Plant Archives Vol. 16 No. 2, 2016 pp. 834-838 *ISSN 0972-5210*

ISOLATION AND IDENTIFICATION OF BACTERIA FROM SOME SPOILED FRUITS

Lalita Chaudhary* and T. S. Dhaka

Department of Botany, D.A.V. (PG) College, Muzaffarnagar - 251 001 (U.P.), India.

Abstract

An investigation was carried to study the different bacterial sp. present in various spoiled fruits. Eleven bacteria, which caused spoilage of fruits were isolated from different spoiled fruits by serial dilution-agar plating method and enumerated. The isolated bacteria were identified as *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Enterobacter aerogens, Bacillus subtilis, Staphylococcus aureus, Shigella dysenteriae, Bacillus cereus, Klebsiella pneumoniae, Staphylococcus epidermidis* and *Bacillus megaterium* on the basis of cultural, morphological and biochemical characteristics. *Bacillus* was the commonest genera present in all tested spoiled fruit samples.

Key words : Spoiled fruits, *Bacillus,* nutrient agar medium.

Introduction

Fruits play a vital role in human nutrition by supplying necessary growth factors such as vitamins and essential minerals in daily diet which help to live a healthy life (Al-Hindi *et al*., 2011). India is the fourth largest producer of fruits in the world, yet due to losses in the field, during storage, transit or trans-shipment, during handling processes of the crop from the grower to the whole sale dealer and to retailer and finally to consumers (Chukwuka *et al*., 2010 and Zubbair, 2009), they become inadequate (Barth *et al*., 2013). The succulent nature of fruits and vegetables makes them to be easily invaded by microbes. The high concentration of various sugars, minerals, vitamins and amino acids also provide a good platform for the successful growth and survival of various microorganisms (Bhale, 2011). Most microorganisms that are initially observed on whole fruit or vegetable surfaces are soil inhabitants (Andrews and Harris, 2000; Janisiewicz and Korsten, 2002).

Spoilage refers to any change in the condition of food in which the food becomes undesirable or unacceptable for human consumption (Akinmusire, 2011). Bacterial spoilage first causes softening of tissues as pectins are degraded and the whole fruit may eventually degenerate into a slimy mass. Starch and sugars are metabolized next and unpleasant odours and flavours develop along with lactic acid and ethanol (Rawat, 2015). Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas and Katsoudas, 2005). Present investigation was carried out to study the presence of various bacteria responsible for the post harvest decay and deterioration of economically important fruits.

***Author for correspondence :** E-mail: lalitadav@gmail.com

Materials and Methods

Eight types of various unwashed and unprocessed spoiled fruits *viz*. apple, corn, grapes, guava, mango, orange, papaya and pomegranate were collected in plastic zip bag from local vegetable market of Muzaffarnagar and brought in the laboratory for further analysis.

The bacteria were isolated from spoiled fruits by using serial dilution agar plate method (Aneja, 2009). The spoiled fruits were crushed into presterilized mortar and pestle with distilled water to form suspension, which was serially diluted from 10^{-1} to 10^{-5} dilutions. 100µL of food suspension from each dilution was spreaded over nutrient agar medium (NAM) plates. The NAM was supplemented with amphotericin B (10µg/mL) before pouring to prevent fungal growth. The inoculated petriplates were incubated at 37^0C for 24 hours for bacterial growth. After incubation the morphologically different colonies of bacteria were isolated and subcultured. The bacterial isolates were maintained and stored on NAM slants at 4^0C for further use.

Table 1 : Cultural and morphological characteristics of bacteria isolated from different spoiled fruits.

S. no.	Isolate no.	Medium	Colonial morphology	Gram reaction	Shape and arrangement	Endospore present/ absent	Motility
1.	FRb*1	NAM	White, flat, large, smooth, opaque, moist and glistening	_	Short rods in scattered arrangement	_	+
2.	FRb*2	NAM	Yellow, circular, smooth and soft	+	Cocci in irregular clusters	_	_
3.	FRb*3	NAM	Thin, blue-grey, spreading growth	_	Short rods in scattered arrangement	_	+
4.	FRb*4	NAM	White, glistening, thick, mucoid with dark center and light edge	_	Short rods in scattered arrangement	_	+
5.	FRb*5	NAM	White, dry, attached, spreading with crenate margin	+	Long rods with round edges in scattered arrangement	+	+
6.	FRb*6	NAM	Golden yellow, large, circular, convex, smooth, shiny and opaque	+	Cocci in bunches	_	_
7.	FRb*7	NAM	Greyish, large, circular, convex, thin and smooth	_	Short rods in scattered arrangement	_	+
8.	FRb*8	NAM	White, waxy, opaque and abundant growth	+	Rods in chains	+	+
9.	FRb*9	NAM	White, translucent, slimy and raised growth	_	Short rods in scattered arrangement	_	_
10.	FRb*10	NAM	Off-white, shiny and irregular shaped with opaque zone around the colonies	+	Cocci in bunches	_	_
11.	FRb*11	NAM	White and circular	+	Rods in scattered arrangement	+	+

FR-fruit, b*-bacteria, NAM-nutrient agar medium, +-present and _-absent.

The bacterial isolates were identified on the basis of morphological and biochemical characteristics according to the Bergey's Manual of Systematic Bacteriology (Clauss and Berkeley, 1986). The cellular morphology of isolated bacteria was studied by Gram staining. The bacterial species were further identified on the basis of endospore staining**,** motility test and biochemical characteristics *viz*. carbohydrate fermentation, Indole production, Methyl Red, Voges-Proskauer, Citrate utilization, Catalase, Oxidase, Urease, Hydrogen sulphide production, Starch hydrolysis, Casein hydrolysis and Gelatin liquefaction (Cappuccino and Sherman, 2005).

Results and Discussion

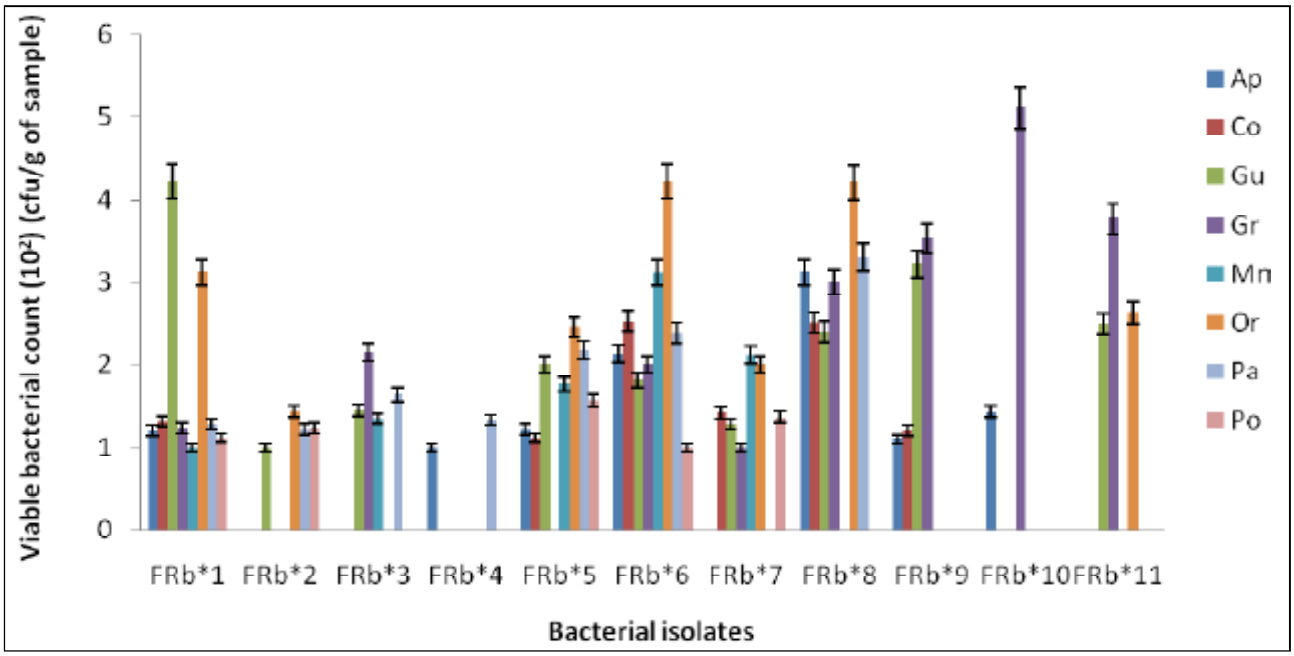
Eleven bacterial species were isolated from various spoiled fruits and enumerated. Fig. 1 shows the viable count of bacterial isolates (numbered FRb1- FRb11) on NAM plates. The morphological and biochemical characteristics of isolated bacteria are given in tables 1 and 2. The bacterial isolates FRb1 and FRb6 were present in all tested spoiled fruits. The other dominating bacterial isolates were FRb5, FRb8 and FRb7.

The food associated bacteria isolated from various samples of different spoiled fruits identified on the basis of cultural, morphological and biochemical characteristics are *Escherichia coli* (FRb1), *Micrococcus luteus* (FRb2), *Proteus vulgaris* (FRb3), *Enterobacter aerogens* (FRb4), *Bacillus subtilis* (FRb5), *Staphylococcus aureus* (FRb6), *Shigella dysenteriae* (FRb7), *Bacillus cereus* (FRb8), *Klebsiella pneumonia* (FRb9), *Staphylococcus epidermidis* (FRb10) and *Bacillus megaterium* (FRb11). The dominant members of the bacteria isolated from spoiled fruits belong to the genera *Bacillus*. Some of the above bacteria were isolated by other workers also.

Table 2 : Identified bacteria on the basis of biochemical characteristics.

S. no.	Biochemical tests	Bacterial isolate no.										
		FRb*1	**FRb*2**	**FRb*3**	**FRb*4**	**FRb*5**	**FRb*6**	**FRb*7**	**FRb*8**	**FRb*9**	**FRb*10**	**FRb*11**
1.	Carbohydrate fermentation • Glu • Suc • Lac • Man	 AG A AG _	 _ _ _ _	 AG AG _ _	 AG AG AG _	 A A – A	 A A A A	 AG A _ AG	 A A _ A	 AG AG AG AG	 A A A _	 A A _ A
2.	Indole production	+	_	+	_	_	_	+	_	_	_	_
3.	Methyl red	+	_	+	_	_	+	+	_	_	+	_
4.	Voges proskauer	_	_	_	+	+	+	_	+	+	+	_
5.	Citrate utilization	_	+	_	+	+	_	+	_	+	_	+
6.	Catalase	+	_	+	+	+	+	+	_	+	+	+
7.	Oxidase	_	_	_	_	_	_	_	+	_	_	_
8.	Urease	_	+	+	_	_	_	_	_	+	+	_
9.	H_2S production	_	_	+	_	_	_	_	_	_	_	_
10.	Starch hydrolysis	_	_	_	_	+	_	_	+	_	_	+
11.	Casein hydrolysis	+	_	_	_	+	_	_	_	_	_	_
12.	Gelatin liquefaction	_	+	+	_	+	+	_	+	_	+	+
Bacteria identified		*Escherichia coli*	*Micrococcus luteus*	*Proteus vulgaris*	*Enterobacter aerogens*	*Bacillus subtilis*	*Staphylococcus aureus*	*Shigella dysenteriae*	*Bacillus cereus*	*Klebsiella pneumoniae*	*Staphylococcus epidermidis*	*Bacillus megaterium*

FR-fruit, b*-bacteria, Glu-glucose, Suc-sucrose, Lac-lactose, Man-mannitol, AG-acid & gas, A-acid, +-present and _-absent.



FR-fruit, b*-bacteria, Ap-apple, Co-corn, Gr-grapes, Gu-guava, Mn-mango, Or-orange, Pa-papaya, Po-pomegranate, S.D.-standard deviation and cfu/g-colony forming unit per gram.

Fig. 1 : Viable count (cfu/g) of bacteria isolated from different spoiled fruits.

Raja *et al*. (2012) investigated that *Pseudomonas* sp. and *Bacillus* sp. were dominantly found in both local and super market samples of spoiled vegetables and fruits. Kumar *et al*. (2011) collected 30 spoiled food samples form Paonta sahib, isolated and characterized seven bacterial isolates *viz*. *Bacillus*, *Klebsiella*, *Pseudomonas, E.coli*, *Lactobacillus*, *Staphylococcus* and *Micrococcus* on the basis of morphology and biochemical reactions. They reported that the *Bacillus, Klebsiella* and *Pseudomonas* were the dominating species in the spoilage of every categories of food material. Oviasogie *et al*. (2015) reported that a number of bacterial species associated with the fruit spoilage belonging to genera *Bacillus*. Ismail and Zhang (2004) isolated *Bacillus* species and *E. coli* from spoiled papaya by serial dilution method.

References

Akinmusire, O. O. (2011). Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. *Advances in Environmental Biology*, **5(1)** :157-161.

Al-Hindi, R. R., A. R. Al-Najada and S. A. Mohamed (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *African Journal of Microbiology Research*, **5(4)** : 443-448.

Andrews, J. H. and R. F. Harris (2000). The ecology and biogeography of microorganisms on plant surfaces. *Annual Review of Phytopathology*, **38** : 145–180.

Aneja, K. R. (2009). Expriments in Microbiology, *plant pathology and Biotechnology* fourth ed. *New Age International Publishers*, Daryaganj, New Delhi.

Barth, M., T. R. Hankinson, H. Zhuang and F. Breidt (2013). Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) Fruits In Sokoto State. *Nigerian Journal of Basic and Applied Sciences*, **21(3)** : 193-196.

Bhale, U. N. (2011). Survey of market storage diseases of some important fruits of Osmannabad District (M.S.). *India Science Research Reporter*, **1(2)** : 88-91.

Cappuccino, J. G. and N. Sherman (2005). *Microbiology : A Laboratory Manual*, seventh ed. Pearson Education. Inc. and Darling Kindersley (India). 143- 203.

Chukwuka, K. S., I. O. Okonko and A. A. Adekunle (2010). Microbial Ecology of Organisms Causing Pawpaw (*Carica papaya* L.). Fruit Decay In Oyo State, Nigeria. *American-Eurasian Journal of Toxicological Sciences*, **2(1)** : 43-50.

Clauss, D. and R. C. W. Berkeley (1986). Genus *Bacillus* Cohn 1872. In Bergey's manual of determinative bacteriology, Sneath P H A Baltimore. *MD: Williams Wilkins*, **2** : 1105-1141.

Ismail, M. and J. Zhang (2004). Post-Harvest *Citrus* diseases and their control, outlooks on pest management. *Peouen*, **15** : 29-35.

Janisiewicz, W. J. and L. Korsten (2002). Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology*, **40** : 411–441.

Kumar, A., V. Bhushan, S. Verma, G. Srivastav and S. Kumar (2011). Isolation and Characterization of Microorganisms

Responsible for Different Types of Food Spoilages. *International Journal of Research in Pure and Applied Microbiology*, **1(2)** : 22-31.

Oviasogie, F. E., A. G. Ogofure, A. Beshiru, J. N. Ode and F. I. Omeje (2015). Assessment of bacterial pathogens associated with orange spoilage. *African Journal of Microbiology Research*, **9(29)** : 758-1763.

Raja, M. M., A. Raja, M. S. Hajee and S. A. Mohamed (2012). Screening of bacterial compost from spoiled vegetables and fruits and their physiochemical characterization. *International Food Research Journal*, **19(3)** : 1193-1198.

Rawat, S. (2015). Food spoilage : Microorganisms and their prevention. *Asian Journal of Plant Science and Research*, **5(4)** : 47-56.

Tournas, V. H. and E. Katsoudas (2005). Mould and yeast flora in fresh berries, grapes and citrus fruits. *International Journal of Food Microbiology*, **105** : 11-17.

Zubbair, N. A. (2009). Determination of microbial characteristics of selected fruits sold in major markets in Ilorin metropolis. *Afr. Sc*., **10(2)** : 1595-6881.