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RESEARCH ARTICLE

ISOLATION, PURIFICATION AND CHARACTERIZATION OF ASSOCIATED ORGANISMS USUALLY AVAILABLE IN CHICKEN SANDWICH

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ABSTRACT

The microbiological quality of 4 types of chicken sandwich samples were collected from different well known shops of Chittagong City Corporation, Bangladesh which were used as test samples to isolate and identify the associate contaminated bacteria. The isolated bacteria were identified following the standard bacteriological methods: morphology, gram staining and biochemical tests. Three types of bacteria were identified and characterized by differents tests as *Lactobacillus delbrueckii, Salmonella bongori* and *Yersinia pestis.* The results of microbiological assessment in the laboratory and the corresponding questions that asked to the food handlers and food servers also suggested that the microbial safety of the investigated fast foods depends not only on the environmental conditions but also on the personnel hygiene. These results also indicate poor microbiological quality of the meat-based ready-to-eat fast food items sold in the First Food Shops. For the first time we reported the microbiological safety by identifying the microorganisms usually contaminated the chicken sandwiches available in the four renowned shops of the Chittagong.

INTRODUCTION

Bakery products are an important part of food expenditure. It is considered as a important staple foods in most country and cultures. It is a valuable source of nutrients in our diet providing us with most of our food calories and approximately half of our protein requirements (Kent, 1983). For several thousands of years, man has used wheat and other cereals to produce bread with an average consumption of about 65 kg of bread per capita per year in Europe. In 2009 Hunt and Robbins reported that bakery products accounted for 9 per cent of the average weekly food consumption (Hunt and Robbins 2009). Anon (2000) estimated the consumption of bread in the UK was still 41.5 kg per person in 1990 (Anon, 2000). Baur (2001) estimated the western European bread market to be 23.000 million French francs (Baur, 2001). But microbiological spoilage is often the major factors limiting the shelf life of bakery products. Spoilage from microbial growth causes economic loss for both manufacturers and consumer. These losses could be due to many individual cases such as, packaging, sanitary practice in manufacturing, storage conditions and product turnover. Rachel Needham *et al*. (2004) tested the microbial spoilage caused by bacteria, yeast and fungi and enzymic spoilage caused by lipoxygenase can be differentiated from one another and from unspoiled bread analogues after 48 hours using cluster analysis, prior to signs of visible spoilage. The spores of *Bacillus subtilis* for examples are heat resistant; 55 per cent remain active in amylase after 20 minutes at 65°C (Needham *et al.,* 2004). This microorganism, which is present in raw ingredients, e.g. flour, sugar, and yeast, causes rope in bread (Smith, 1993). Ropey bread is characterized by discoloration from brown to black, the release of a rotten fruit odor and having an extremely moist, stringy bread crumb (Rosenkvist and Hansen, 1995). Ozay Mentes *et al.* (2005) studied the effect of two different sourdoughs, produced with *Lactobacillus plantarum* and *Lactobacillus alimentarius* (Mentes *et al.,* 2005). Sana M'hir *et al*. (2007) collected thirty samples of fermented wheat dough microflora from different Tunisian bakeries. Carla and Maria (2009) evaluated the ability of lactic acid bacteria to inhibit *Aspergillus*, *Fusarium* and *Penicillium*, the main contaminants in bread (M'hir *et al.,* 2007).

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MATERIALS AND METHODS

Four Samples of Chicken Sandwich were collected from the reputed fast food manufacturer of Chittagong City, Bangladesh. After collection, the samples were enclosed in polythene bags with proper labeling carefully and then taken to the experimental Microbiology Laboratory of Chittagong

University, Bangladesh in order to prevent or avoid cross contamination. The samples were analyzed in triplicate as soon as possible.

Liquid assay

For bacteriological examination, the samples were prepared by using dilution technique. The microbial colonies were isolated by serial dilution plate procedure (Foster *et al.,* 1958). For this purpose, 5gm of the chicken sandwich was taken in 90ml of the sterile distilled water in a sterile conical flask. It is used as the 10^{-1} dilution. Further dilutions were prepared up to 10^{-6} from the 10^{-1} dilution with sterile distilled water. For the isolation and enumeration of microorganisms particularly bacteria, nutrient agar media was used through out the study.

Identification of microorganisms

Bacterial isolates were identified by the microscopic, cultural and biochemical characteristics according to the Bergeys Manual of Systemic Bacteriology.

Isolation of Discrete colonies

The discrete bacterial colonies were carried out immediate after counting of the colonies. On the basis of their morphology, several different colonies were selected for this purpose. The selected colonies were marked and their characteristics were studied depending on the various points viz. form, elevation, margin, surface, color etc. (Eklund and Lankford, 1967; Bryan, 1950). Then the marked and observed bacterial colonies were transferred to nutrient agar slant aseptically for further purification.

Maintenance and preservation of the isolates

The purified bacterial isolates were maintained on nutrient agar slants during the course of investigation. The culture tubes (slant) were kept in polythene bags. The bags were tied up and preserved in a refrigerator at $4^{o}C$ as stock culture, and these isolates were maintained by transferring to the fresh medium periodically.

Preparation for light microscopic examination

The following methods (Eklund and Lankford, 1967; Bryan, 1950) were used for this purpose. The morphological characteristics of bacteria during staining, much importance was given on the preparation of slides (Eklund and Lankford, 1967; Bryan, 1950). Thin smears were prepared on extremely clean slides and freshly prepared filtered stains were used as per schedule to stain bacterial samples. The simple staining was performed as reported (Eklund and Lankford, 1967) but for gram staining, (Hucker and Conn, 1927) modified method was used (Eklund and Lankford, 1967; Hucker and Conn, 1923). Again for spore staining (Conklin, 1934) modification of (Wirtz, 1908) method was followed (Wirtz, 1908).

Cultural and biochemical examination

The cultural examination of the bakery food samples was done according to the standard method. The identification of bacteria were performed using the following established methods: catalase test (Claus, 1995), deep glucose agar test by Khan, (1962), indole test (Sneath *et al.,* 1986), motility test (Eklund and Lankford, 1967), oxidase test (Collins *et al.,* 1991), VP test (Bryan, 1950) and methyl red test (Bryan, 1950).

The physiological characteristics of all the pure isolates i.e. their growth at different temperature, pH, salt tolerance and heat resistance were studied by the following procedure: growth at different temperatures[18] and growth at different pH values (Williams *et al.,* 1971).

RESULTS AND DISCUSSION

As presented in the Table 1, the following questions were asked to the respective persons of all the experimentally selected shops and the results are summarized in the Table. From the findings, it can be suggested that the food handlers in the shop are not so much concerned about maintaining the hygienic condition.

Table 1. The answers to the questionnaires asked to the food handlers during collection of the food sample

Name of the Company	No of persons Interviewed	Hand washing		Proper dressing		Personal cleanliness		Education	
		Yes	No	Yes	No	Yes	No	Yes	No
Sample 1	3	3	0	3	0	2	1	2	1
Sample 2	3	1	2	0	3	3	0	1	2
Sample3	3	2	1	0	3	2	1	1	2
Sample4	3	1	2	3	0	2	1	1	2

Information from food handlers

A set of questionnaires was asked to the food handlers for additional supports of the investigation about hygienic conditions of the fast food shops. In every case at least 3 food handlers were asked. Their answers were then analyzed and compared with the laboratory findings.

The bacteriological condition of different portions of chicken sandwich was studied. The bacteria were identified by careful observation of colony characteristics. Among the experimental sandwich used in the analysis of the four different company and 3 types of bacteria were isolated and identified from the sample. All the samples are collected freshly and the work was done within 2-5 hours after collecting the sample. From the 4 samples *Lactobacillus delbrueckii*, *Salmonella bongori* and *yersinia pestis* was identified. Collins *et al.* (1991) isolated Bacillus strains from bread and bakery products (Collins *et al.,* 1991). Bailey *et al.* (1993) reported that *Bacillus subtils, Bacillus licheniformis* caused economic losses to the baking industries (Bailey 1993). Smith *et al.* (2003) demonstrated the microorganisms of concern in minimally processed bakery product when *salmonella sp, Staphyloccus aurees* and *Bacillus sp* were present (Smith *et al.,* 2003). The microbiological analysis of different types of chicken sandwich suggested that in most of the fast food samples, higher number of bacterial count made the food unsafe and unhygienic. Through the fried portion gave the least number of bacterial counts, and the raw portion (lettuce) was the most responsible one. The storage conditions of all the shops were unhygienic. In some cases fast

Table 2. Morphological, biochemical and cultural Characteristics of the Isolate No. 7

Place of collection	Chittagong, Bangladesh.
Vegetative Cells	Rod shaped, cells formed short chain
Size	0.5 – 0.8 x 1.0-10µm
Spores	Non spore former
Gram stain	Gram positive
Acid-fast stain	Non acid fast
Flagella stain	Not done
Motility test	Non motile
Agar colonies	Circular colonies
Agar slant	Echinulate growth
Nutrient broth	Flocculent growth
Glucose broth	Flocculent growth with turbidity
Asparagine broth	Little sediment growth
Catalase activity	Catalase negative
Oxidase test	Oxidase negative
Deep glucose agar	Moderate surface growth within the media
Oxygen relation	Facultative anaerobic
Indole test	Negative
Nitrate reduction test	Negative
Inorganic medium	No growth
Citrate medium	Negative
Gelatin liquefaction	Not liquefied
Hydrogen sulphide (TSI)	Produced
Urease test	Negative
Coagulated egg albumin	Moderately proteolytic
Milk agar plate	Not hydrolyzed
Starch agar plate	Negative
Methyl red	Positive
V. P. test	Negative
Growth at	0^0C 10^0C 20^0C 30^0C 37^0C 40^0C 45^0C - + ++ +++ ++++ ++++ ++
Fermentation	Acid and no gas form : Glucose, Arabinose, Mannitol, Xylose No change: Lactose, Sucrose, Starch, Rhamnose, Cellulose, Inolin.

Identification : The morphological, cultural and biochemical characteristics of isolate No. 7 was compared with standard description of 'Bergey's Manual of Determinative Bacteriology'-8th Ed.[21] and found closely related with the genus *Lactobacillus*. The isolate was provisionally identified as *Lactobacillus delbrueckii* (Leichmann) Beijerinck, 1901; but it differs with described species *Lactobacillus delbrueckii* only sucrose fermentation.

Table 3. Morphological Biochemical and Cultural Characteristics of the Isolate No. 23

Place of collection	Chittagong, Bangladesh.
Vegetative Cells	Rod shaped
Size	0.7 – 1.5 x 2-5 µm
Spores	Non spore former
Gram stain	Gram negative
Acid-fast stain	Non acid fast
Flagella stain	Not done
Motility test	Motile
Agar colonies	Circular with light grey coloured
Agar slant	Echinulate growth
Nutrient broth	Flocculent growth
Glucose broth	Flocculent growth turbidity
Asparagine broth	Little sediment growth
Catalase activity	Positive
Oxidase test	Negative
Deep glucose agar	Moderate surface growth within the media
Oxygen relation	Facultative anaerobic
Indole test	Negative
Nitrate reduction test	Positive
Inorganic medium	No growth
Citrate medium	Positive
Gelatin liquefaction	Liquefied
Hydrogen sulphide (TSI)	Produced
Urease test	Negative
Coagulated egg albumin	Moderately proteolytic
Milk agar plate	Not hydrolyzed
Starch agar plate	Hydrolyzed strongly
Methyl red	Positive
V. P. test	Negative
Growth at	0^0C 10^0C 20^0C 30^0C 37^0C 40^0C 45^0C - ++ ++ +++ +++ ++ ++
Fermentation	Acid and gas form: Glucose, Mannkitol. Acid and no gas form: Arabinose, Maltose, Rhamnose, Xylose. No change: Lactose, Sucrose.

Identification: The morphological, cultural and biochemical characteristics of isolate No.23 was compared with standard description of 'Bergey's Manual of Determinative Bacteriology' - 8th Ed.[21] and found closely related with the genus *Salmonella*. The isolate was provisionally identified as *Salmonella bongori* (Reeves *et al,* 1989); but it differs with described species *Salmonella bongori* only in Sucrose and Lactose fermentation (no change).

Table 4. Morphological Biochemical and Cultural Characteristics of the Isolate No. 23B

Place of collection	Chittagong, Bangladesh.
Vegetative Cells	Coccoid to short rod shaped
Size	0.5-0.8 x 1-2 µm
Spores	Non spore former
Gram stain	Gram negative
Acid-fast stain	Non acid fast
Flagella stain	Not done
Motility test	Non motile
Agar colonies	Circular, opaque to yellow
Agar slant	Echinulate growth with yellow substrate colour
Nutrient broth	Pellicle growth
Glucose broth	Flocculent growth turbidity
Asparagine broth	Little sediment growth
Catalase activity	Positive
Oxidase test	Negative
Deep glucose agar	Heavy surface growth
Oxygen relation	Aerobic
Indole test	Negative
Nitrate reduction test	Negative
Synthetic medium	Scanty sediment growth
Citrate medium	Negative
Gelatin liquefaction	Liquefied
Hydrogen sulphide (TSI)	Produced
Urease test	Negative
Coagulated egg albumin	Strongly proteolytic
Milk agar plate	Not hydrolyzed
Starch agar plate	Hydrolyzed strongly
Methyl red	Positive
V. P. test	Negative
Growth at	0^0C 10^0C 20^0C 30^0C 37^0C 40^0C 45^0C - + ++ +++ +++ ++ ++
Fermentation	Acid and no gas form: Glucose, Fructose, Xylose, Maltose, Arabinose, and Inulin. No change: Sucrose, Galactose, Lactose, Rhamnose and Raffinose.

Identification:
The morphological, cultural and biochemical characteristics of isolate No. 23B was compared with standard description of 'Bergey's Manual of Determinative Bacteriology'-8th Ed.[21]and found closely related with the genus *Yersinia*. The isolate was provisionally identified as *Yersinia pestis* (Lehmann and Neumann) Van loghem,1944; but it differs with described species *Yersinia pestis* only in gelatin liquefaction and hydrogen sulphide production.

foods were kept within glass under lighting for long period of time. This condition is very dangerous because it can create an optimum growth temperature for food borne microorganisms and thus food could be spoiled and unsafe for consumption. During serving the handlers did not maintain proper hygienic condition, which might act as a major source of microbial contamination. The organisms gaining access to bakery foods was not only the indication of deterioration and spoilage of food but also a warning signal of the presence of many food borne pathogens, especially the gastrointestinal ones.

Government should pay attention in this matter. Action is required in the form of food inspection services supported by different analytical laboratories and health education of food handlers (Salam, 1994). The study has revealed many important issues relating to contamination of foods by pathogenic organisms. The most important factor that influences the contamination of food is personal hygiene. In all the cases, it was found that shoppers do not wash their hand properly more or less before serving foods (Table 1). Pathogens may exist on the finger tips that cannot be washed off simply by hand washing. These pathogens may along with other organisms be able to be transferred to the foods. They do not use enough soap or detergent for washing.

Conclusion

The bacteriological condition of different samples and their safety assessment revealed that most of the foods were unacceptable. The microbial safety of the investigated chicken sandwich depends not only on the environmental conditions but also on the personal hygiene. The study was confined only to certain shops in Chittagong city, so the result does not represent the whole country. Detailed study is required concerning more areas, increasing sampling sites and their numbers. For bacterial growth in bakery foods, storage duration plays an important and vital role, and long storage duration favors more bacterial growth, so always try to avoid the fast food storage for long time. As most bacteria are able to produce toxins, it is recommended for strict monitoring and certification of the bakery foods, hoping to maintain quality of bakery foods and ultimately to ensure good health.

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