OCCURRENCE OF STAPHYLOCOCCUS AUREUS IN SOME PHILIPPINE FOODS AND ITS ENTEROTOXINS IN SMOKED FISH (SARDINELLA LONGICEPS C.V.)

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ABSTRACT

Some dried and salted Philippine foods analyzed for the occurrence of S. aureus yielded average counts of $8.2 \times 10^3 - 6.5 \times 10^4$ /g sample. Eighteen lots of market samples of smoked-cured takban (tinapa) were all found positive for S. aureus with four samples containing enterotoxin A in amounts ranging from $3.24 - 5.52 \mu g/g$ and one sample containing $5.08 \mu g/g$ enterotoxin D in addition to enterotoxin A. The presence of S aureus in a number of our dried and salted foods suggests the poor handling and storage methods given such commodities. Further, smoked fish, a commonly consumed product, can be a potential cause of food poisoning.

Introduction

Staphylococcus aureus Rosenbach continues to be one of the primary foodborne disease organisms despite extensive research on its growth requirements, epidemiology and control. Food poisoning caused by the organism is characterized by law mortality and relatively short duration, but the frequency of its outbreaks and the severity of its symptoms mark the disease as an important foodborne hazard. The true incidence of staphylococcal food poisoning is unknown locally since it is not a reportable disease. Most cases are never seen by a physician because of its relatively short duration. Only outbreaks involving large numbers of people as may occur at picnics, group dinners or public institutions are likely to establish the cause of illness.

The types of food most frequently implicated include ham, sausage, bacon and other cured meats, hamburger, bakery goods such as cream-filled pastries, custards, bread puddings, cakes and sandwiches, chicken and tuna salads, meat pies, potted meat, cheese, spray-dried eggs and milk, and precooked frozen foods. In the Philippines, dried and salted foods are either in plastic bags, covered jars, wooden boxes or in wide-mouth containers, with or without covers, and are stored at room temperature. Since the organism is tolerant to high sodium chloride (NaCl) and low water contents, holding the products at room temperature may allow the bacterium to multiply and produce toxin.

Smoke-cured Sardinella longiceps C.V. (tamban), commonly known as "tinapa", is a popular viand in the Philippines. It contains 4-5% salt and 54-60% moisture (Cabuslay, 1976; Guevarra, 1973); hence, could support growth of *S. aureus*. Shewan (1945b) showed that the smoking process is smoke-cured fish destroys the ordinary spoilage bacteria such as *Achromobacter* and *Pseudomonas*, but not the micrococci.

This study was undertaken to primarily determine the presence of S. aureus in some dried and salted Philippine foods, and the type(s) and amount of enterotoxin in market samples of "tinapa".

Materials and Methods

Salted shrimp fry (alamang) and fish (bagoong), dried fish (daing, dilis baot), dried squid (pusit), smoked fish (tinapa), fermented soybean (tahure), salted black beans (tausi), dried meat (tapa), and salted eggs all bought from the market were analyzed for the presence of *S. aureus*. The samples were prepared and plated following essentially the Association of Official Analytical Chemists (AOAC, 1970) methods. Cultures were examined for gram reaction, cell shape and arrangement, glucose and mannitol utilization and gelatin liquefaction. Subjected to the same tests were four enterotoxigenic strains of *S. aureus* (Food Research Institute (FRI)-100, FRI-472, S-6, 196E). These were supplied by Prof. M.S. Bergdoll, FRI, University of Wisconsin, Madison, Wisconsin, USA and were used as reference in characterizing the local isolates.

For the enterotoxin study, 18 lots of market samples were screened for the presence of coagulase positive *S. aureus*. Lots found positive in the screening were used in the extraction and detection of enterotoxin using the method of Fund (1972) and Fung and Wagner (1971). Standard curves for staphylococcal enterotoxins A, B and D, the toxins and their respective antisera likewise provided by Prof. Bergdoll, were also prepared using the above methods. The pH and moisture content of all lots and NaCl concentration of enterotoxin-containing samples were determined following the AOAC methods.

Results and Discussion

S. aureus was detected in all samples tested (Table 1) with numbers ranging from $8.2 \times 10^3 - 6.5 \times 10^4$ /g sample. Of the commodities analyzed, "tause" had the lowest count while "daing" had the highest count. Since there are no rigid standards in the manufacture of these food products, the numbers may vary from time to time depending on the processing, handling and storage of these commodi-

Sample Lot No.		No. of organisms		Coagulase		Production		Enterotoxin	
	Iso lates	per g sample x 10 ⁷	Reduction typel	Clothing time (min)	µg/g A	D	pН	Moisture %	NaCl ² %
1	А	4.2	4	20	4.62	_	6.00	59.46	6.21
2	В	4.0	3	40	_	_	5.75	59.27	_
3	С	5.1	_	_	_	_	5.95	55.22	_
4	D	4.9	_	-	_	_	6.1	61.53	
5	Ε	2.6	~		_	_	6.3	64.84	-
6	F	3.7	_		_	_	6.0	59.74	_
7	G	5.8	4	20	3.2	_	6.1	55.43	4.47
8	J	3.5	-	_	_	-	6.4	63.95	_
9	К	4.0	•	_	_	_	6.0	60.88	_
10	L	2.2	_	_	_	_	5.8	66.25	
11	Μ	4.4	_	_	_	_	6.0	53.21	_
12	Н	4.0	4	40	4.14	5.08	6.0	54.19	5.48
13	Ι	2.8	3	40	5.52	_	6.0	56.30	2.31
14	N	3.3	_	_	_	10110	6.3	59.68	_
15	0	4.6	_	_	_	_	6.0	57.49	-
16	Р	2.9	_	_	_	_	6.0	59.09	-
17	Q	4.6	_	~	_	_	6.1	57.36	-
18	R	3.5	_	_	_	_	5.9	58.94	-

Table 2. Some factors affecting growth and enterotoxin production of presumptive coagulase positive Staphylococcus aureus isolated from market samples of smoke-cured tamban

¹Extent of clotting of plasma interpreted according to types of reaction given by Turner and Schwarts (1958). ²Only toxin-containing samples were analyzed for NaCl content.

The pH of the samples ranged from 5.75-6.4, the moisture content from 53.21-66.25%, and the salt concentration from 2.31-6.21% (Table 2). On certain food products, *S. aureus* grew at pH values as low as 4.5-4.7 and as high as 9.5-9.8 although it produced enterotoxin at a narrower range of 5.0-9.0 (Tatini, 1973; Troller, 1976). Meat samples with 40-60% moisture also harbored staphylococci (Segalove and Dack, 1951) and sliced Canadian bacon with 0.3-3.2% salt supported enterotoxin production of *S. aureus* (Thatcher et al. 1962). It is evident thus that our smoke-cured *S. longiceps* for sale in the market could support the growth and enterotoxin production of *S. aureus*. We recommended that it be considered a semi-perishable food and that it be kept under refrigeration temperatures to prevent the multiplication and toxin production of *S. aureus*.

Summary

Market samples of some salted and dried foods were analyzed for the presence of *S aureus*. The numbers of *S. aureus* per gram of tested food sample after 72 hr. incubation at ambient room tempreature $(28^{\circ}C-30^{\circ}C \text{ on mannitol salt agar were 8.2 x 10^3, 1.3 x 10^4, 2.1 x 10^4, 2.4 x 10^4, 2.7 x 10^4, 3.2 x 10^4, 3.8 x 10^4, 4.3 x 10^4, 4.6 x 10^4, 5.4 x 10^4 and 6.5 x 10^4 for tause, pusit, salted egg, tahure, dilis, tapa, haot, bagoong, alamang, tinapa and daing, respectively.$

Eighteen lots of market samples of smoke-cured tamban were screened for the presence of coagulase positive and possibly enterotoxigenic strains of S. aureus. The staphylococci were present in all samples; however, only 27.8% of the isolates was coagulase positive.

Enterotoxin A alone was detected in three sample lots while both enterotoxins And D were present in one sample lot. All the other samples, including one lot which yielded coagulase-positive staphylococci did not contain enterotoxin A, B or D. The level of enterotoxin A ranged from $3.24-5.52 \ \mu g/g$ while enterotoxin D concentration was $5.08 \ \mu g/g$.

Chemical analyses of the samples indicated that the NaCl and moisture levels as well as the pH were well within the ranges that support growth and enterotoxin production of *S. aureus*. Samples which contained contained enterotoxin A had pH of 6.0-6.1 with 2.31%-6.21% and 5.419%-59.46% NaCl and moisture levels, respectively. Enterotoxin D was detected in smoke-cured tamban with pH of 6.0, 5.48% NaCl and moisture content of 54.19%.

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