BACTERIOLOGICAL INVESTIGATIONS OF PRAWN CANNERIES

I. INCIDENCE OF AEROBIC SPOREFORMERS

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A detailed bacteriological survey of the prawn canneries of Cochin area was carried out to study the nature and type of micro-organisms present in the factory environs and their role in causing contamination of the canned products. About 26% of the total of 1030 strains isolated were found to be gram positive spore-formers of the *Bacillus* type, the cooling water being their major source. Similar types of organisms formed the major group often met with in defective canned prawn samples picked up from the factories for examination, thus establishing a correlation between bacterial characteristics and load of cooling water and can contamination.

INTRODUCTION

One of the most important problems facing the canning industry is the bacterial spoilage of canned products. Fortunately, the actual incidence of spoilage of commercially canned foods is fairly low. But when it does happen, the identification of the type or types of the casuative microbes enables us in devising methods for their elimination. In actual practice, the bacterial contamination of the product can happen in more than one way. The various types of organisms harboured in the slime and guts of the fish find slow entry into the flesh after death. During subsequent processing, extraneous contamination is a possibility unless due care is bestowed. This extraneous contamination is mainly by bacteria associated with the surface of the utensils and equipments with which the material comes into contact during transportation and processing. Further, the water used at various stages of processing is also a potential source of contamination of the products.

Nambiar & Iyer (1970) have studied the bacterial flora of canned prawns.

They found gram positive spore-formers of the *Bacillus* type to be the predominating spoilers in these. Iyer *et, al* (1966) have shown that external contamination from unclean surfaces and other environmental conditions was a serious problem to be reckoned with in prawn processing industry. Amano (1947 a, b, c) made bacteriological investigations of the Japanese baby clam canneries and found that the blackening and swelling of canned products are related to bacterial contamination. Reimann (1957) showed that infection usually takes place during cooling of the cans after processing and since the cooling water was usually chlorinated so that all vegetative cells were killed, post-processing infection would consist mainly of spore-formers. The present study was undertaken to get a complete picture of the types of microorganisms present in the canning factory environs and also to find out the probable sources of contamination of canned prawns.

Materials and Methods

Prawn canning factories situated in and around Cochin formed the field centres for this study. Samples of raw material, water, ice, swab and processed cans were collected from the canning factories at frequent intervals. Raw material samples (samples before peeling, after peeling, after washing, after blanching and after grading) were taken under aseptic conditions in sterile petri dishes and brought to the laboratory under ice. Tap water, blanching solution, filling brine, water used for cooling the cans immediately after seaming, water used for cooling the cans after retorting and samples of ice used in the factories were taken under aseptic conditions. Swab samples were taken from the utensils, table surfaces and also from the interior of the cans before filling using sterile swabs and sterile buffered water (APHA, 1958). Processed cans were collected after the cooling process and incubated at 37°C for 10 days.

The samples were pour plated using Nutrient Agar (NA) with distilled water as diluent. The samples were also inoculated into Thioglycollate broth and the tubes were incubated at room temperature for 48 hours. In the case of processed cans, 1 ml of the filling brine was inoculated into Thioglycollate broth and incubated at room temperature for 48 hours. Typical well isolated colonies from the NA plates were transferred into Distilled Water Tryptone media Strains from the Thioglycollate broth were also isolated and purified. The pure strains were streaked on to NA slants and these cultures were used for further studies on morphology and biochemical characteristics (Nambiar and Iyer, *loc cit.*

Results and Discussion

Ten leading canning factories were included in the study. 29 samples of raw material, 110 swab samples, 160 water samples, 15 ice samples and 112 cans were analyzed. From these samples 1030 strains were isolated. The classification of the strains according to morphology and gram reaction is shown in Table I.

About 40% (410 numbers) of the strains were found to be motile. Biochemical characteristics like catalase production, reduction of nitrate to nitrite, production of indole, hydrogen sulphide and acetyl methyl carbinol, liquefaction of gelatin, hydrolysis of starch, utilization of citrate as sole source of carbon, growth at 56°C and fermentation of sugars viz; glucose, lactose, sucrose, mannitol and maltose were studied for all the 1030 strains. The results are tabulated in Table II. The gram positive sporeformers were classified into different species according to Bergey's Manual (1957) and

Table I Micro-organisms isolated from canning factory environments

Samples	No. of samples analysed	No. of strains isolated	Gram + ve spore-formers.	Gram + ve non-sporeformers.	Gram + ve cocci.	Gram —ve cocci.	Gram —ve rods.	Gram —ve coccoids
Raw material	29	72	13	10	9	18	20	2
Water	160	510	170	59	120	60	99	2
Ice	15	65	8	3	20	8	24	3
Swab	110	375	68	40	84	57	116	10
Cans	112	8	7	—	1	—	—	—
Total	426	1030	266	112	234	143	259	16

details given in Table III. Out of the 112 cans examined only 8 showed bacterial growth. Among the 8 strains isolated, 7 were gram positive sporeformers and 1 was gram positive cocci. The species-wise distribution of the gram positive spore-formers isolated from cans was: *Bacillus brevis* - 3; *B. pumilus* - 2; *B. firmus* - 1; and *badius* - 1.

Table II Biochemical characteristics of micro-organisms isolated from canning factory environments

Total strains studies 1030

Reaction	% of strains showing positive reaction
Catalase production	100.0
Reduction of nitrate to nitrite	59.2
Production of indole	3.3
Production of H_2S	43.2
Production of acetyl methyl carbinol	17.5
Liquefaction of gelatin	47.0
Hydrolysis of starch	27.7
Utilization of citrate as sole source of carbon	52.0
Growth at 56°C	22.0
Fermentation of sugars	
i) Acid alone	
a) Glucose	70.2
b) Lactose	24.5
c) Sucrose	62.4
d) Mannitol	40.0
e) Maltose	60.8
ii) Acid and gas	
a) Glucose	7.8
b) Lactose	4.6
c) Sucrose	7.5
d) Mannitol	7.2
e) Maltose	7.8

Table III Species-wise classification of gram+ve spore-formers

Total number isolated. 266

Species	Percentage
Bacillus subtilis	19.5
,, *pantothenticus*	18.4
,, *megatherium*	15.7
,, *brevis*	12.4
,, *pumilus*	7.5
,, *cereus*	4.5
,, *larvae*	3.4
,, *licheniformis*	3.1
,, *badius*	3.1
,, *firmus*	2.6
,, *pubvifaciens*	2.6
,, *circulans*	2.3
,, *lentus*	1.1
,, *pasteuri*	< 1.0
,, *macerans*	< 1.0
,, *anthracis*	< 1.0
,, *polymyxa*	< 1.0
Unidentified	< 1.0

From the results it is obvious that about 26% of the strains isolated from the factory environs are gram positive sporeformers of the *Bacillus* type. Majority of the spore-formers was isolated from the water samples, especially the cooling water. Table IV shows the results of analysis of cooling water samples. 33.7% of the strains isolated from cooling water samples were gram positive sporeformers. The cooling water samples are usually chlorinated so that almost all the vegetative cells are killed and this may lead to the predominance of gram positive spore-formers. Also, most of the processors use the same cooling water throughout the day with frequent addition of ice to keep it cool. Examination of cooling water samples at frequent intervals throughout a day have shown that the

Table IV Micro-organisms isolated from cooling water

Total samples analysed: 110

Total strains isolated: 285

Group	Number	Percentage
Gram+ve sporeformers	96	33.7
Gram+ve non-sporeforming rods	42	14.7
Gram+ve cocci	72	25.2
Gram—ve cocci	18	6.3
Gram—ve rods	57	20.0

percentage of gram positive spore-formers increased after each cooling process and subsequent addition of ice. Analysis of ice samples has shown that more than 12% of the strains isolated were gram positive spore-formers (Table I). Iyer and Chaudhuri (1966) have reported that ice formed a major source of contamination depending on the nature of water used for its preparation.

The swab samples from utensils, table surfaces and interior of the cans before filling mainly contained gram negative rods (about 31% of the total strains isolated from the swab samples). 22.4% of the strains were gram positive cocci, gram positive sporeformers constituting only 18.0%. Of the gram positive spore-formers, majority was from the interior of the cans. Improper washing of the cans before filling might be the reason for the predominance of gram positive spore-formers inside the cans.

The raw material samples contained less gram positive spore-formers. Majority of the strains isolated from raw material samples were gram negative rods (27.7%) and gram negative cocci (25.0%). In the case of filling brine, majority of the

strains isolated was gram positive non-spore-forming rods and gram positive cocci. These findings clearly show that the chances of contamination of the processed cans by gram positive spore-formers from the raw material and filling brine are rare.

Among the gram positive spore-formers isolated from factory environs, the predominating types were *Bacillus subtilis* (19.5%), *B. pantothenticus* (18.4%), *B. megaterium* (15.7%), *B. brevis* (12.4%) and *B. pumilus* (7.5%) (Table III). Similar types of organisms were found to predominate in bacteriologically defective cans also (Nambiar and Iyer, *loc cit*). This is an indication of the chances of contamination of cans from environs. The contamination can take place either before processing or during the process of cooling after processing. Heat resistance studies of the spores of 12 gram positive spore-formers isolated from defective cans have shown that none of them could withstand more than 10 minutes at 121°C, the usual processing temperature (Table V). Also, the raw material and filling brine samples were found to contain less gram positive spore-formers. These findings lead to the conclusion that the chances of contamination by gram positive spore-formers entering the cans before processing are rare, except where there is gross under-processing. Another important source of contamination is the cooling water. Bacteria present in the cooling water may enter the cans during cooling through defective seams. Our findings have shown the cooling water to be a potential source of gram positive spore-formers. Since gram positive spore-formers were found to be the predominating types in defective cans the most probable source of contamination of the cans seems to be the cooling water.

Table V Heat resistance of gram+ ve spore-formers

Number of strains studied:

Temperature	% of strains showing complete destruction within 5 min.	10 min.	15 min.	20 min.
115°C	16.6	66 6	91.6	100
121°C	83.3	100	—	—

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