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Research Article

Screening and Characterization of Protease Producing Bacillus Spp from Spoiled Vegetables and Fruits

E. Venkata Nagaraju* and Goli. Divakar

Department of Biotechnology & Microbiology, Acharya & B.M. Reddy College of Pharmacy, Soldevanahalli, Hesaraghatta, Banglore, Karnataka, India.

ABSTRACT

Ever increasing population and industrialization has resulted in sudden increase in pollution. Because of the detrimental effects of pollution on humans, animals and plants, the ever increasing pollution is causing concern all over the world. The microbial biodiversity is important on many grounds ranging from aesthetic considerations to its usefulness, particularly for biotechnology. The fastest growing segments are enzymes for feed and fuel production. Abundant amount of waste materials are produced by vegetables and fruit processing industries, which pose considerable disposal problems and ultimately leads to pollution. Vast varieties of micro-organisms are present in the environment which can be exploited for the utilization of waste material. Our Studies were carried out to Screen protease producing Bacillus spp from spoiled vegetables and fruits from different places around Bangalore and Mysore. Collected samples were processed by using Total Microbial Count, Standard Plate Count and Coli form count. Bacillus spp were dominantly found in both local and super market samples and identified by using Morphological character and Biochemical characters.

Keywords: Protease, Bacillus spp, Spoiled vegetables, Biochemical tests, Bangalore.

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 which have the capability of producing proteases.

Proteolytic enzymes play an important part in the metabolism of almost all organisms (plants, animals, fungi, bacteria, and viruses). Investigation of proteases is a central issue in enzymology due to both their immense physiological importance and wide application in research and economical activities. Proteases are one of the most important groups of industrial enzymes and are used in a variety of industrial applications as laundry detergents, pharmaceuticals, leather products, as meat tenderizers, protein hydrolyzates, food products, and even in the waste processing industry [1].This enzyme accounts for 30% of the total worldwide production of enzymes .

The genus Bacillus contains a number of industrially important species and approximately half of the present commercial production of bulk enzymes derives from the strains of Bacillus sp. [2]. These strains are specific producers of extracellular proteases and can be cultivated under extreme temperature and pH conditions to give rise to products that are, in turn, stable in a wide range of harsh environments [3].

Bacillus sp. Mostly produces proteases,Bacterial proteases are active in narrow pH range (Ph 5.0-8.0) and have relatively low thermo tolerance [4].This property is advantageous for controlling their activity during the production of food hydrolysis. Bacterial neutral proteases generate less bitterness in hydrolyzed food proteins than animal proteases and, hence, are valuable for use in food industry .The aim of the present study was to isolate the Bacillus sp from spoiled vegetables and fruits from

different places around Bangalore and Mysore, screen the protease producing bacillus sp.

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 and Apple were collected from local market and super markets around Bangalore and Mysore.

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 -80^{0C} where as pure cultures strains were incubated at 50^{0C} 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.

Catalase test

Isolates were grown in Nutrient Agar Medium for 24-48 hours at 37^{oC}.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 37^{oC}. 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^{0C} 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.

RESULTS AND DISCUSSION

Studies were carried out to Screen protease producing Bacillus spp from spoiled vegetables and fruits. Samples were collected from local market and Super Market in and around bangalore, Mysore. Collected samples were analyzed by using Total Microbial Count, Standard Plate Count by spread plate technique, Coli form Count by pour plate technique and protease screening by casein. 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 produced higher proteases 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[5] .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[23], the bacteria such as Bacillus spp. were found in the samples and shown in (Table-1). 60% of the local market sample contained Bacillus spp.. On the other hand only 20% of the super market sample showed Bacillus spp. were isolated from vegetables and fruits collected from various places. These result showed that microbiological qualities of the vegetable and fruit were better in super market sample when compared with local market and also shows high protease activity.

TABLE 1:

S.No	CHARACTERISTICS	*BACILLUS SPP.*
1	Morphology	Rod shaped
2	Grams Staining	+Ve
3	Motile	+Ve
4	Spore	+Ve
5	Indole Test	-Ve
6	Methyl red Test	-Ve
7	Vogespraskauer test	-Ve
8	Nitrate reduction test	+Ve
9	Starch Hydrolysis test	+Ve
10	Casein Hydrolysis test	+Ve
11	Gelatin Hydrolysis test	+Ve
12	Catalase test	+Ve
13	Oxidase test	+Ve

Fig. 1: casein hydrolysis test

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