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Evaluation and Screening of Bacterial contaminants Isolated from Bakery Products

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Abstract

Bakery products are a valuable source of nutrients in our diet. However, bakery products are subject to microbiological spoilage. The objective of the present study was to investigate the occurrence of bacterial contaminants in ready to eat bakery products collected from local market in Nagpur city. Bacterial contaminants were isolated from five different types of bakery products such as Bread, Salty biscuit, Sweet biscuit, Doughnut and Pastry. During the present study, the bacterial isolates obtained from the bread were mainly species of *Bacillus* such as *Bacillus subtilis* and *Bacillus megaterium*. A total of ten types of *Bacillus* spp. were isolated from five bakery products which includes *Bacillus subtilis* and *Bacillus megaterium*. From bread, Sweet biscuit, Salty biscuit and Doughnut both *Bacillus subtilis* and *Bacillus megaterium* were isolated while from Pastry only *Bacillus subtilis* was isolated. Antibiotic susceptibility of the bacteria was carried out by taking 5 different types of antibiotics discs on Mueller Hinton agar plates. It was observed that the organisms, *B. subtilis* and *B. megaterium* were found to be resistant to penicillin while sensitive against remaining tested antibiotics. However, *Bacillus megaterium* from pastry was found to be resistant to the tested antibiotics.

Keywords: Bakery Products, Bacterial Contaminants

Introduction:

Bakery products are an important part of a balanced diet and available on supermarket shelves. Bakery products are a valuable source of nutrients in our diet such as carbohydrates, proteins, lipids, vitamins and minerals. However, bakery products are subject to microbiological spoilage. Bakery products are prepared in sterile conditions but can be contaminated by exposure to airborne contaminants as well as utensils contact. Bakery industry in India is the largest of the food industries. Because of urbanization and increased demand for ready to eat products bakery products now become essential food items of the majority of population (Kent, 1983).

Bakery products are subjected to spoilage problems. These include physical, chemical and microbial spoilage. Since the most common factor of bakery products is water activity and microbiological spoilage (Saranraj and Geetha, 2012). Improper handling also introduces contamination as bakery workers are major source of contaminants. Frazier and Westhoff, (1988) found 96.3% positive bacterial culture from workers of a bakery industry. Foods are served after passing through a long chain of steps involved in production, processing, distribution and marketing.

According to Hunt and Robbins (2009), bakery products accounted for approximately 9 per cent of total food expenditure, with bread king the most important. The freshly baked bread is free of viable microorganism but is subject to contamination from the air, during cooling and before wrapping. During slicing, contamination may take place from microorganisms in the air, on the knives, or on the wrapper.

Members of the genus *Bacillus* bring about bacterial spoilage of bread known as rope. This is of major economic to the baking industry. Spores of bacteria able to cause ropiness in bread will survive the baking process. Ropiness is the most important spoilage of bread when the climatic conditions favour growth of bacteria. It is mainly caused by *Bacillus subtilis* but *Bacillus licheniformis*, *Bacillus magaterium* and *Bacillus cereus* have also been associated with ropy bread. Spoilage of bread by rope formation may constitute a health risk, high numbers of *Bacillus subtilis* and *Bacillus licheniformis* in foods may cause a mild form of food illness (Frazier and Westhoff, 1988; Adesetan et al., 2013).

All types of flour, especially wheat flour, are contaminated with *Bacillus* spores as a result of soil contamination (Pandey and Palni, 1997), cultivation and processing methods (Farmiloe et al., 1954; Voysey, 1989). Other origins of the *Bacillus* contamination have been reported to be raw materials and bakery equipment (Bailey and VonHoly, 1993; Rosenkvist and Hansen, 1995). The objective of the present study was to investigate the occurrence of bacterial contaminants in ready to eat bakery products collected from local market in Nagpur city.

Material and Methods:

Sample collection:

Five different types of bakery products such as Bread, Salty biscuit, Sweet biscuit, Doughnut and Pastry were collected from local bakery shops in Nagpur. The purchased samples were added in to sterile poly ethylene bag and transported to the laboratory for isolation of bacteria. The microbial analysis was done within 1-3 h of sample collection.

Isolation of Bacteria:

The serial dilution agar plate technique was used for the isolation of bacteria from bakery products. The Hicrome Bacillus Agar was used for isolation of bacteria. In the serial dilution agar plate technique, 1 g bakery product sample was suspended in 9 mL of saline solution and homogenized in the Erlenmeyer flask for about 5 min using shaker at 160 rpm. The homogenized sample was serially diluted 10^{-1} to 10^{-6}. A 0.1 mL of aliquot was spread plated on Hicrome bacillus Agar from appropriate dilutions and incubated at 35°C for 36 to 48 h (Acco et al., 2003).

Identification of Bacterial Isolates:

The isolated bacteria were identified on the basis of morphological, cultural and biochemical characteristics by using Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1974; Collee and Marr, 1996).

Antibiotic susceptibility of the Bacterial Isolates:

A total 6 different types of antibiotics (HiMedia Laboratories Pvt. Limited) were used in the present study (Table 1). Each *Bacillus* spp. was inoculated into nutrient broth and incubated at 35^{0}C for 8 hours. A sterile swab was dipped into the bacteria suspension, pressed on side of the test tube to allow excess drip-off, and used to evenly streak the entire surface of the Mueller – Hinton agar plate. The inoculum was allowed to dry for 5 minutes with lid in place. Sterile forceps then used to place the multiple antibiotic discs on the media plates and the plates were incubated at 37ºc for 18hours. After incubation, the zone of inhibition for each antibiotic was measured (Bauer et al., 1966).

Results and Discussion:

Bacterial contaminants were isolated from five different types of bakery products such as Bread, Salty biscuit, Sweet biscuit, Doughnut and Pastry. During the present study, the bacterial isolates obtained from the bread were mainly species of *Bacillus* such as *Bacillus subtilis* and *Bacillus megaterium*. A total of ten types of *Bacillus* spp. were isolated from five bakery products which includes *Bacillus subtilis* and *Bacillus megaterium*. From bread, Sweet biscuit, Salty biscuit and Doughnut both *Bacillus subtilis* and *Bacillus megaterium* were isolated while from Pastry only *Bacillus subtilis* was isolated (Table 2). Antibiotic susceptibility of the bacteria was carried out by taking 5 different types of antibiotics discs on Mueller Hinton agar plates. It was observed that the organisms, *B. subtilis* and *B. megaterium* were found to be resistant to penicillin while sensitive against remaining tested antibiotics. However, *Bacillus megaterium* from pastry was found to be resistant to the tested antibiotics.

The present findings correlated with the findings of Thompson et al., (1998); Ogundare and Adetuyi (2003) who also reported the presence of *B. subtilis*. Gram-positive bacteria were found to be more prevalent than Gram negative bacteria in bakery products. Species of *Bacillus* such as *B. subtilis* and *B. megaterium* have been reported to be the major cause of rope spoilage in breads (Smith *et al.*, 2004; Guynot *et al.*, 2005). *Bacillus* spp. have been reported to be the major source of contamination of bakery products during post-preparation handling which may lead to severe outbreak of food poisoning as a result of enterotoxin production by these bacteria, as reported by Smith *et al.* (2004). This may be due to two reasons owing to the poor hygienic conditions in the bakery and by not taking the proper care during post-preparation handling that might result in the contamination of the bread. *Bacillus* spp. was heat resistant and survives baking and under favourable conditions, grows to level associated with toxin production. The survival of spores during baking depends on the type of products, the internal temperature reached during baking, as well on the thermal resistance of the spores (Kaur, 1986).

Antibiotic susceptibility of the bacteria was carried out by taking 5 different types of antibiotics discs on Mueller Hinton agar plates. It was observed that the organisms, *B.subtilis* and *B. megaterium* were found to be resistant to penicillin while sensitive against remaining tested antibiotics. However, *Bacillus megaterium* from pastry was found to be resistant to the tested antibiotics (Table 3, 4). The ability of *Bacillus* species to resist dessication allows their survival on dried products such as cereal and flours. *B. cereus* is widely distributed in the environment and can be isolated from soil, water and vegetation (Adams and Moss, 1995). Isolation of *Bacillus subtilis* and *Bacillus cereus* from bakery equipments conforms to the work of (Viljoen and Holy, 1997) that isolated *Bacillus* species from equipment surfaces, air, raw materials, and hands of workers, and (Nazir and Islam, 2007) who isolated *Bacillus* and *Staphylococcus* from bakery foods in Bangladesh. *Bacillus* species was also isolated from flour and ropy bread as the main species involved in bread spoilage (Sorokoluva et al., 2003) and reported the source of contamination to be from raw materials and bakery equipment. Toxigenic *B. cereus* has been isolated from Nigeria flour based foods (Yusuf et al., 1992). A major source of

B*acillus* contamination is from raw ingredients (Holy and Allan, 1990).

Table 1: Antibiotics used in the study

Antibiotics	Abbreviation	Concentration
Gatifloxacin	GF	5 mcg
Penicillin G	P	10 units
Erythromycin	E	15 mcg
Clindamycin	CD	2 mcg
Ciprofloxacin	CF	5 mcg

Table 2: *Bacillus* Contamination in Bakery Products

Sample	*B. subtilis*	*B. megaterium*
Bread	+	+
Salty biscuit	+	+
Sweet biscuit	+	+
Doughnut	+	+
Pastry	+	-

Where, + = Present
- = Absent

Table 3: Antibiotics Resistance pattern of *B. subtilis* isolated from Bakery products

Samples	Gatifloxacin	Erythromycin	Clindamycin	Ciprofloxacine	Penicillin
Bread	27 mm	28 mm	23 mm	27 mm	R
Salty Biscuit	27 mm	19 mm	23 mm	28 mm	R
Sweet Biscuit	20 mm	28 mm	14 mm	18 mm	R
Doughnut	25 mm	25 mm	22 mm	25 mm	R
Pastry	19 mm	11 mm	12 mm	16 mm	R

Where, R= Resistant

Table 4: Antibiotics Resistance pattern of *B. megaterium* isolated from Bakery products

Source	Gatifloxacin	Erythromycin	Clindamycin	Ciprofloxacine	Penicillin
Bread	25 mm	25 mm	24 mm	26 mm	R
Salty Biscuit	24 mm	24 mm	23 mm	26 mm	R
Sweet Biscuit	26 mm	28 mm	16 mm	24 mm	R
Doughnut	29 mm	28 mm	31 mm	32 mm	R

Conclusion

In conclusion, the result of the study suggests that bakery products supplied by retailers are associated with a very low rate of bacterial contamination. The organisms like *B. subtilis* and *B. megaterium* were isolated from bakery products indicating poor sanitary control and measures and poor hygienic conditions.

B. subtilis are reported in all 5 different bakery products while *B. megaterium* was reported in 4 bakery products except pastry. Antibiotic resistance profile showed that both the bacteria *B. subtilis* and *B. megaterium* were found to be resistant to penicillin.

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