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Screening of bacterial compost from spoiled vegetables and fruits and their physiochemical characterization

Mohamed Mahroop Raja, M., Raja, A., Mohamed Hajee, S. and Sheik Mohamed, A.

Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamilnadu, India

Abstract: Studies were carried out to Screen the bacterial compost from spoiled vegetables and fruits and their physiochemical characterization. Samples were collected from local market and super markets in and around Madurai, Tamil nadu, India. Collected samples were processed by using Total Microbial Count, Standard Plate Count and Coli form count. *Bacillus* spp. and *Pseudomonas* spp. were dominantly found in both local and super market samples were identified by using Morphological character and Biochemical characters. The dominant isolates such as *Bacillus* sp and *Pseudomonas* sp were processed and involved in composting process to determine moisture content, pH, energy sources such as carbon, nitrogen, phosphorous and potassium content. Compost production can be justified for high value crops such as vegetables and fruits.Thier may be potential relationship with biologically very active compound used to suppress the plant diseases.

Keywords: Compost, moisture, crop and plant disease

Introduction

Consumption of fruit and vegetable products has dramatically increased in the United States by more than 30% during the past few decades. It is also estimated that about 20% of all fruits and vegetables produced is lost each year due to spoilage. According to a USDA-Economic Research Service study in 1995, 18.9 billion pounds of fresh fruits and vegetables were lost annually due to spoilage, which was 19.6% of all US losses of edible foods that year (Kantor *et al*., 1997). Most microorganisms that are initially observed on whole fruit or vegetable surfaces are soil inhabitants, members of a very large and diverse community of microbes that collectively are responsible for maintaining a dynamic ecological balance within most agricultural systems. Vectors for disseminating these microbes include soil particles, airborne spores, and irrigation water.

Most bacteria and fungi that arrive on the developing crop plant either are completely benign to the crop's health or, in many instances, provide a natural biological barrier to infestation by the subset of microorganisms responsible for crop damage (Andrews and Harris, 2000; Janisiewicz and Korsten, 2002). The even smaller subset of bacteria responsible for causing spoilage to the edible portion of the crop plant is the subject of this section. Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field during harvesting and post harvest handling, or during storage and distribution (Day, 2000). Those same types of soil-borne spoilage microbes that occur on produce are the same spoilage microorganisms that are present on harvesting equipment, on handling equipment in the packinghouse, in the storage facility, and on food contact surfaces throughout the distribution chain.

Many fruits and vegetables present nearly ideal conditions for the survival and growth of many types of microorganisms. The internal tissues are nutrient rich and many, especially vegetables, have a pH near neutrality because the principal storage polymer is starch. Spoilage microorganisms exploit the host using extra cellular lytic enzymes that degrade these polymers to release water and the plant's other intracellular constituents for use as nutrients for their growth (Miedes and Lorences, 2004). Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas, 2005b). At the same time, tough new environmental laws mandated that industries could no longer simply dump their waste products onto the surrounding land or discharge them into nearby rivers. To meet these

*Corresponding author.
Email: *mahroop_raja07@yahoo.co.in*

laws, many industries began their own recycling and composting programs. Today, most compost is processed in large facilities designed to handle a specific type of raw material. Agricultural compost is usually produced and used on the same farm that generated the raw materials. Agricultural compost facilities use materials readily available on nearby farms (Yun *et al*.,2000). These include animal manure, used stable straw, spoiled fruits and vegetables, field refuse, vineyard and orchard prunings, rotted hay, and other agricultural waste products.

Materials and Methods

Collection of sample

During the study period, Fifteen samples of spoiled vegetable and fruit samples such as Tomato, Spinach, Coriander, Potato, Cabbage, Beet, Capsicum, Cauli-flower, Brinjal, Peas, Lemon, Sweet Lime, Banana, Chickoo and Apple were collected from local market and super markets around Madurai, Tamil Nadu, India.

Enumeration of microbes

The samples were rinsed thoroughly with distilled water and serially diluted up to 10-7. Dilution was made depending on cell density. The highest three dilutions were taken for analyzing the total microbial count by using Nutrient agar medium at 37^0C for 24 hours. Standard Plate Count (SPC) was carried out by Spread Plate Technique & Coli form Count (CC) was carried out by Pour Plate Technique.

Isolation and preservation of bacteria

According to Bergey`s Manual of Determinative Bacteriology, the microorganisms were Isolated. In long term preservation, Glycerol stocks were prepared and stored at -800^0C where as pure cultures strains were incubated at 500C for 48 hours. 0.5 ml of each pure culture was transferred into cry tubes accompanied by 40% glycerol. The samples were mixed gently and stored at -80°C.

Nutrient agar plate

Morphological and cultural characteristics such as abundance of growth, pigmentation, optical characteristics, form, size, margin and elevation were studied on Nutrient agar plates.

Gram staining

A loop full of overnight culture was placed on the slide. Smear was prepared by spreading the drop with a toothpick. The heat fixed smear was first stained with crystal violet for 60 sec. After rinse the slide, it was flooded with Grams iodine solution and was kept for 60 sec. Slide was again washed under the tap water and added 95% alcohol for 30sec. After wash the slide, it was stained with safranin for 60sec. It was again rinsed under tap water and dried on paper towels. The cells were examined under the light microscope.

Motility determination

A small amount of Vaseline was placed at each corner of clean cover glass. Two loopful of the 24 hours culture of the organism was placed at the center of the cover glass. A depression slide was pressed over the cover glass, such that the depressions cover the culture drop and quickly inverted. The completed preparation was observed microscopically.

Examination of endospores

Isolated microorganism grown on Luria Bertani Broth medium for 3-4 days were suspended in 3-5 µl of sterile 0.09% NaCl on a Microscopic slide and covered with a cover slip. Endospores were observed as shiny bodies in the cells under the phase contrast microscope.

Indole production test

Using sterile technique, inoculate isolated organisms into appropriate tubes and one tube serves as control. Incubated tubes for 24 hrs at 37°C. After incubation followed by addition of kovac's reagent were determined the indole positive or negative isolates.

Methyl red test

The isolated organisms were inoculated into test tubes containing MR-VP broth . Incubated tubes for 24 to 48 hours at 37°C. After incubation, the methyl red indicator in which is indicates the positive or negative isolates at PH 4 .

Voges-proskauer test

The isolated organisms were inoculated into MR-VP broth and incubated at 37°C for 24 hrs. After incubation, Barrit's reagent A& followed by B were added to determine positive or negative isolates.

Gelatin hydrolysis

Gelatin is protein may act as a nutrient source for many microorganisms. When gelatin is enzymatically hydrolyzed into short peptide and aminoacid. It loses its ability to become gel even at low temperature. The isolates were inoculated into gelatin deep tubes by stab inoculation. It was incubated at 37°C for about 48 hours. After incubation, the tubes were placed in refrigerator at 4ºC for 30 minutes. Cultures that remain liquefied by gelatinase which showed positive

result and that remained solid which showed negative result.

Carbohydrate utilization analysis

The carbohydrate tests are based on the principle of pH change and substrate utilization. During the incubation of organisms undergo metabolic changes which were indicated by a spontaneous color change in the media. The organisms were analyzed for the utilization of 12 carbon sources like Lactose, Xylose, Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Mellibiose, Sucrose, L-Arabinose, Mannose, Inulin, Sodium Gluconate, Glycerol, Salicin, Glucosamine, Dalicitol, Inositol, Sorbitol, Manitol, Adonitol, α Methyl D-Glucoside, Ribose, Rhamnose, Cellobiose, Melezitose, α Methyl D-mannoside, Xylitol, ONPG, Esculin, D-Arabinose, Citrate, Malanate and Sorbose 50µl of the broth containing the sample was inoculated into the well as provided in the kit and incubation was carried out at 37ºC for 24 hrs.

Catalase test

Isolates were grown in Nutrient Agar Medium for 24-48 hours at 37ºC.After incubation, 3% hydrogen peroxide was poured onto the colonies. Formation of air bubbles indicate the presense of catalase enzyme

Oxidase test

Isolated microorganisms were grown in nutrient agar medium for 24-48 hours at 37oC. A filter paper was placed into a Petri dish and was wetted with 1% solution of tetramethyl-p-phenylenediamine. One large colony was taken with a loop and tapped lightly onto the wet fitler paper. Formation of a blue-purple colour was taken as the evidence for oxidase activity.

Nitrate reduction test

Nitrate broth was prepared and sterilized and inoculated with the isolates and incubated at 37^{0}C for 24 hours. After incubation presence of nitrate was tested by adding few drops of sulphanilic acid and alpha Napthalamine reagent to each of the tubes. A distinct red colour turned brown which indicates the reduction of nitrate.

Starch hydrolysis test

About 15-20 ml of sterile starch agar medium was transferred aseptically into the sterile Petri dish. The isolated colonies were streaked on sterile starch agar plates and incubated at 37°C for 48 hours. After incubation, gram's iodine was added in to the culture plates to determine the starch hydrolyses activity or not.

Casein hydrolysis test

20 m1 of the sterile skim milk agar medium were transferred aseptically into sterile Petri plate and the medium was allowed to set. Culture were inoculated and incubated for 24-48 hours at room temperature. The opaque zone surrounding the microbial growth in casein milk powder indicates the protease activity.

Carbohydrate fermentation

A Durham's tube was placed in l0ml of the culture medium and autoclaved for 15 minutes at 121^{0}C. A loop full of 18-24 hours culture were inoculated into the medium and incubated at 37^{0}C for 24-48 hours. The presence gas production which indicates the carbohydrate fermentation.

Microbial composting

The experiment was carried out in heaps of the size 2x2x2 feet. Each Heap was filled with 10kg of substrate. This substrate was retained until fine tilt is reached.

Culture application

Two organisms of bacteria were used for the composting of vegetable and fruit waste in this present studies were designated as follows.

Heap + *Bacillus sp* + vegetable and fruit waste → *Bacillus* sps compost

Heap + *Pseudomonas sp* + vegetable and fruit waste → *Pseudomonas sp*

} Bacterial compost

Heap+ vegetable and fruit waste for control (Conventional compost).

The pure culture broth was sprayed on each compost pit and turned on whenever necessary to provide adequate aeration. The parameters such as moisture content, pH and Carbon - Nitrogen ratio, potassium and phosphorous were analyzed during different periods of composting

Compost analysis

Samples were withdrawn from the 20cm depth of pits for every 10 days interval up to 50 days. Those samples were analyzed for microbial load and nutrient contents. The microbial load was estimated by using serial dilution method.

Estimation of organic carbon

The organic carbon content was estimated by wet digestion method (Walkley and Black, 1974). 1 gm of oven dry sample was taken in 500ml clean and dry conical flask. 30m1 of potassium dichromate and

20m1 of concentrated sulphuric acid were added to the conical flask and it was kept for 30 minutes. It was diluted with 200m1 of distilled water. 10ml of ortho phosphoric acid and 2m1 of di phenyl amine (indicator) were added to the flask. The above content was titrated against standard ammonium ferrous sulphate in the burette. The end point was noted when the blue colour turned to green.

Estimation of the total nitrogen

The nitrogen content was determined by the micro-kjeldhal method (Umbriet *et a1*., 1974). The catalyst was prepared by powdering and mixing 1gm of copper sulphate, 8 gm of potassium sulphate and 1 gm of selenium dioxide.10 mg of powdered sample was taken in micro-kjeldhal flask. A pinch of catalyst and 0.5 ml of concentrated sulphuric acid was introduced into the micro-kjeldhal flask. The flask was gently heated in a digestion rack until fumes of sulphuric acid entered. It was then strongly heated until the digest in the flask turned into an apple green colour. After cooling, the digest was made up to 20 m1of distilled water. 2 ml of diluted digest of water, 2ml of colour reagent and 3 ml of 2N NaOH were added. After 15 minutes, the absorbance of the solution was noted at 490 nm against the reagent blank in a spectrophotometer. The quantity of nitrogen in the sample was determined with reference to a standard graph prepared using NH_4Cl and expressed in percentage.

Estimation of phosphorous

The phosphorous estimation was followed by modified Vogel's method. 100 mg sample was dissolved in 100 ml of distilled water. From this 5 ml was taken in a 50 ml volumetric flask and 2 ml of ammonium molybdate mixture was added and appeared Light blue colour. The optical density of the developed light blue colour was read at 640nm in a calorimeter. The amount of phosphorus in the sample was calculated and expressed in percentage.

Estimation of potassium

The potassium content in the samples was estimated by 50 ml of 1 N ammonium acetate solution in 100 ml conical flask already contained 5 gm of soil sample. Placed the stopper tightly and shake vigorously for 30 min. After 30 min, the filtered solution was analyzed by using flame photometer.

Estimation of soil moisture content

Soil moisture content of the sample was determined from the weights of shade dried and oven dried sample. A known quantity of shade dried sample was taken and kept inside the hot air oven at 800c for 48hrs. Weight of the oven dried sample was also taken. By using this formula, moisture content of the sample was calculated as follows.

$$\text{Moisture content of sample (\%)} = \frac{\text{Weight of shade dried sample - weight of oven dried sample}}{\text{Weight of shade - dried sample}}$$

Table 1. Morphological, physiological and biochemical characteristics of the bacterial isolates

S.No.	Characteristics	*Bacillus* spp.	*Pseudomonas* spp.
1	Morphology	Rod	Rod
2	Gram staining	+	–
3	Motile	+	–
4	Spore	+	–
5	Indole Test	-	–
6	Methyl red test	-	–
7	Voges praskauer test	-	–
8	Nitrate reduction test	+	+
9	Citrate	-	+
10	Cellulose	+	+
11	Starch	+	–
12	Casein	+	–
13	Gelatin	+	–
14	Catalase	+	+
15	Oxidase	+	+

Measurement of pH

pH of the sample was estimated by using the pH indicator papers. 20gm of sample was taken and 40m1 of distilled water was added and mixed well. After 10 min, few ml of saturated Barium sulphate was added to settle down the impurities. New pH indicator paper was dipped and then compared with universal indicator paper. The nearest pH range was marked as the pH of the sample.

Results and Discussion

Studies were carried out to Screen the bacterial compost from spoiled vegetables and fruits and their physiochemical characterization. Samples were collected from local market and Super Market in and around Madurai, Tamilnadu, India. Collected samples were analyzed by using Total Microbial Count,

Table 2. Physio chemical Parameters of Bacterial Compost of *Bacillus* sp and Conventional Compost (Control)

Parameter	*Bacillus* spp. Days							Control Days						
	0	10	20	30	40	50	60	0	10	20	30	40	50	60
Moisture content %	50	54	57	61	63	59	56	50	50	52	55	57	57	54
pH	5.9	5.7	6.3	6.6	6.8	7	7.2	5.8	5.8	5.9	6.0	6.1	63	6.5
Carbon %	19.76	19.53	19.16	18.92	18.70	18.63	18.55	19.76	19.71	19.67	19.49	19.25	19.18	19.00
Nitrogen %	0.86	0.89	0.96	1.07	1.15	1.20	1.23	0.86	0.86	0.93	1.00	1.09	1.14	1.17
C:N %	22.97	21.94	19.95	17.68	16.2	15.52	15.08	22.97	22.91	21.15	19.49	17.6	16.8	16.25
Phosphorous %	0.21	0.25	0.29	0.33	0.38	0.41	0.43	0.21	0.22	0.26	0.30	0.34	0.36	0.38
Potassium %	0.24	0.29	0.34	0.38	0.42	0.44	0.46	0.24	0.26	0.29	0.33	0.37	0.39	0.41

Table 3. Physio chemical parameters of bacterial compost of *pseudomonas* spp. and conventional compost (control)

Parameter	*Pseudomonas* Days							Control Days						
	0	10	20	30	40	50	60	0	10	20	30	40	50	60
Moisture content %	50	53	55	58	61	55	51	50	50	52	55	57	57	54
pH	5.9	5.8	6.2	6.5	6.7	6.7	6.9	5.8	5.8	5.9	6.0	6.1	63	6.5
Carbon %	19.76	19.61	19.21	19.04	18.93	18.84	18.71	19.76	19.71	19.67	19.49	19.25	19.18	19.00
Nitrogen %	0.86	0.88	0.94	1.03	1.11	1.17	1.20	0.86	0.86	0.93	1.00	1.09	1.14	1.17
C:N %	22.97	22.28	20.43	18.43	17.00	16.10	15.59	22.97	22.91	21.15	19.49	17.6	16.8	16.25
Phosphorous %	0.21	0.23	0.27	0.31	0.36	0.38	0.40	0.21	0.22	0.26	0.30	0.34	0.36	0.38
Potassium %	0.24	0.27	0.31	0.36	0.40	0.41	0.43	0.24	0.26	0.29	0.33	0.37	0.39	0.41

Standard Plate Count by spread plate technique and Coli form Count by pour plate technique. The range of local market sample contained in the range of 5-6 log Cfu/ml. The high range of log Cfu/ml of Total viable count were present in local market sample because of unhygienic condition occurred during the exposure of transport facility and improper storage condition in the local market. Total viable count as 8.7, 8.6, 7.5, 7.4 and 6.3 log 10 Cfu /ml for various sample collected from various retail market (Kawo *et al*., 2005). Low contaminants were found in the super market sample which was treated with chlorinated water before its transportation to retailer.

Collected samples were processed by using Morphological and Biochemical character to identify the dominant microorganisms found in the sample. According to Bergey's Manual Determinative Bacteriology, the bacteria such as *Bacillus* spp. and *Pseudomonas* spp. were found in the samples (Table 1). 60% of the local market sample contained *Bacillus* spp. and *Psedumonas* spp. On the other hand only 20% of the super market sample showed *Bacillus* spp. and *Pseudomonas aeruginosa* were isolated from vegetables and fruits. These result showed that microbiological qualities of the vegetable and fruit were better in super market sample when compared with local market (Troiler, 1993).

The effect of bacterial compost on moisture content was determined by dominant isolated such as *Bacillus* spp. and *Pseudomonas* spp. on 14th day of compost.63% of moisture content was determined by *Bacillus* sp and 60% of moisture content was determined by *Pseudomonas* spp. (Table 2 and 3). Bacterial organisms can found in the soil due to eradication and fertilization with manure and sludge in the farming area (Barrington *et al*., 1997).

The pH content of the bacterial compost was gradually increased from initial to final stage (Table 2 and 3).The pH content of the Bacillus spp. and Pseudomonas spp. in compost were obtained on 40th day and determined as 7.2 and 6.9 pH respectively. Listeria monocytogenes were found in 6.7 pH of the fertilizing land for vegetable farming (Hassen *et al*., 2001).

The Microorganisms of the compost were needed energy source like Carbon, Nitrogen, Potassium and Minor elements were essential to make enzyme and their activity. The nutrient content of the Carbon, Nitrogen, Phosphorus and Potassium in *Bacillus* spp. compost was analyzed once in 10 days and found to be 19%, 1.15%, 0.43% and 0.46% respectively (Table 2 and 3) where as in Pseudomonas spp. compost was found in 18.93%, 1.11%, 00.4% and 0.43% respectively. In the same way the nutrient content of the control was analyzed once in 10days and found as 19.25%, 1.09 %, 0.38 % and 0.41% respectively. The Bacteria, Actinomycetes and Fungi of the compost heap like any other living things need both carbon from carbohydrate and nitrogen from protein in the form of compost substrate subjected to thrive and reproduce. All microbes must have access to a supply of elements which cells were made (Barrington *et al.*, 2002).

Composting is a very good organic way of enriching the soil. Compost can be made easily in pots from your backyard with garden and kitchen refuse. Materials like leaves, lawn cutting, pine needles, weeds, carrot tops, spoiled fruit and vegetable, animal manure and the like, can be used to make good compost. The decomposition of the organic material forms bacteria and fungi in the soil. This helps in converting unavailable nutrients like nitrogen to ammonia and nitrates making it usable for the plants. The fertility of the soil depends upon three components: nitrogen, phosphorus and potassium. Nitrogen increases the growth of lush foliage. Phosphorus helps with strong roots and sterns. Potassium protects the plants from disease and cold. These nutrients are needed for every plant that stays alkaline for more than a year will help in raising a healthy and abundant crop.

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