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Isolation and Identification of Food Spoiling Bacteria

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Abstract

Raw fruits and vegetables have been known to serve as vehicles of human disease for at least a century.In the present study a survey of different hospitals and clinics of Srinagar was done to investigate the cases of food borne diseases in the valley. It was found that *Staphylococcus aureus* was predominant in banana and grapes, which is known to cause Staphyloccocal food poisoning.

Key words : *Bacterial contamination, food spoilage,Staphylococcus aureus.*

Introduction

Food is one of the most important requirements for life of single cell microbes to human. Except plants and some algae most of the organisms are dependent to others for their nutritional requirements and use varied food materials. Microbes are present almost everywhere in the environment (except fire) and use a vast range of food substances. All microbes comes under different categories on different characters they have, they may be harmful, beneficial or neutral. Among these under harmful category we consider pathogenic microbes which can cause diferent disease in humans, animals as well as in plants.

In humans the most common source of disease is due to spoiled food which is result of microbial growth, mainly by bacteria and their byproducts. There are more than 250 food borne diseases, many of which cause other symptoms other than vomiting and diarrhea. Bacterial contamination is most common cause of food poisoning, followed by viruses and parasites. Among the common bacterial species, *Camphylobacter, Salmonella, E.coli, Staphylococcus aureus, Shigella, Clostridium perfringens, Botulinum cereus* are encountered most frequent. In the present study a survey of different hospitals and clinics of Srinagar was done to investigate the cases of food borne diseases in the valley.

Material And Methods

Sample collection

Vegetables, fruits and meat samples were collected from the roadside market and were packed in presterile autoclavable polybags to bring in lab. Sample were washed with 100 ml sterile distill water for 2-3 times. 10 ml of this water was then taken into presterile tube and was considered as test samples.

Isolation and identification of bacterial species

Isolation and identification of bacterial species responsible for food spoilage was done following the standards methods of APHA(1998).

Results

The results of the present study is given table 1-4 while the data collected from Govt. Hospital for food and water related disorders is given in table 5.During the course of study total number of colonies obtained from different samples were 607. In case of banana (Orange 54, yellow 13, white 47) , in case of grapes

(Orange 60, yellow 03, creamy 150) while in case of tomato (Orange3, yellow 7, creamy 150) and in case of meat (yellow 20 , white 100) number of colonies were found (table 1).On the basis of different parameters viz. morphology colour and size 11 groups were prepared. One representative of each group was taken for further assay.

Gram staining reveal that all the representatinve isolates were 11 gram positive (table 2) Cells which were found round shaped i.e. Coccus and clustered like grapes were Staphylococcus sps. while those which were coccus in shape but individual in arrangement were of micrococcus Sps. Series of biochemical test were performed for each bacterial colony. Catalase test (positive 9, negative 2) , mannitol test (positive 9, negative 2)

Bile esculin test and 6.5 % NaCl (positive1, negative 1) Table 3 and Fig 1 (a -c).

For determining spoilage rate fresh food articles (Banana, meat and tomato) were brought from market. Bacterial colony present on the surface of thease food articles cultured onnutrient agar medium.These food articles were then kept at room temperature (40^{o} C) for determining the bacterial species responsible for spoilage. After 24 hour meat sample showed bacterial growth, produced bad odour and lesion appereance While in case of banana all the above conditions were seen after 48 hrs. Tomato relatively showed only bacterial growth and foul odour on 3rd day.These material was taken and were treated as sample for obtaining bacterial growth of media plates.

After incubation same bacterial colonies were observed which shows that same bacterial species are responsible for spoilage of food.

Discussion

Raw fruits and vegetables have been known to serve as vehicles of human disease for at least a century. In1899 Morse linked typhoid infection to eating celery. Warry(1903) attributed an outbreak of typhoid fever to eating watercress grown in soil fertilized with sewage. Pixley (1913) recorded two cases of typhoid from eating uncooked rhubarb which was grown in soil known to have been fertilized with typhoid excreta. Melick (1917) recovered typhoid bacilli from mature lettuce and raddish harvested from soil that has been inoculated at the time seeds were planted. Similiarly in the present Staphylococcus aureus was predominant in banana and grapes, which is known to cause Staphyloccocal food poisoning.

Staphylococcus aureus is known to be carried in the nasal passages of healthy food handlers and has been detected on raw produce (Abdelnoor *et al.* 1983). According to Hauschild, 1992 cases of botulism that have been linked to fresh produce are very rare.De Roever, 1998 reported outbreaks involving cooked/ processed vegetable products 9 e.g. garlic in oil, mushrooms).

Pao and Brown (1998) studied human pathogens associated with the surface of citrus from seven commercial packing house. They found no generic *E. coli* on fruit .In the present study orange samples which were serially diluted and plated were contaminated with *S. aureus, S, bovis, B, cereus and E .facecalis.*

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Table 1: Morphology and number of bacterial colonies in different food samples

SAMPLES	ORANGE	YELLOW	WHITE	CREAMY	TOTAL
BANANA	54	13	47	--------	114
GRAPES	60	3	--------	150	213
TOMATO	3	7	--------	150	160
MEAT	--------	20	100	--------	120

Table2 Number of bacterial colony showing Gram- Positive and Gram Negative test

SAMPLE	COLONY	GRAM REACTION	SHAPE	ARRANGEMENT	INFERENCE
BANANA	ORANGE	POSITIVE	COCCUS	CHAIN LIKE	*STREPTOCOCCUS*
	YELLOW	POSITIVE	COCCUS	CHAIN LIKE	*STREPTOCOCCUS*
	WHITE	POSITIVE	COCCUS	GRAPE BUNCH LIKE	*STAPHYLOCOCCUS*
GRAPES	CREAMY	POSITIVE	COCCUS	GRAPE BUNCH LIKE	*STAPHYLOCOCCUS*
	ORANGE	POSITIVE	COCCUS	GRAPE BUNCH LIKE	*STAPHYLOCOCCUS*
	YELLOW	POSITIVE	COCCUS	GRAPE BUNCH LIKE	*STAPHYLOCOCCUS*
TOMATO	YELLOW	POSITIVE	ROD	INDIVIDUAL	*BACILLUS*
	CREAMY	POSITIVE	COCCUS	GRAPE BUNCH LIKE	*STAPHYLOCOCCUS*
	ORANGE	POSITIVE	COCCUS	GRAPE BUNCH LIKE	*STAPHYLOCCCUS*
MEAT	YELLOW	POSITIVE	COCCUS	GRAPE BUNCH LIKE	*STAPHYLOCOCCUS*
	CREAMY	POSITIVE	COCCUS	GRAPE BUNCH LIKE	*STAPHYLOCOCCUS*

Stain +ve -Positive test ; Stain -ve- negative test; Coccus- round
Bacilus- rod; Individual single; Irregular- grapes like

Table 3; List of biochemical test and organism identified

SAMPLE	ISOLATE	G.R.	SHAPE	PIGMENT	Biochemical test				INFERENCE
					CATALASE	MANNITOL	BILE TEST	NACL TEST	
BANANA	B.B.O1	+	COCCUS	ORANGE	-	------	+	-	MAY BE S. BOVIS
	B.B.C2	+	COCCUS	CREAMY	-	-----	+	+	E.FAECALIS
	B.B.Y3	+	COCCUS	YELLOW	+	+	------	------	S. AUREUS
GRAPES	G.B.C4	+	COCCUS	CREAMY	+	+	----	------	S. AUREUS
	G.B.O5	+	COCCUS	ORANGE	+	+	------	------	S.AUREUS
	G.B.Y6	+	COCCUS	YELLOW	+	+	------	-------	S. AUREUS
TOMATO	T.B.Y7	+	ROD	YELLOW	----	-	---	-----	B. CEREUS
	T.B.C8	+	COCCUS	CREAMY	+	+	------	-----	S. AUREUS
	T.B.O9	+	COCCUS	ORANGE	+	+	-----	-----	S.AUREUS
MEAT	M.B.Y10	+	COCCUS	YELLOW	+	+	-----	----	S.AUREUS
	M.B.C11	+	COCCUS	CREAMY	+	+	------	------	S.AUREUS

B.B. O1 - banana bacterial orange isolate 1
B.B. C2 - banana bacterial orange isolate 2
B.B. Y3 - banana bacterial orange isolate 3
G.B. C4 - banana bacterial orange isolate 4
G.B. O5 - banana bacterial orange isolate5
G.B. Y6 - banana bacterial orange isolate 6
T.B. Y7 - banana bacterial orange isolate 7
T.B. C8 - banana bacterial orange isolate 8
T.B. O9 - banana bacterial orange isolate 9
M.B.Y10 - banana bacterial orange isolate 10
M.B. C11 - banana bacterial orange isolate 11
G.R. -Gram reaction

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Table 4: Rate of Spoilage of different food samples

SAMPLES	TEMP	DAYS	B.G	ODOUR	LESIONS
BANANA	40	2ND	+	+	+
MEAT	40	24 HRS	+	+	+
POTATO	40	3 RD	+	+	-

Table 5: Food and water related disorder of the year 2004 collected from Govt. Hospital Srinagar

DISORDER	CASE	AGE GROUPS					RECORDED IN MONTH											
		0-15	15-30	30-45	45-60	60-75	J	F	M	A	MY	JN	JU	AU	S	O	N	D
Colitis	26	6	9	5	5	1	-	-	2	-	-	3	12	5	3	3	-	-
Diarrhea	12	4	3	1	1	3	1	-	3	2	-	3	3	-	-	-	-	-
Dysentry	30	5	10	10	3	2	-	1	1	-	3	5	15	2	1	2	-	-
Gastroentritis	10	--	3	5	2	--	2	-	-	4	1	3	-	-	-	-	-	-
Acute abdomine	15	---	9	3	2	1	5	2	-	-	3	2	1	-	-	2	-	-

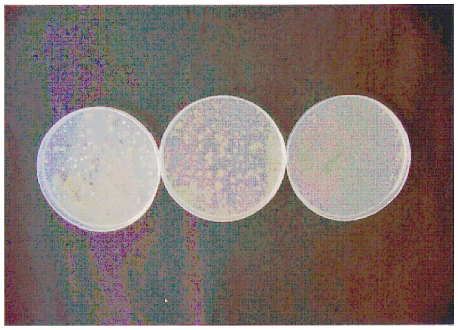
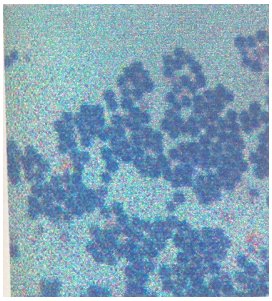
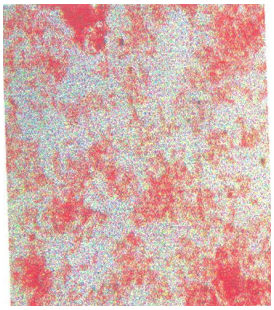


Fig : 1: Slide showing gram staining results

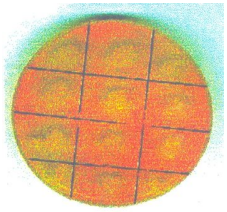


Gram positive



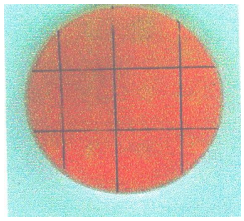
Gram Negative

Fig : 2: Slide showing gram staining results



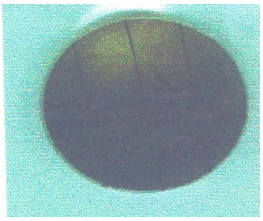
Mannitol positive test

Presence of yellow zone around bacterial colony



Mannitol negative test

Absence of yellow zone



Positive bile esculin test

Light brown colour changes to blackish brown

Fig : 3: Evaluation of isolate for different biochemical test