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Enumeration of Anaerobic Bacteria from Marketed Table Grape to Determine the Health Hazard in Urban Areas of Jessore, Bangladesh

Mizanur Rahman[1], Sazin Islam[2*], Md. Mahabub Hasan[2], Md. Jahid Al Jakaria[2], Mahmuda Sultana[2], Mohammad Rocky Khan Chowdhury[2]
[1]*Faculty of Agriculture, First Capital University of Bangladesh, Chuadanga 7200, Bangladesh*
[2]*Department of Public Health, First Capital University of Bangladesh, Chuadanga 7200, Bangladesh*

Abstract: *This study was conducted to identify contaminants in marketed white table grape for avoiding health hazard. White table grape samples were collected from Jessore, Bangladesh. The samples were inoculated into Nutrient Agar (NA) and Trypticase (Tryptic) Soy Agar (TSA) medium to obtain primary culture for bacteria. Following the inoculation the for bacterial colonies were found after incubation for 24 hours in 37 ^{0} C. Colony for bacteria were streaked into NA and TSA medium and incubated for 24 hours in 37 ^{0} C. Then pure cultures were identified by gram-staining technique, biochemical tests, carbohydrate fermentation tests and differential tests. Biochemical tests weredone byCatalase test, Oxidase test, Coagulase test, Urease activity test, Gelatin liquefication test, Citrate utilization test, Indole test, Methylene red test, and Voges-Proskauer test. Carbohydrate fermentation tests were included the utilization ofGlucose, Lactose, Sucrose, Maltose, L-rhamnose, L-arabinos, D-mannitol, Salinic, Glycerol, Cellbiose, Trehalose, Raffinose and Starch. Differential tests were initiated with Coagulase test, Novobiocin test and Hemolysis test. The morphologicalcharacteristics, cultural characteristics and results from different tests of isolated were compared with standard parameter and found to closely related to the genus Staphylococcus epidermidis.*

Keywords *–Staphylococcus epidermidis, Grape, Health Hazard, Bangladesh*

I. Introduction

Grapes were a type of fruit that grow in clusters of 15 to 300, and can be crimson, black, dark blue, yellow, green, orange and pink. "White" grapes were actually green in color, and were evolutionarily derived from the purple grape. Grapes were typically an ellipsoid shape resembling a prolate spheroid. (Waterhouse et al. 2002).

The microbial species recovered from grapes may be divided in several groups according to their technological significance in grape and wine production. Under this view, microorganisms were characterized as a function of their effect on grape and wine quality. However, these fungi have not the ability to grow in wines and their effect on wine quality is due to grape damage. Contrarily, the microorganisms of the WMC, do not damage grapes (except in sour rot) but were able to survive or grow on wine, depending on the efficiency of adequate processing measures. Thus, based on the concept already suggested by the authors (Loureiro and Malfeito-Ferreira, 2003; Malfeito-Ferreira, 2011), the partners of this consortium may be grouped in: (i) easily controllable or innocent species, without ability to spoil wine when good manufacturing practices (GMP‘s) were applied; (ii) fermenting species, responsible for sugar and malic acid conversion; and (iii) spoilage sensustric to species responsible for wine alteration even when GMP‘s were believed to be applied.

The most frequent grape contaminant species were *basidiomycetous*, oxidative or weakly fermentative species, regarded as innocuous or easily controllable yeasts. Their inability to grow, or their limited growth, in musts and wines explains their recognized irrelevance technological terms. In the worst scenario, apiculate, oxidative or film-forming yeasts have been traditionally regarded as detrimental to wine quality due to the production of unwanted volatiles or to the induction of stuck fermentations, which may be prevented by appropriate technological measures (Malfeito-Ferreira, 2011). However, some reports have mentioned the possible beneficial role of contamination yeasts due to volatile production impartingpeculiar flavor characteristics, dependent or not on enzymatic activities releasing aroma precursors or hydrolysingpectolytic molecules (Fernández et al., 2000), or to factors (mouthfeel, body) independent of ester production (Jolly et al., 2006). Even *basidiomycetes (Sporobolomycesroseus)* and A. pullulans were found to produce wine flavor compounds after grape inoculation in vitro (Verginer et al., 2010), but results lack from validation under technological conditions. Must co-inoculation with non-*Saccharomyces* yeasts has been used to benefit wine quality, such as decrease in volatile acidity or improvement of mouth feel sensations (Ciani et al., 2002).

II. Materials and Methods

2.1 Sample Collection
Grape samples were collected from Jessore, Bangladesh between January to February, 2015 for isolation and identification of bacterial and fungal species.

2.2 Sample Preparation
Collected grapes were rested for 7 days in a nylon covered glass jar for decomposing by bacteria and fungus. After decomposing 5 of sample grapes were dissolved in 250ml distilled water to make a sample suspension.

2.3 Media Preparation
Following media were prepared for isolation procedure-Nutrient agar (NA), Trypticase (Tryptic) Soy Agar (TSA).Sterilization of all tubed media was accomplished at 15lb pressure (121°C) for 15 minutes. All of these media were available commercially in powdered form, with specific instructions for their preparation and sterilization.

2.4 Bacterial Isolation
The procedures for the isolation and identification were based on the Nordic Committee on Food Analysis methods.Bacteria wereisolated on NA and TSA media by spread plate method. 20ml of each media was poured on 5 different petri plates. Petri plates were rested for solidify into laminar air flow for several minutes. 1ml sample was spreaded on every petri plate containing medium by using L-shaped glass rod. After spreading plates were left in incubator at 37oC for 24 hours. After growth in all plates, bacterial culture was streaked on NA and TSA media for isolating single colony, bacterial culture was also streaked in NA and TSA slant.

2.5 Gram stain
Heat-fixed smears were prepared from the sample. The slides were placed on the staining rack. Flood The smears was flooded with crystal violet and rested for 30 seconds 4. Rinsed with water for 5 seconds. Then smear was covered with Gram's iodine mordant and rested for 1 minute. Rinsed with water for 5 seconds. Next the smear was decolorized with 95% ethanol for 15 to 30 seconds. The decolorizer was added drop by drop until the crystal violet fails to wash from the slide and then rinsed with water for 5 seconds. The smear was counter-stained with safranin for about 60 to 80 sec and rinsed with water for 5 seconds. Smear was blot dried with bibulous paper. Smear was examined under oil immersion. Organisms were stained blue to purple.

2.6 Catalase Test
A clear slide was taken and 1 drop of hydrogen peroxide was added. A single colony was taken and added on slide. Then wait for 2-3 minutes. (Prescott 2002)

2.7 Oxidase Test
A disk was taken. 2-3 drops of reagent (tetra-methyl-p-phenyl-diaminedihydro chloride) was added on the disk. A loop full of bacteria colony was added on the disk. (Prescott 2002)

2.8 Citrate Utilization Test
5ml of Simmons citrate media was taken in to sterile test tube. A loop full of bacteria was inoculated in to the tube. Then incubate at 37°c for 24 hours. (Prescott 2002)

2.9 Indole Test
A loop full of overnight growth bacteria was inoculated in to the TSB. Incubate for at least 48 hours. Kovac´s reagent was added to the media. (Prescott 2002)

2.10 Methylene-Red Test
Using sterile technique, inoculate each experimental organism into its appropriately labeled tube of medium by means of a loop inoculation. The last tube was served as a control and all culture were incubated for 24 to 48 hours at 37ºC. (Prescott 2002)

2.11 Vogues Proskauer Test
A loop full of organisms was inoculated in to the MR-VP medium in the tube. Barrits A (alpha-napthol)+ Barrits B (potassium-hydroxide) was added to the medium. Keep it 20 to 30 minutes. (Prescott 2002)

2.12 Gelation Liquification and Carbohydrate Fermentation
Gelation liquification and carbohydrate fermentation methods as demonstrated by Collee and Miles (1989).

III. Results

3.1 Physical Observation

Table 1: Biochemical Identification

Features	Characteristics on Media
Vegetative cells	Non-flagellate, Non-motile, Non-spore forming.
Gram Staining	Gram Positive
Colony shape on NA and TSA media	Small, circular
Colony Color on NA and TSA media	White

3.2 Biochemical Identification

Table 2: Biochemical Identification

Tests	Results
Coagulase	-
Indole production	+
Methyl Red reaction	-
Voges-Proskauer reaction	+
Citrate Utilization	-
Urease activity	+
Catalase	+
Oxidase	-
Gelatin Liquefication	-

3.3 Carbohydrate Fermentation

Table 3: Carbohydrate Fermentation

Tests	Results
Glucose	+
Lactose	+
Sucrose	+
Maltose	+
L-Rhamnose	-
L-Arabinos	-
D-Mannitol	-
Salinic	-
Glycerol	+
Cellobiose	-
Trehalose	-
Raffinose	-
Starch	-

3.4 Differential Tests

Table 4: Differential Tests

Tests	Results
Coagulase test	Negative
Novobiocin	Sensitive
Hemolysis test	Negative

IV. Discussion

McGovern P. E. (2003) isolated both anaerobic species (e.g. *Staphylococcus epidermidis*) and aerobic species (e.g. *Botrytis cinerea*) from white table grape. The study was similar with that observation in the sense of aerobic microorganisms isolation.

The morphological, cultural and biochemical characteristics of isolated is compared with the "Bergey's Manual of systematic bacteriology" 2nd edition, 3rd volume (The Sirmicute pp394) and found to closely related to the

genus *Staphylococcus epidermidis*. The isolated bacterial species *Staphylococcus epidermidis* is a saprophytic microorganism in a variety of hosts and normal micro-flora of human skin.

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