel in TBE buffer with a 1kb (bioline) ladder. Bands were visualized in a UV illuminator The obtained products were observed in the gel documentation system UV transilluminator. All the obtained data were analyzed by the statistical tool IBMS SPSS software.

Results and Discussion

Physical Characteristics of Waste Samples

Tthe moisture content of the samples collected from Gulistan-e-Johar, Garden east, Garden west, Pahar Ganj north Naziabad, Iqra university north campus sector 7, Golimar Chowrangi, Kachra Kundi with Saifee college boundary wall North Nizamabad, near Dr. Ziauddin hospital North Nizamabad campus, Saddar Cantt station and people's Chowrangi Karachi, Pakistandump site was about 66.6%, 102%, 241.3%, 57%, 60%, 7.1%, 69%, 16.66%, 23% and 42% respectively. Maximum moisture content (241.3 %) was obtained from Garden west, while minimum moisture content (7.1 %) was noted from Golimar Chowrangi. The study reported that the rank of dampness content of gathered samples diverse from 25.09 - 78.19 %. However, the highest wetness content (78.19 %) was observed in the samples collected from Islamic University Campus Bangladesh garbage and garbage from Islamic University Campus Kushtia samples, whereas the least moisture content (25.09 %) was reported from the soil surface pond side of Islamic Campus, Bangladesh sample (Zaved et al., 2008). Physio-chemical environment plays a crucial role in microbial growth like the availability of pH, temperature, incubation time, and carbon supply. Microbial inhabitants of different soils are connected through their moisture content. The highest microbial concentration is set up in an area of elevated humidity and the best point for the actions of bacteria that required oxygen regularly is 50 -75 % of the soil dampness-holding ability (Alexander, 1978). Numbers of the genera *Pseudomonas, Achromobacter*, and *Bacillus* are set up in mainly aerobic soils; where the environment is anaerobic *Clostridium* will occur (Berkeley & Campbelt, 1972). The current study reported that pH of the soil samples from different regions including, Gulistan-e-Johar (7), Garden East (7.2), Garden West (6), Pahar Ganj (7.5), Iqra University North Campus (7), Golimar Chowrangi (7.6), KachraKundi with Saifee collegeboundary wallNorth Nazimabad (6), near Dr. Ziauddin Hospital North Nazimabad campus (8), Saddar Cantt station (7.4) and People's Chowrangi Karachi dump site (7). The observed pH of samples was found to be slightly acidic to alkaline that might be due to the degradation of waste material. Microbes are capable to accept soil feedback between pH levels 4 and 10, but the encouraging pH for the greater part is the alkaline side of neutrality (Berkeley & Campbelt, 1972).

Isolation of Microbes from Municipal Solid Waste

In the waste soil sample, the average cfu/ml for all the bacterial cultures was 2. 2 x 10^7 cfu/ml. The total microbes were obtained via the spread plate technique using a nutrient agar plate. The current study reported that the total number of organisms by spread plate method was 9.6×10^4, 8.8×10^4 from the waste dump site of the soil sample (Emimol. et al., 2012). Another study revealed an average cfu/g was 3.7×10^6 cfu/g at the temperature of 15°C (Hamid et al., 2019).

Isolated colonies were chosen and recognized as the source of morpho-cultural uniqueness, gram staining and biochemical properties according to the explanation of Bergey's manual of Determinative Bacteriology. The chosen microbial colonies were examined via light microscopy and various characteristics were recorded (Table 2).

All the microbes were characterized by the following biochemical test (Table 3). Based on microscopic examination, cultural characteristics, biochemical analysis and carbohydrate fermentation test the isolated microbes were identified: SSI_1, SSI_5 and SSI_8 as *Staphylococcus epidermidis*, SSI_2 and SSI20 as *Staphylococcus aureus*, SSI_3, SSI_9, SSI_{12}, SSI_{17}, SSI_{18} and SSI_{19} as *Bacillus* sp, SSI_4 as *Salmonella* sp, SSI_6 and SSI_{10} as *Pseudomonas* sp, SSI_7 as *Listeria s*p, SSI_{11} as *Klebsiella s*p, SSI_{13} as *Candida* sp, SSI_{14} as *Aspergillus* sp, SSI_{16} as *E.coli* sp. In our study percentage distributions of *Bacillus sp* were high in the waste sample (Figure 1).

Another study performed on sewage sludge compost reported that Bacillus is the most dominant in composting process (Fujio & Kume, 1991). The Bacillus species was the most dominant species in the degradation of organic waste.

Table 2. Colony forming unit and Microscopic Examination of soil sample isolates (SSI) Microbes

SSI	CFU/mL	Isolate	Gram reaction	Arrangement
SSI_1	2.4×10^8	SSI_1	positive	cocci in grape-like clusters
SSI_2	2.8×10^7	SSI_2	positive	cocci in grape-like clusters
SSI_3	1.8×10^7	SSI_3	positive	terminal spores rods in chains
SSI_4	3.0×10^8	SSI_4	negative	short rods
SSI_5	2.0×10^7	SSI_6	negative	rods in pairs and single

CFU: colony forming unit.

The colony morphology on different mediums showed that on Cetrimide agar *Pseudomonas* sp produces bluish-green pigmentation. On EMB agar *E.coli* showed dark greenish metallic sheen colonies, on MSA *Staphylococcus aureus* showed golden yellow colonies and *Staphylococcus epidermidis* showed pink colonies, on MacConkey agar *Klebsiella* produced pink lactose fermented colonies, *Salmonella* sp produced black colonies on salmonella-shigella gar, on Bile Aesculin agar *Listeria* sp produced black colonies, on Nutrient agar *Bacillus* showed white colonies, *Candida* sp showed white colonies on yeast extract peptone dextrose agar, *Trichoderma* sp produced light green conidia cover the entire media and the initial growth of *Aspergillus* sp shown as white in color. It was also observed by (Faryal et al., 2006; Ohgiwari et al., 1992; Prakasam et al., 2017; Thapa et al., 2015).

Table 3. Biochemical characteristics of soil sample Isolated (SSI) Organisms.

Isolate	Catalase test	Oxidase test	Indole	Methyl Red	Voges-Proskauer	Citrate	TSI				Nitrate Test
							Slant	Butt	Gas	H_2S	
SSI_1	+	-	-	-	+	-	-	-	-	-	+
SSI_2	+	-	-	+	+	+	-	-	-	-	+
SSI_3	+	-	-	-	+	+	K	A	+	+	+
SSI_4	+	-	-	+	-	-	K	A	+	+	+
SSI_5	+	-	-	-	+	-	-	-	-	-	+
SSI_6	+	+	-	-	-	+	K	K	-	-	+
SSI_7	+	-	-	+	+	+	-	-	-	-	-
SSI_8	+	-	-	-	+	-	-	-	-	-	+
SSI_9	+	-	-	-	+	+	K	K	+	+	+
SSI_{10}	+	+	-	-	-	+	K	K	-	-	+
SSI_{11}	+	-	-	-	+	+	A	A	+	-	+
SSI_{12}	+	-	-	-	+	+	K	A	+	+	+
SSI_{16}	+	-	+	+	-	-	A	A	+	-	+
SSI_{17}	+	-	-	-	+	+	K	A	+	+	+
SSI_{18}	+	-	-	-	+	+	K	A	+	+	+
SSI_{19}	+	-	-	-	+	+	K	A	+	+	+
SSI_{20}	+	-	-	+	+	+	-	-	-	-	+

SSI: Soil sample isolates, Positive test = +; Negative test = -.

Screening of Isolates for Extracellular Enzymes Production

The production of extracellular enzymes by isolated microbes from waste dump site collections is shown in table 4. The isolated microbes were streaked on starch agar plates using the Gram's iodine method and observed for the zone of clearance around their colonies (Figure 2). The isolates were screened for proteolytic activity on skimmed milk agar and showed a zone of hydrolysis around the colonies.

The isolates were screened for cellulase activity by using the carboxymethyl cellulose congo red plate technique, four isolates showed positive results (Figure 2). The microbes were tested for lecithinase production on Egg yolk agar, SSI_{18} showed a zone for lecithinase activity (Figure 2D). Extracellular lecithinase *bacillus sp.* isolated from the water was reported by (Bal et al., 2009). The isolates SS_{I9}, SSI_{12}, SSI_{17}, SSI_{18}, SSI_9, and SSI_3 which were *Bacillus sp.* able to produce two extracellular amylase and protease enzymes was also reported in (Sánchez-Porro et al., 2003). *Bacillus* sp SSI_3 was able to produce three extracellular enzymes was also reported in (Emimol et al., 2012; Khatiwada et al., 2016). The isolates *Aspergillus* SSI_{14}, *Trichoderma* SSI_{15} showed positive results for cellulase production was also reported in (Gautam et al., 2012). *Trichoderma* sp also produced amylase enzyme (Chávez et al., 2004). The strains SSI2 and SSI_{20} have shown amylase-producing activity. It was also reported that *Staphylococcus* isolated from soil samples showed a positive result for amylase activity(Duza & Mastan, 2013). The isolate SSI_6 & SSI_{10} showed positive results for two extracellular enzymes amylase and protease. Extracellular protease and amylase production by *Pseudomonas* was also reported by (Dutta et al., 2016; Gaur et al., 2010). SSI_7 showed a positive result for protease activity. Production of protease by *Listeria monocytogenes* was reported by (Shumi et al., 2004).

Trichoderma sp also produced amylase enzyme (Chávez et al., 2004). The strains SSI2 and SSI_{20} have shown amylase-producing activity. It was also reported that *Staphylococcus* isolated from soil samples showed a positive result for amylase activity(Duza & Mastan, 2013).

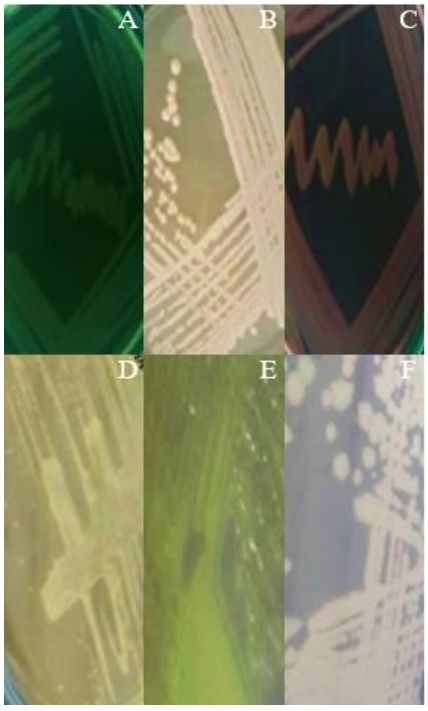

Figure 1. Isolated microbes on different agar mediums. Legend: a) *Pseudomonas* sp on Cetrimide agar, b *Candida* sp on TPD agar, c) *Staphylococcus epidermidis* on Mannitol Salt agar, d) *Staphylococcus aureus* on Mannitol Salt agar, e) *E.coli* on Eosin Methylene Blue agar, f) *Bacillus* sp on nutrient agar.

Table 4. Qualitative analysis of Soil sample isolates (SSI).

Isolate	Amylase	Protease	Cellulase	Lecithinase
SSI_1	-	-	-	-
SSI_2	+	-	-	-
SSI_3	+	+	+	-
SSI_4	-	-	-	-
SSI_6	+	+	-	-
SSI_7	-	+	-	-
SSI_9	+	+		-
SSI_{10}	+	+	-	-
SSI_{11}	-	-	-	-
SSI_{12}	+	+	-	-
SSI_{13}	-	-	+	-
SSI_{14}	-	-	+	-
SSI_{15}	+	-	+	-
SSI_{16}	-	-	-	-
SSI_{17}	+	+	-	-
SSI_{18}	+	+	-	+
SSI_{19}	+	+	-	-
SSI_{20}	+	-	-	-

The isolate SSI_6 & SSI_{10} showed positive results for two extracellular enzymes amylase and protease. Extracellular protease and amylase production by *Pseudomonas* was also reported by (Dutta et al., 2016; Gaur et al., 2010). SSI_7 showed a positive result for protease activity. Production of protease by *Listeria monocytogenes* was reported by (Shumi et al., 2004).

Metal Tolerance Assay

The metal tolerance assay revealed that the highest tolerance (50 mm) was obtained for the cadmium chloride 500mM concentration, whereas the minimum tolerance (14 mm) was obtained for the medium provided iron sulphate.

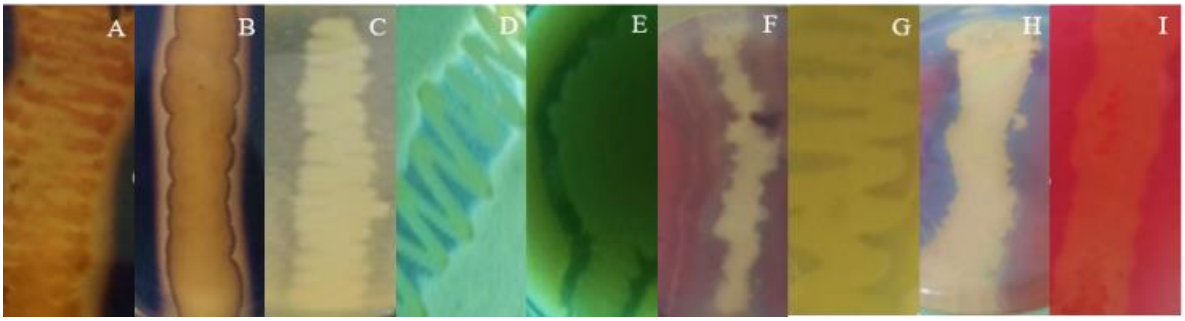

Figure 2. Screening of isolates for enzymatic activities (a-b) amylase activity, a) *Staphylococcus sp,* b) *Bacillus sp,* (c-e) protease activity, **c**) *Bacillus sp*, d) *Pseudomonas sp*, e) *Bacillus sp*,, (f-l) f) *Aspergillus sp*, g) *Bacillus sp.*, h) *Trichoderma sp,* and i) Lecithinase activity of *Bacillus sp*.

All the isolated microbes of the MIC (Minimum Inhibitory Concentration) ranged from 0.3 to 5 mM for Zn, Cd (0.6-5 mM). In the current study, the most lethal metal (lowest MIC) is Zn while the least lethal metal is Cu and Fe.

Studies performed on isolates of Otamiri River showed high MIC values for Fe and Zn and entirely the micro-organisms had low MIC values for Pb (Mgbemena et al., 2012). Another study revealed that the isolated bacteria from Barrackpore, Dhapa and Kolkata in India municipal solid waste had shown the most poisonous metal (with the lowest MIC) is Cd while the least poisonous metal is Pb (Singh, 2015). MIC is the maximum concentration of the heavy metals needed to prevent the development of microorganisms. Therefore, lower MIC values point out more lethal metals and elevated MIC values point toward less lethal ones (Mishra & Mishra, 2015).

Solid waste carries various components for example paper waste and kitchen waste. Food garbage, textile waste and metals that's why municipal solid waste is suitable surroundings where the micro-organisms can build up resistance to toxic heavy metals (Silver, 1996). Differences in the heavy metals results are because of the level of metal contamination and the nature of organic structures (Bezverbnaya et al., 2005).

Bioconversion of Municipal Solid Waste

Enzymes have immeasurable exploitation in the biodegradation of waste. In the current study, 11 bacterial and fungal strains capable of producing amylase, protease, cellulase and lecithinase enzymes respectively were applied to waste bio-degradation test for 90 days.

The bacterial strains including, *S.aureus* (SSI_{20}), *Bacillus* (SSI_6), *Pseudomonas* (SSI_6), *Bacillus* (SSI_3 **),** *Bacillus* (SSI_{19}), *Bacillus* (SSI_{17}), *Bacillus* (SSI_{18} **)** and Listeria (SSI_7) were shown weight loss (%) of waste 27 %, 68 %, 30 %, 25 %, 13.3 %, 15.3 %, 24 % and 34 % respectively. The fungal strains *Candida* (SSI_{13}), *Aspergillus (*SSI_{14}) and *Trichoderma* (SSI_{15}) were observed to weight loss (%) of waste by 13 %, 83 % and 20 % respectively. Among the 11 bacterial and fungal strains studied, *Aspergillus* (SSI_{14}) showed the highest degradation potential losses 83 % and 68 % weight loss was also observed in *Bacillus* (SSI_6). The study revealed that waste is crashed by the potential microbes and the heaviness of the waste reduction because microorganisms break down the waste into uncomplicated molecules. The percentage of weight reduction of unwanted waste was improved with the increase in degradation.

The second-highest weight loss percentage was found to be 68 %. Comparable results were obtained in another study conducted by (Zaved et al., 2008). The detection of low-density polyethylene biodegrading soil fungi and described *Aspergillus flavus* loss the weight by 30%, *Aspergillus niger by* 20%, *Aspergillus japonicus by* 36%, *Mucor* sp by 16%, *Penicillium* sp 24%, and *Fusarium* sp 32% following 30 days incubation. They reported that *T. viride* reduced the weight by 12.51 % and 20 % after 60 days of incubation (Gautam et al., 2012). Bacterial species concerned with the biodegradation method include *Bacillus, Pseudomonas, Klebsiella, Rhodococcus, Flavobacterium, Escherichia, Azotobacter* and *Alcaligenes* (Sangale et al., 2012).

Molecular Identification of Potential Microbes

The study confirmed the presence all 10 microbial strains via 16SRNA amplification showing in Figure 3. The study confirmed the presence of all 10 microbial strains via 16SRNA amplification shown in Figure 3.

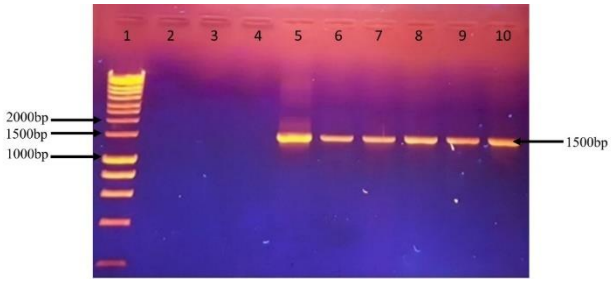

Figure 3. Representation of DNA bands of microbial strains over UV illuminator. Legend: Lane 1= 1 kb ladder, Lane 5 = Isolate 9, Lane 6 = Isolate 18, Lane 7 = Isolate 1, Lane 8= Isolate 10, Lane 9 = Isolate 7, Lane 10= Isolate 3.

The study reported that universal primers 27F and 1492R for the isolated cellulase-producing bacteria from soil samples and visualized about 1400bp bands (Patagundi et al., 2014). Another study reported the universal fungal primers ITS-1F and ITS86R for the amplification of fungal community DNA and captured the 350bp band in the UV illuminator (Figure 4) (Vancov & Keen, 2009).

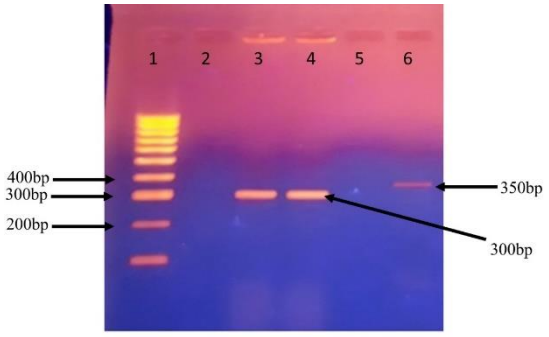

Figure 4. Representation of DNA bands of targeted microbial strains over UV illuminator. Legend: Lane 1= 1 kb ladder, Lane 5 = Isolate 9, Lane 6 = Isolate 18, Lane 7 = Isolate 1, Lane 8= Isolate 10, Lane 9 = Isolate 7, Lane 10= Isolate 3.

Conclusion

Microbes can grow up in a broad array of humidity ranks. In this research, it was observed that the moisture content of the samples was collected from different sites. Isolates were screened for solid waste degradation over 90 days. *Aspergillus* (SSI14) 83% and *Bacillus* (SSI6) 68% showed effective degradation results.

In the future, our research findings can be utilized as tremendous microbial apparatus in the field of biodegradation to tackle solid waste management. Various biodegradation of solid waste methods is available, but the environment-friendly technique is a breakdown of waste exploiting industrial enzymes producing microorganisms.

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