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Separation And Characterization Of Microbes Secluded From Vegetable Market Waste For The Manufacture Of Industrial Enzymes And Waste Deprivation

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Abstract

There is an emerging drift to the utilization of new technologies today, which mostly aims in focusing on the biotic progressions ensuring the reprocessing and also the effective application of the biological residues that may be processed by a number of diverse microbes for their basis of nutrients and energy. In this current study, the separation of strains of various bacteria from four diverse vegetable waste dumping sites was accomplished by the very detached of assessing the hydrolytic measurements of the bacterial strains and also their appropriateness for utilization in biological alteration of particular substrates. The 23 species that isolated were then separated in a suitable particular culturing medium for analyzing the manufacture of numerous hydrolyte-based enzymes (protease, lipase, and so on). It was then identified that six species showed action with all substrates and capacities to produce all hydrolytic enzymes. The species that exhibited the maximum hydrolytic dimensions by effective biological possible were then designated, genotypically characterized, and was recognized to be *Bacillus licheniformis*, *Pseudomonas* sp., *Bacillus subtilis*, *Planococcus* sp., *Bacillus cohnii*, and *Serratia* sp. The inhibiting and combined actions inside the species, heavy metal acceptance, tolerance to aromatic pollutants, and their susceptibility to different antibiotics were documented. These outcomes have enlarged the possibility of discovering environmentally significant microbes from vegetable market waste dumping sites and this isolate might be essential basis to explore the industrially valuable bacterial metabolites.

Keywords: Wastage dumping; Hydrolytic enzymes; 16SrRNA gene; Antagonism

1. Introduction

One among the maximum plentiful renewable and sustainable sources on earth is agro waste disposal materials and residues. Biomass deposition in enormous amounts each year results not just in degradation of the external environment, but also in depletion of potentially appropriate information that could be extracted and turned into a variety of value-added items. These solid

wastes are potentially filled with an immense resource of energy and carbohydrates that could then be wasted if they were dumped in open waste pits and landfills without being handled as such (Nigam; Pandey, 2009; Okonko et al., 2009).

Based on these type and composition of disposal, it will be possible to produce items of greater digestibility and nutritional value that are intended for both the human and animal food (Nortey et al., 2007; Okonko et al., 2009). Moreover, it will also make it easier to use them in large-scale digestion process as a sustainable source of carbon. Another choice could be its use as a highly important raw material for the manufacture of elevated goods. There has been an increasing trend in the usage of novel technological advances, focusing primarily on biological systems, efficient recycling and the efficient need for organic matter (Nigam; Pandey, 2009; Okonko et al., 2009).

Biotechnological methods like the brewing process provide excellent prospects for the reusage of agricultural by-yields available in large quantities and wastes for the synthesis of another yields (Vishwanatha et al., 2010; Muthulakshmi et al., 2011). These compounds are then usually regarded as the correct substrata for the fluid substrate brewing process (Pandey et al., 1992). Polymer, for example, polysaccharides, proteins and lignin, that may be substantially processed as a resource of energy by different classes of microbes by enzymatic hydrolytic processes are the essential biodegradable molecules available biologically. Although the microbe generating hydrolytic enzymes like microbes, fungus, and mushrooms has a wider range of properties the use of native waste microbial population ensures its successful function, operating in the setting to that it has developed (Ponce; Moreira; Roura, 2008).

The huge amount of manufacturing methods involves the utilization of various bacterial enzymes, and there is novel realm of usage that has been continually introduced. Enzymes are able to change and improvise the nutritional, functional as well as the sensory assets of constituents and goods, and it was also utilized as an alternative to conventional chemical methodologies (Pandey et al., 1992; Kirk; Borchert; Fuglsang, 2002).

Hence, for the reasons mentioned above, the effective usage of these agro wastes might help numerous determinations like value-added harvests, wastage organization and synthesis of various enzymes by possible manufacturing use. The work aims in isolating and characterizing the microbial species from vegetable waste dumping of Chennai vegetable market and their capability to yield hydrolytic enzymes and its potential to be used as conglomerate microbes in the process of biological conversion of the agro wastage.

2. Materials and methods

2.2 Sources of microbes

Microbes were properly secluded from vegetable waste dumping (soil mixed with vegetable waste) of Chennai vegetable market. All of these isolated specimens were then taken below sterile circumstances, packed in polypropylene model bags and then elated aseptically to the concerned laboratory. The content of humidity and pH of specimens were documented. Until utilization, they were preserved under sterile circumstances at the normal room temperature.

2.3 Evaluation of the moisture content in percent and the pH of specimens

Freshly test specimens of waste also were deposited in filter paper, recording the initial weight. Afterwards, the specimens were preserved in a 110 °C hot air oven. The sample solutions were then measured multiple times until a suitable equilibrium was reached. The sample's relative humidity was determined using the formula given by AWPA below (AWPA, 1986).

$$MC(\%) = \frac{W\text{-}w}{w} X\,100$$

Where, MC = moisture contented, W = original weight and w = constant weight after oven dried, pH of specimens was then evaluated in Electrometric technique by the aid of pH meter utilizing amalgamation glass electrode.

2.4 Isolation of microorganism:

Ten gram of weighed specimens was mixed in Homogenizer with 90 mL of the peptone water and serial dilution. The obtained samples were then plated onto Nutrient Agar Plates and subjected cultivation at 35 °C for 24-48 hrs. The various groups with defined structural features were then extracted after the gestation period and transported to Nutrient Broth (NB) by immunization to separate the microbes from the dumping of various agricultural wastes.

2.4.1 Microorganism culture

All the microorganism cultures were then maintained at 4 °C in nutrient agar. All the obtained cultures were sub-cultured at 15 days interval.

2.5 Enzymatic Specific media

2.5.1 Extracellular protease action

On a modified screening medium (MSM) containing (per liter): (skim milk powder-10g, yeast extract-3.0g, ammonium sulfate-6.7g, NaCl-0.5g, K_2HPO_4-0.7g, $MgSO_4$ 7H2O-0.5g) (Lee, 2009) with pH-7.5 and nurtured for 48 hrs, the qualitative assessment of proteolytic enzymes was then assessed. The development of the clearance zone across the colonies indicates the development of the protease hydrolytic enzyme. Positive isolates for the caseinase expression were further analyzed.

2.5.2 Caseinolytic movement test

The species attained were separated for its caseinase action on MSM augmented with 1 percent casein. Positive caseinolyte enzyme action was then noticed through the creation of white halo zone nearby the strains which were then selected for further study (Joo et al, 2002).

2.5.3 Lipolytic activity analyze:

Qualitative lipase action assesses on Tween agar medium

Tween medium that contains(per liter): 10 grams of weighed peptone, 0.1g $CaCl_2$.2 H_2O, 5.0g NaCl, 10 mL Tween 80 and 20g agar- agar at pH of 7.4 (Davender Kumar, 2012). The very creation of a white precipitate near the well resultant owing to the deposition of the quartzes of the Ca^{2+} salt designed through fatty acid enlightened through the enzyme, designates +ve lipolytic behavior.

2.5.3.1 ROA plate assay

The selected lipase positive strains were subjected for secondary screening on modified Rhodamine amended medium, in accordance to the U.T. Bornscheuer et al., 2002 to ensure true lipases producers and esterase maker. The basal medium utilized (per liter) encompassed: 5.0g of nutrient broth, 20g of NaCl, 4.0g of agar-agar and 250 µL of Tween - 80. The medium's pH degree was then changed to 7.2; it was then sterilized by autoclaving and chilled to approximately 60 °C. Then 30 mL of olive oil (originally warmed at 80 °C for 20 minutes in a culture oven) and 10 mL of Rhodamine B solution were exposed to filtering sterilization (0.22 µm) and applied to the basal media by continuous agitating and emulsifying agent by combining it with a sonicator for 1 minute. To decrease foaming, the final pure culture formulated was then exposed to 10 mins of rest at 60 ° C, and then 20 mL of media was dumped through every petri dishes. The Agar plates comprising Rhodamine B and trioleoylglycerol were opaque and pink in color. The enzyme lipase output was controlled by irradiating the culture plates at 350 nm with UV light. The bacterial growth with "true lipases" showed orange fluorescence after being subjected to 48 hours of incubation.

2.5.4 Amylolytic test

The attained medium was then examined for the capability to dissect starch on Modified starch agar medium (1.0% soluble starch, K_2HPO_4 0.1%, yeast extract 0.4% and $MgSO_4 \times 7H_2O$ 0.15%, pH 7.2) (Beffa, 1996). After 24 h period of incubating, the amylase enzyme producers were then identified by flooding the culture plates with 1% iodine in 2% KI solution. The zone of clearance around the well designated a +ve amylase action.

2.5.5 Cellulolytic activity test

The species were then separated for the production of cellulase action on modified screening medium (carboxymethylcellulose - 0.5g, $NaNO_3$ - 0.1g, K_2HPO_4 - 0.1g, KCl - 0.1 g, $MgSO_4$ - 0.05g, Mold extract - 0.05g, Agar - 1.6g, Glucose - 0.1g, Distilled H_2O - 100ml) (Ziad, 2008). After 24 hours of incubating the petri plates, they were then swamped with 0.1% Congo red solution and left uninterrupted for about 15 mins. For the visualization of zone of clearance formed by the cellulase +ve species the petri plates were detained utilizing the 1M NaCl solution. A +ve cellulase action was noticed through observing the creation of a yellow-halo towards a red-color background.

2.5.6 Lecithinase activity assay

As per Oladipo et al. (2008), the development of lecithinase was evaluated on a changed medium in which 10 percent corn millet substituted in an actual broth. Lecithinase was identified as per the steps of the scientific method through Nandy et al. (2008), in that 1 ml of every microbial culture was immunized to test tubes comprising corn millet broth at a cellular thickness of 6 * 108CFU/ml and nurtured at 37 ºC for 24 hrs. The cultures were centrifugated at 2500 rpm for 15 minutes after incubation to attain a cellular free filtrate and 100 µl of the filtrate was transported to 10 mm wells directly developed in the egg yolk agar dishes and nurtured at 37 °C for 24 hours. Opaque zones were calculated as lecithinase creation measures and the mean values were utilized as a lecithinase action parameter.

2.5.7 Dnase activity assay

Both samples were inoculated with toludiene blue test agar comprising DNase and methyl green test agar contains DNase. Due to the extreme metachromatic properties of toludiene blue, DNA hydrolysis on DNase test agar comprising toludiene blue is detected as a slight pink halo of the initial blue color across the colonies. While the hydrogenated region becomes colourless from green on DNase test agar with methyl green. Toludiene blue produces a blue color in response to DNA and a green color forms with methyl green. Both media have achieved consistent findings.

2.6 Molecular Identification of hydrolytic strains

The selected hydrolytic microbial strains were molecular authenticated by 16S r DNA analysis. The genomic DNA was removed from the microbiome and afterwards the extremely purified DNA collected was amplification in a thermocycler at circumstances: 35 sets of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, which would be sustainability performance for amplifying the 16S r RNA sequences and use the primers (A.F. Fouad, 2002). FORWARD (16F27):5 -AGAGTTGATCCTGGCTCAG-3; FORWARD (16R1522):5 -AGAGGAGGTGATCCAGCCGCCA-3; FORWARD (16R1522):5 -AGAGGAGGGTGATCCAGCCAGCCGCCA-3. In an extremely automated gene sequencer, DNA sequencing was done. In Genbank databases (BLAST), the sequences were then read and contrasted to the other sequencing to evaluate the microbial class and its phylogenetic analysis. The sequences were further contrasted when using BLAST program (National Center for Biotechnology Information) with another published sequences in the GenBank database (Michel, 2000).

2.7 Antagonism test

Each of the strains were subjected to growth at room T and then consequently examined through the cross-streaking technique at standard room T and at 37 °C. As defined by Gillers & Govan et al., the cross-streaking technique was carried out with two adjustments. The strains to be evaluated were immunized diametrically around replicated nutrient agar plates as a 1.5-cm-wide stripe. At either room T or 37 °C, the petri dishes were exposed to incubation overnight. Since the inhibitory zones created were greater and stronger, a broader streaking of an actual inoculation has been used. The inoculation was then extracted by a glass slide after incubation period and the enduring viable development was slaughtered by introduction to UV light for 30 minutes. When using a wire loop, the indicator species were individually smeared at right angles to the initial inoculum. The striped dishes were then hatched at room T or 37 °C overnight, or where the pointer strains passed the standard inoculant, the suppression was registered. This protocol was conducted until they evaluated each of the varieties towards each other.

2.8 Metal tolerance test

Waste dumping soil from vegetable and domestic sources has various numbers of ions of heavier metal in varying concentration. In order for the isolation of the bacteria having tolerance to heavy metal-based ions, we patterned for an individual MICs utilizing numerous metal-based ions like the chromium(Cr), cadmium(Cd), lead(Pb), copper(Cu), cobalt(Co) and nickel(Ni). The pasteurized agar media (Beef extract-10g, Peptone-10g, Sodium chloride-5g, Glucose-1g, Agar-20g per liter of distilled water, pH-7.5) was then processed for microbial development with metal concentrations that range among 50 µg/ml and 500 µg/ml by an increase of 50 µg/ml. Then the process was followed in the context where the apparent development was missing to evaluate the MIC for each metal with such a significant rise of 10µg/ml (Shakoori et al., 1998). C1 xV1 =C2 xV2, where C1 is the metal intensity in the stock solution, V1 is the density of the formulated stock solution utilized, C2 is the particular concentration of the metal throughout the agar and V2 is the volume of the agar. 20 ml of agar medium then was squeezed through each petri dish and then the amount of metal stock solutions was determined through the formula: The hydrolyzing species chosen were then smeared with sterile chains on the surface of the agar medium supplemented the metal salts. At a T of 37 °C, the plates were then fully enclosed and subjected to incubation for about 7 days. Then the striped plates were inspected for microbial development. The MIC for that metal for that species is the unique concentration of the metal ion at that there was no development identified. Plates without any of these metal concentrations were used for reference.

2.9 Aromatic pollutants Assay

The growth of these strains was qualitatively experienced over significant recalcitrant chlorinated aromatic pollutants like carbazole, naphthalene, dibenzothiophene, benzo(α)pyrene, fluorine and pentachlorophenol as an only carbon resource in pasteurized agar media with 1 mM concentration at pH-7.5.

2.10 Antibiotic sensitive test

The cup test technique (Cooper, 1955) was utilized for antibiotic sensitive experiment. Then the pasteurized agar media was formulated for microbial development. The petri dishes were then immunized with microbial interruption via spreading plate technique. On every of the cups were finished through pasteurized cup borer. A different quantity of Standard antibiotic viz, Penicillin (10 µg), Ampicilin (10 µg), Tetracycline (30 µg), Gentamicin (10 µg), Chloramphenicol (10 µg), Kanamycin (30 µg), Nalidixic acid (30 µg), Streptomycin (10 µg) was dispensed on cups of pre-inoculated petri plates. For every antibiotic concentration an isolated plate with pre-inoculated microorganisms were utilized. All these petri dishes were nurtured at 37±2 °C for 48 hrs. The result of suppression zones of the isolates was then

construed as sensitive (S), intermediate (I) and resistant (R) accordingly to the references to the normal against each of the antibiotic and also towards every microbial. The petri plates that did not contain any these antibiotic concentrations were utilized as reference plates. All of the experimentations were performed utilizing triplicates.

2.11 Wastage deprivation probable of designated microbial specimens

A tube of 10 ml of sterile distilled H_2O was applied to 24 hours of old microbial culture and then combined well for suspensions. After that, 5ml of this test organism was immunized with the disinfected garbage and blended well. Control trials with immunization were also conducted.

The capacity for decomposition of waste of microbes was examined using the process of weight loss. The litter information was obtained under aseptic conditions in sealed litter bags. Samples are collected were taken to the laboratory and then disinfected for 15 minutes in a sterilizer at 121 °C at 15 lb/inch2. Disinfected wastage specimens were then balanced and bacterial isolates were immunized and held for 7, 15 and 21 days at 38 °C, respectively. Specimens of wastage were splashed under running water after a definite time of incubation to eliminate inoculated microbes, and then it was desiccated and weighed. The weight losing was measured and the deterioration potential of microbes was estimated from that information.

3. Results and discussion

3.1 Separation and the hydrolytic profiling evaluation

The separation and the characterization of the obtained microbial species with hydrolytic profiling from different vegetable waste dumping pits of Chennai vegetable market regions were assumed in these present works. Microbial development is based upon the numerous physico-chemical factors like the composition of media, pH, T, carbon source, incubation time etc. So diverse circumstances based upon that microbe grew in organic habitat must be studied and understood before progressing to enormous growth for utilizing it as a decomposer. Therefore, the subsequent constraints were engaged into attention.

3.2 Effects of the moisture % and pH on wastage dumping specimens

Microbe can develop significantly in a wider ranging of the moisture extent. In our work, we identified the moisture constituent of an obtained sample from Chennai vegetable market waste dumping site was about 64% to 67%. Microbial populations of diverse soils are nearly linked to their moisture constituent. The higher level of microbial thickness was present in the regions that have fairly higher moisture contented and the higher extent for their actions of the aerobic microbe often ranges between 50 to 75 percent of the soil moisture landing capability (Alexander, 1977). The soil moisture determined the growth and development of microorganisms, their diversity and also the activity of soil enzymes (Borowik, 2016).

In this study the effective pH of the obtained samples was 7.2 to 7.9 and also the pH is a main feature for developing microbe in artificial medium. Optimization of pH was then executed to all chosen medium viz. Nutrient Agar (NA) and Extracellular enzyme screening mediums. NA, Nutrient Broth (NB) at pH 7.2 and was identified to be suitable for the highest progress of the microbial species. From these results, it was observed that pH of an obtained sample was 7.2 to 7.9 and probable for these reasons, the obtained species were also identified to progress well in, in-vitro circumstance at pH 7-8 in the entire enzyme screening medium. The samples from the vegetable wastes were neutral but were uncovered to pollutants owing to its various suburban position of a bigger city the pH was raised up to 7.9, but pH may not efficiently show a major part alone for the microbial variety in soil (Sun-Ja Cho et al., 2016). Microorganisms can endure soil pH reaction among the pH stages 4 and 10, and the more promising pH for the mainstream is fair an alkaline cross to impartiality. Soil microbes viz.,

Bacillus, and Streptomyces etc., are some of the most prolific producers of the metabolites that acts towards the numerous co-existing phyto-pathogenic fungal and also the human pathogenic microorganisms (Pathma et al., 2011).

3.3 Separation and hydrolyte profile evaluation

Twenty-three isolates of bacterial was attained from four diverse sample sites. These species then were scrutinized on specified culture medium with response to hydrolytic enzymes viz. protease, lipase, amylase, cellulose, lecithinase and DNase production. The strains SSK1, SSK2, SSK3, SSK4, SSK5 and SSK6 were showed activity with all substrates and capacities to produce all hydrolytic enzymes (Table. 1). As per Muthulakshmi *et al.* (2011), the outcomes that were attained suggested that the hydrolytic profiling of all isolated species are straightly associated to the substrate from that it has been obtained. The probability of identifying such a species within a specific hydrolytic property enhances meanwhile the isolate is accomplished from wastage richer in the source of the enzyme of attention (Vishwanatha et al., 2010). The municipal dumping position is the potentially capable resource for broad spectra of antibacterial and the industrially relevant enzyme delivering microbes because of rare species and its valuable biological active substances for ecofriendly deprivation of wastage (Saha, 2014).

Table 1: Qualitative assay for enzyme production

S.No.	Strain	Enzymes					
		Protease	Lipase	Amylase	Cellulase	Lecithinase	DNase
1.	*Bacillus cohnii*	+++	+++	++	++	-	-
2.	*Bacillus licheniformis*	++	++	++	+++	-	+
3.	*Bacillus subtilis*	++	+	++	+	-	++
4.	*Planococcus sp.*	++	++	+	+	+	+
5.	*Pseudomonas sp.*	+++	++	++	+	-	++
6.	*Serratia sp.*	++	+++	++	+	-	+

'+' indicates visible colony growth

3.4 Characterization and Molecular Identification of the hydrolytic the strains

The selected hydrolytic strain's viz. SSK1, SSK2, SSK3, SSK4, SSK5 and SSK6 was characterized using 16S ribosomal DNA genes examination and it was recognized phylogenetically as, *Bacillus cohnii, Bacillus licheniformis, Bacillus subtilis, Planococcus* sp., *Pseudomonas* sp., *Serratia* sp. respectively (Table 2). The bacterial cultures were given to NCBI GenBank and Accession no. was attained (GenBank Accession Nos: MH992754, MH992711, MH992702, MH992755, MH992636, MH995509). Diverse species of microbial populations has been perceived at fluctuating phases of the wastage deprivations from the preceding analytical studies (Nakasaki et al., 2005). The isolation dump sites from where the samples had been obtained were representations of different composting stages with the diverse cultivation environments but still low bacterial diversities had similarly been reported by certain other authors using similar isolation and identification methods from bacterial rich environments (Ryckeboer *et al*., 2003, Xiao *et al.*, 2011). In agreement with our outcomes, similar reports available for *Bacillus licheniformis* isolated from a tannery effluent and were studied for the concomitant creation of important enzymes like the protease and the enzyme lipase (Sangeetha et al., 2010). (Bhaumik et al., 2015) stated that cellulases, xylanases and amylases produced by *Bacillus licheniformis* under solid-state fermentation for the

development of biofuels. *Bacillus cohnii* an obligate alkaliphilic bacillus strain has many traits that could be used in different enzymes production like alkaline protease, amylase (Nilgün, 2012, Ghorbel, 2009). Production of the protease and lipase was reported in *Pseudomonas* sp. with both the enzyme being secreted concomitantly (Sathyavrathan, 2013). In addition, novel amylase and lipase-producing *Pseudomonas* sp. study was reported by Lamia Khannous, 2014. In contract to the outcomes that were attained, few researchers have designated the manufacturing of extracellular enzymes produced by *Planococcus* sp. as well as protease, lipase and amylase (G.S.Nagvenkar, 2006). *Serratia* species are naturally found in the environmental sources such as the H_2O, soil, animals and also the exteriors of floras (Venil; Sangeetha kamatshi; Lakshmanaperumalsamy, 2009). Additionally, *Serratia* strains have also been identified in spoiled the foods and also vegetables and receptor source. Many investigators claim that many of *Serratia* species separates from different resources are possible of fabricating extracellular enzymes that is amylase, protease and so on, signifying which may be efficiently used as biological control and biological degrading substances (Venil; Sangeetha Kamatshi; Lakshmanaperumalsamy, 2009).

Table 2: Identified strains from vegetable waste dumping.

S.No.	Strain	Identified species	Accession number
1.	SSK1	*Bacillus licheniformis*	MH992754
2.	SSK2	*Pseudomonas* sp.	MH992711
3.	SSK3	*Bacillus subtilis*	MH992702
4.	SSK4	*Planococcus* sp.	MH992755
5.	SSK5	*Bacillus cohnii*	MH992636
6.	SSK6	*Serratia* sp.	MH995509

3.5 Antagonism test

To evaluate the antagonism between the microbial species for their future use in various aspects, the cross-streaking approach was used. There are mentioned descriptions of the antagonistic behavior inside the isolated bacteria. *Planococcus* sp. with all the other species, it has an inhibitory action, so it is not possible to create a consortium that uses this strain as those other isolated strains. *Pseudomonas* sp. is the most potential strain as it has antagonistic effect with none of these other isolates. *Bacillus cohnii* and *Serratia* sp. have antagonistic effect by a certain isolate and these species alongside *Pseudomonas* sp. could be verified in diverse mixtures for formulating consortium. Compatibility testing among the chosen species is important in describing the composition of the prospective mixed crop. The mixtures of such non-antagonistic species that might generate the optimal wastage hydrolysis might be used in future assays, in agreement with the opinion of the antagonistic activity testing. Similar results had been reported by some authors (Fagbemi and Ratu, 2016), who found that consortia of *Bacillus* spp., *Serratia* spp., and *Pseudomonas* spp. shows promising combinations for bioremediation.

The deteriorating capacity of a various microorganisms is generally high, as per certain authors (Loperena et al., 2009), then some of the discrete species that make it up, because this volume also depending on co-operation agreement within bacterial consortiums. All other different pairs would therefore be ideal for the acquisition of an efficient consortium of bacteria, enhancing the hydroxylation of residues of interest for use in the biosynthesis process and the

reuse of agro-based industrial by-products and recyclable management, both as a clean crop and as a mixed crop, in the form of a microbial consortium.

3.6 Metal tolerated study

Six heavier metals (Cr, Cd, Pb, Cu, Co and Ni) were chosen for evaluation of metal resisted capacity of the separated microbial species (SSK1, SSK2, SSK3, SSK4, SSK5 and SSK6). The tolerance study levels dictated that amongst six investigated heavier metals, highest resistance was exhibited by Cr depicting microorganism's progress up to 480 μg/ml and less tolerated by Co displaying no significant development greater than 80 μg/ml. MIC was marked when the strains were unsuccessful in growing on the petri plates even after ten days of cultivation. The outcomes displays that the MIC ranging from 90 μg/ml to 200 μg/ml for Pb and Cu, Cr (120 - 480 μg/ml), Cd (90-160 μg/ml), Co (80-250 μg/ml) and Ni (90-180 μg/ml) (Table 4). In this work, the greatest resistance of Cu and Pb originate in *Bacillus subtilis* and *Serratia* sp., while the highest Cr tolerance is observed in *Bacillus licheniformis*. In this analytical work the utmost toxic metals (having the lesser MIC) are cd whereas the least toxic metals confirmed is Pb (Table 3).

MIC was again observed when these segregates declined its development on the surfaces even after 10 days of cultivation [18]. Mergeay et al. [19] measured minimal inhibitory concentrations (MICs) of many various metals and noticed the maximum deadly metal (having the lowermost MIC) was mercury while the smallest harmful metal was Mn [19]. The bacterial resistance at every absorption of heavier metal was represented through streak plate examination. The wastewater coming from the household sewage provides the required atmosphere where the microbes can build tolerance to the harmful chemicals. The existence of the minimal number of extra metals inside the solid waste will cause the development of resistance to metal ions microbes. The resistance of microbes to the heavy metal then becomes due to the different of detoxifying processes which are formed by the microbial resistance including the complexation through exo-polysaccharides, bonding with the microbial cellular envelopes, metal decrease and metal effluence etc. These processes are often fixed inside the plasmid genes that promote the transition of deadly metal tolerance from one nucleus to other [20]. Such heavier metal tolerant bacterium may be a possible source for the phytoremediation of heavier metal's contamination. Because the heavy metals displayed similarities in their toxic mechanisms, different metallic tolerances are very widely observable occurrences amongst these noted metal ions resistant strains [21].

Table 3: Metal tolerance of isolated bacterial strains

S.No.	Strain	MIC for various metals in μg/ml					
		Cr	Cd	Pb	Cu	Co	Ni
1.	*Bacilluslicheniformis*	480	100	150	90	130	100
2.	*Pseudomonas* sp.	190	130	160	130	160	90
3.	*Bacillussubtilis*	130	160	200	110	120	130
4.	*Planococcus*sp.	190	110	180	110	190	110
5.	*Bacillus cohnii*	170	90	90	130	80	180
6.	*Serratia*sp.	120	90	130	200	250	110

3.7 Aromatic pollutants Assay

The visible colony growth study was qualitatively examined by significant intractable chlorinated aromatic pollutants like the Naphthalene, Carbazole, Dibenzothiphene, Benzo(α)pyrene, Fluorene, Pentachlorophenol on the isolated bacterial strains (SSK1, SSK2, SSK3, SSK4, SSK5 and SSK6) (Table 4). This test results shows that among six isolates, *Serratia* sp. grown on all the aromatic pollutants and maximum visible colonies observed on Naphthalene, Carbazole, Benzo(α)pyrene, Pentachlorophenol. *Pseudomonas* sp. and *Bacillus cohnii* both showed similar results by showing their visible colonies on aromatic pollutants except Benzo(α)pyrene and Pentachlorophenol. *Bacillus licheniformis* showed a moderate growth on most of these aromatic pollutants except naphthalene and *Planococcus* sp. showed the least visible colonies. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in nature which is generated from both anthropogenic processes and natural sources. The bioaccumulation of PAHs has paid a serious attention to health of humans and aquatic life. The microbial removal has been noted to be the most significant process for the eradication of the PAHs (Fagbote and Olaufekum, 2010). Arulazhagan et al. (2010) used a consortium of halotolerant bacterium contains *Ochrobactrum* sp., Stenotrophomonas maltophilia and Enterobacter cloacae to degrade the PAHs such as fluorene, naphthalene, phenanthrene and anthracene. At 30 g/l concentration of pollutants, >90% were degraded by the consortium of bacteria within 4 days. Fortnagel and co-workers (1990) used the aromatic compounds such as DF, catechol, biphenyl, benzoic acid, 2, 2', 3-trihydroxybiphenyl, salicylic acid3-hydroxybenzoic, gentisic phenylglyoxylic, phenylmalonic, and 2-hydroxyphenylglyoxylic acid, etc., against *Pseudomonas* sp. HH69. Growth of the *Staphylococcus auriculans* DBF63 was analyzed with different aromatic pollutants such as DF, phenoxathiin, phenanthrene, FN and anthracene. The strain was able to metabolize the pollutants such as FN, DF and anthracene, but failed to metabolize phenanthrene and phenoxathiin. The cooxidation ability of other polycyclic aromatic compounds was also analyzed. The succinate or DF was co-oxidized with the carbazole, naphthalene and dibenzothiopene. No growth was observed with phenazine, DD and sulfone (Monna et al., 1993).

Table 4: Qualitative growth of isolated bacterial strains on Aromatic Pollutants

S.No.	Strain	Polycyclic aromatic hydrocarbons (PAHs)					
		Naphthalene	Carbazole	Dibenzothiphene	Benzo(α)pyrene	Fluorene	Pentachlorophenol
1.	*Bacilluslicheniformis*	+	+++	++	++	+++	++
2.	*Pseudomonas* sp.	+++	++	++	+++	+++	+
3.	*Bacillussubtilis*	++	+	++	+	+	+++
4.	*Planococcus*sp.	_	+	+	_	_	+
5.	*Bacillus cohnii*	+++	++	++	+	+++	+++
6.	*Serratia*sp.	+++	+++	++	+++	+	+++

'+' indicates visible colony growth

3.8 Antibiotic susceptibility test

Antibiotic susceptibility analysis aids in determining how efficiently efficient will an antibiotic is towards the testing species. The 6 samples were then analyzed for their sensitive to the eight antibiotics and then results were examined (Table 5). The assay revealed that isolates *Bacillus licheniformis*, *Pseudomonas* sp. *Bacillus subtilis*, *Bacillus cohnii* and *Serratia* sp. Showed high

resistivity to penicillin, ampicillin and tetracycline. Isolating microbe exhibited higher sensitive to nalidixic acid and then it was also disposed to streptomycin, kanamycin and gentamicin. All the isolates showed susceptibility to chloramphenicol. The antibiotic susceptibility analysis of the obtained isolates *Bacillus licheniformis*, *Pseudomonas* sp. *Bacillus subtilis* showed similarity to other isolates *Bacillus cohnii*, *Serratia* sp. [10], but it was more resilient to antibiotics compared to *Planococcus* sp. [11], though on comparing to other strains such as *Serratia* sp. [12].

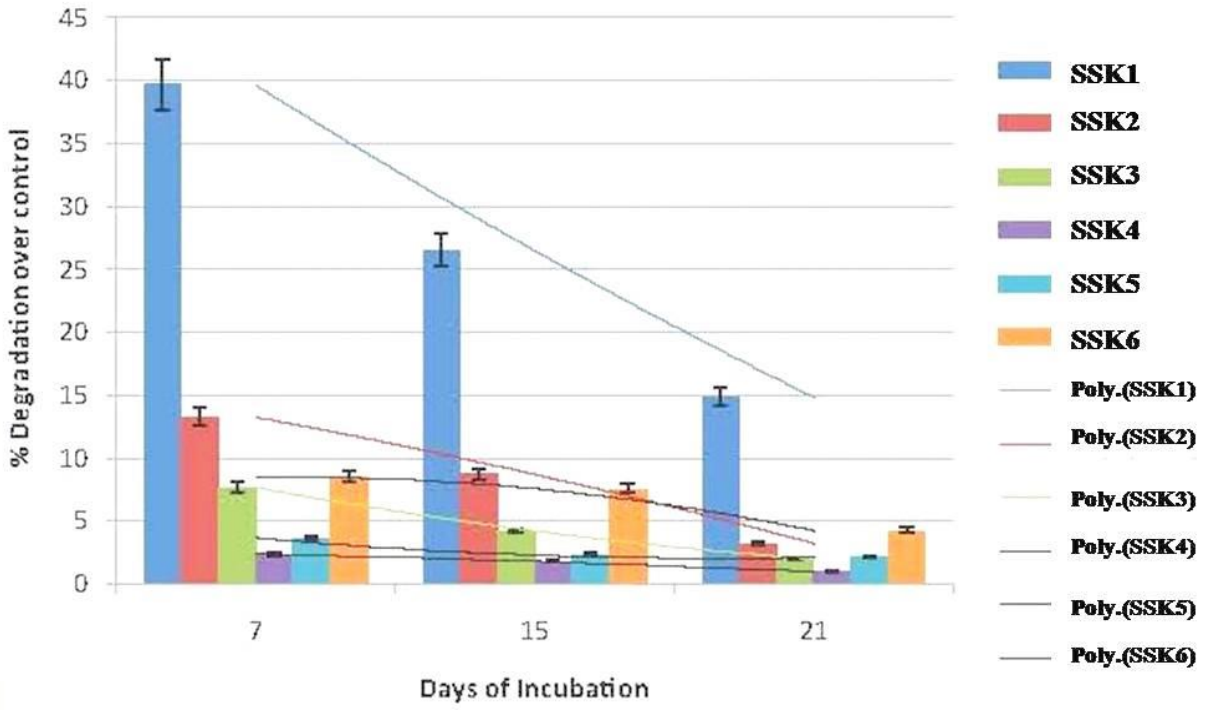
Table 5: Antibiotic susceptibility of isolated bacterial strains

S.No.	Antibiotics	Strain					
		Bacillus licheniformis	*Pseudomonas* sp.	*Bacillus subtilis*	*Planococcuss* p.	*Bacillus cohnii*	*Serratias* p.
1.	Penicillin (10 µg)	R	R	R	R	R	R
2.	Ampicilin (10 µg)	R	R	R	R	R	R
3.	Tetracycline (30 µg)	R	R	R	R	R	R
4.	Gentamicin (10 µg)	S	S	S	S	S	S
5.	Chloramphenicol(10 µg,	S	S	S	S	S	S
6.	Kanamycin (30 µg)	S	S	S	S	S	S
7.	Nalidixic acid (30 µg)	S	S	S	NA	NA	R
8.	Streptomycin (10 µg)	S	S	S	S	S	R

R = resistance; S = susceptible; NA = not available.

3.9 Waste deprivation potential through separated microbe

Figure 1. Waste degradation potential by isolated bacteria



4. Conclusions

Agro dumping wastage is a complex mixture of various substrates and it was a supreme enriched medium for the growth of various microbes. Microorganisms are metabolically

proactive in nature, which, relative to other environmental conditions, contributes to the processing of new enzymes and biological activities compounds. This also is essential for the environmental understanding of waste-derived microbes but also as a source for biotechnology. Our current research has clearly shown that the deposition site of vegetable waste materials is a capable origin of a broad variety of disease developing antibacterial and industrially important enzymes. In addition, novel/rare organisms that could generate useful biological compounds essential for environment-friendly decomposition of wastage could also be an imperious source for biological prospecting and can serve as a possible alternative in application of chemical products.

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