JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2011. Vol. **5**(2), p. 849-952

Isolation and Identification of Bacteria from Rotten Papaya Fruits

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(Received: 18 January 2011; accepted: 18 February 2011)

Sixteen bacterial strains were isolated from seven varieties of rotten papaya fruits. Out of the sixteen isolates obtained through enrichment techniques ten bacterial isolates were present in more percentage and dominated. The present study was undertaken to investigate the microbiological quality of rotten papaya fruits. Seven different varieties samples of these rotten papaya were collected. The samples were analyzed within an hour of procurement. Isolation, enumeration and identification of the prevalent bacteria were carried out following the standard procedures. Analysis of the rotten papaya samples revealed high loads of bacterial food borne pathogens such as *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis, Micrococcus* sp., *Proteus vulgaris, Pseudomonas* Sp., *Flavobacterium* sp., *Lactobacillus* sp., *Serratia marcescens, Aeromonas* sp. Total viable counts of bacteria in all the samples varied between 0.4-3.0x10^4 cfu g^{-1}.

Key words: Rotten papaya fruits, Isolation, Identification, Bacterial strains.

Bacteria are the major group of microorganisms. They are defined as prokaryotes with out chlorophyll 'a'. They are microscopic and exist as single cell. One of the first steps in the identification of bacteria in a food is microscopic examination to as certain the shape, size, aggregation, structure and staining reaction of the bacteria present. Bacterial growth in and on foods often is extensive enough to make the food unattractive in appearance. Pigmented bacteria cause discolorations on the surface of foods; films may cover the surfaces of liquids; growth may make surface slimy; or growth thought the liquids may results in undesirable cloudiness or sediment. The bacteriologist is concerned with the growth and activity of bacteria in foods and with accompanying chemical chances. Knowledge of the factors that favor or inhibit the growth and activity of bacteria is essential to an understanding of the principles of food preservation and spoilage (Frazier and Westhoff, 2003). The present study was carried out to isolate and identify the bacterial organism from rotten papaya fruits.

Collection of sample

Seven varieties of rotten papaya fruits collected from Tamil Nadu Agricultural University, Coimbatore.

Isolation of microorganism

Seven varieties of rotten papaya fruits sample were taken and each variety of 1g was taken and diluted serially upto10^{-6} about 0.1ml of serially diluted sample was taken and done the spread plate technique by using nutrient agar. The inoculated plates were incubated 24hrs for bacteria.

Subculture technique

The isolates of bacterial species was subcultured on nutrient agar plates to check its purity and incubated at 37°C for 24hrs. After completion of incubation periods observed the plates and noted the colony morphology. Single colony was streaked on the Nutrient agar slant.

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Morphological and Physiological characteristic of test organism

The various morphological and biochemical characteristics of pure isolate were examined according to the Dubay & Maheshwari, 2002.

RESULTS AND DISCUSSION

In the present study, ten genera of bacteria were isolated from seven types of decayed sample. Isolates include *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis, Micrococcus* sp., *Proteus vulgaris, Pseudomonas* Sp., *Flavobacterium* sp., *Lactobacillus* sp., *Serratia marcescens, Aeromonas* sp. The results of morphological character and biochemical tests of bacterial isolates were represented in Table 1, 2, 3.

Identification of isolated Bacterial organism

Based on the micrometric technique, Gram staining, and various Biochemical tests, there were ten genera of useful bacteria were isolated from the seven varieties of papaya fruits. The test results were compared with Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The organisms were characterized.

Staphylococcus aureus Gram positive, aerobic and facultative anaerobic, non spore forming, non motile, cocci in chain or pair and capsule producing organism. The size of the organism is 1-2µm diameter. The organism shows positive result to methyl red and catalase but negative to others. They produce acid by fermenting sugar like lactose, sucrose, glucose.

Streptococcus faecalis Gram positive, aerobic and facultative anaerobic, non spore forming, non motile, non capsule producing and cocci shaped organism. Size of the organism is 1µm in diameter. The organism shows positive result to methyl red but negative to others. They produce acid by fermenting sugar like lactose, sucrose, glucose. Thatcher and Clark, 1968 studied the *enterococci* most frequently isolated from foods are *S.faecalis* and *S. faecium*. The *streptococci* is often used in the food industry to describe those enterococci which are used as indicator organisms.

Bacillus subtilis Gram negative, aerobic and facultative anaerobic, spore forming, motile, capsule producing and rod shaped organism. Size of the organism is 2 - 3µm in diameter. The organism shows positive result to oxidase, starch hytrolysis but negative to others. They produce acid

Table 1. Morphological characterization of microbes from rotten papaya sample

S. No	Organisms	Colony formation	Colony Opacity	Colony colour	Cell size	Shape
1	*Staphylococcus aureus*	Circular	Opaque	Yellow	1-2µm in diameter	Cocci
2	*Streptococcus faecalis*	Circular	Opaque	White	1 µm in diameter	Cocci
3	*Bacillus subtilis*	Circular	Transparent	White	2-3µm in diameter	Rod
4	*Proteus vulgaris*	Spindle	Transparent	Pink	1-3µm in diameter	Rod
5	*Lactobacillus*	Circular	Opaque	White	0.5-1.2µm in diameter	Rod
6	*Serratia marcescens*	Circular	Opaque	Pink/Red	1-3µm in diameter	Rod
7	*Micrococcus*	Small Circular	Translucent	Yellow	0.5-1µm in diameter	Cocci
8	*Pseudomonas* Sp.	Irregular	Opaque	Metallic green	1.5-3µm in diameter	Rod
9	*Flavobacterium*	Circular	Translucent	Yellow	1-3µm in diameter	Rod
10	*Aeromonas*	Circular	Transleucent	Gray white	1-2µm in diameter	Rod

by fermenting sugar like sucrose, glucose and negative result for lactose fermentation test. The thermophilic flat sour bacteria that spoil canned vegetables can produce considerable amounts of lactic acid from sugar, and such a culture may be employed for the manufacture of lactic acid. The soil is an important source of *Bacillus* sp[1].

Proteus vulgaricus Gram negative, aerobic and facultative anaerobic, non spore forming, motile, non capsule producing and rod shaped organism. Size of the organism is 1 - 3µm in diameter. The organism shows positive result to indole, MR, urease, catalase but negative to others. They produce acid by fermenting sugar like sucrose, glucose and negative result for lactose fermentation test. Krieg and Holt, 1984 reported that the *Proteus vulgaricus* are typical of enteric bacteria in being present in the intestinal tract of humans and animals. They may be isolated from a variety of vegetable and meat products, especially those that undergo spoilage at temperatures in the mesophilic range.

Lactobacillus Gram positive, anaerobic/ microaerophilic, non spore forming, motile, non capsule producing and rod shaped organism. Size of the organism is 0.5 – 1.2µm in diameter. The organism shows positive& negative result to MR and oxidase result but negative to others. (Holzapfel & Gerber, 1983) reported *Lactobacillus* produce acid by fermenting sugar like lactose, sucrose, glucose. They typically occur on most, if not all, vegetables, along with some of the other lactic acid bacteria. Their occurrence in dairy product is common. *Serratia marcescens* Gram negative, aerobic and facultative anaerobic, non spore forming, motile, non capsule producing and rod shaped organism. Size of the organism is 1 - 3µm in diameter. The organism shows positive result to VP, Citrate but negative to others. They produce acid by fermenting sugar like sucrose, glucose and negative result for lactose fermentation test. (Jay, 1996) it is most prevalent of the food borne sp. it causes spoilage of refrigerated vegetables and meat products.

Pseudomonas sp. Gram negative, aerobic and facultative anaerobic, non spore forming, motile, non capsule producing and rod shaped organism. Size of the organism is 1- 3µm in diameter. The organism shows positive result to VP, Citrate but negative to others. They produce acid by fermenting sugar like sucrose, glucose and negative result for lactose fermentation test. (Jay, 1996) they are typical soil and water bacteria and are widely distributed among foods, especially vegetables, meat, poultry and sea products. They are by far the most important group of bacteria that bring about the spoilage of refrigerated fresh foods.

Table 2. Morphological tests

S. No	Organisms	Gram Staining	Capsular Staining	Spore Staining	Motility	Oxygen required
1	*Staphylococcus aureus*	+	+	_	_	Aerobes & Facultative anaerobes
2	*Streptococcus faecalis*	+	_	_	_	Aerobes & Facultative anaerobes
3	*Bacillus subtilis*	_	+	+	+	Aerobes & Facultative anaerobes
4	*Proteus vulgaris*	_	_	_	+	Aerobes & Facultative anaerobes
5	*Lactobacillus*	+	_	_	+	Anaerobes/ Micro aerophilic
6	*Serratia marcescens*	_	_	_	+	Aerobes & Facultative anaerobes
7	*Micrococcus*	+	+	_	_	Aerobic
8	*Pseudomonas* Sp.	_	_	_	+	Aerobic
9	*Flavobacterium*	_	_	_	_	Aerobes & facultative anaerobes
10	*Aeromonas*	_	+	_	+	Aerobic

Flavobacterium Sp. Gram negative, aerobic and facultative anaerobic, non spore forming, non motile, non capsule producing and rod shaped organism. Size of the organism is 1.5 - 3µm in diameter. The organism shows positive result to Citrate, catalase, oxidase and starch hydrolysis but negative to others. They produce acid by fermenting sugar like glucose and negative result for sucrose, lactose fermentation test. The *Flavobacterium* Sp they present in the spoilage of refrigerated meats and vegetables (Jay, 1996).

Aeromonas sp. Gram negative, aerobic and facultative anaerobic, non spore forming, motile, capsule producing and rod shaped organism. Size of the organism is 1 - 2µm in diameter. The organism shows positive result to indole, MR, VP, Citrate, catalase, oxidase and starch hydrolysis but negative to others. They produce acid by fermenting sugar like glucose and negative result for sucrose, lactose fermentation test. They are normal inhabitants of the intestines of fish and some fish pathogen (Pelczer & Reid,1965).

Table 3. Biochemical test

S.No	Organisms	Ind	MR	VP	Cit	Ure	Cat	Oxi	Sta	Lac	Suc	Glu
1	*Staphylococcus aureus*	_	+	±	_	_	+	_	_	A	A	A
2	*Streptococcus faecalis*	_	+	_	_	_	_	_	_	A	A	A
3	*Bacillus subtilis*	_	_	±	_	_	_	+	+	_	A	A
4	*Proteus vulgaris*	+	+	_	_	+	+	_	_	_	A	A
5	*Lactobacillus*	_	±	_	_	_	_	±	_	A	A	A
6	*Serratia marcescens*	_	_	+	+	_	_	_	_	_	A	A
7	*Micrococcus*	_	_	_	+	+	+	_	_	_	_	_
8	*Pseudomonas* Sp.	_	_	_	_	_	+	+	+	_	_	A
9	*Flavobacterium*	_	_	_	+	_	+	+	+	_	A	A
10	*Aeromonas*	+	+	+	+	_	+	+	+	_	_	A

Micrococcus sp. Gram positive, aerobic and facultative anaerobic, non spore forming, non motile, capsule producing and cocci shaped organism. Size of the organism is 0.5 - 1µm in diameter. The organism shows negative result to indole, MR, VP, oxidase and starch hydrolysis but positive to others. Negative result for sucrose, lactose and sucrose fermentation test. Nelson and Sorrells, 1984 reported the Micrococci are widespread in nature but have been isolated most often from dust and water. They often are found on inadequately cleaned and sanitized food utensils and equipment

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