PART II

SCIENTIFIC AND TECHNICAL

COMMON MICROFLORA INVOLVED IN SPOILAGE OF CANNED PRAWNS

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An elaborate survey was carried out to ascertain the common types of micro-organisms responsible for spoilage in canned prawns. Among nearly 1,500 strains isolated from bacteriologically defective cans, 60% were Gram positive sporeformers of the *Bacillus* type. Other types isolated belonged to Gram positive *cocci*, Gram negative rods, Gram positive non-sporeforming rods, Gram negative *cocci* and *coccoids*. No anaerobe could be isolated. The predominant Gram positive sporeformers were identified as *Bacillus pantothenticus*, *B. firmus*, *B. brevis* and *B. Pumilus* species.

INTRODUCTION

Considerable interest has developed in recent years in the food industry to determine the type of micro-organisms present in canned foods. Nikolaeva *et al* (1967) examined canned fish products for the presence of *Clostridium perfringens* and Chau-Ching-Lin *et al* (1968) studied the flatsour spoilage bacteria in canned *asparagus*. They found that *Bacillus stearothermophilus*, a highly heat resistant organism, was the cause of spoilage of canned *asparagus*.

The pattern and cause of bacterial contamination of canned prawns are the same as those in all heat processed canned foods viz; under-processing which leaves heat resistant, thermophilic spore-formers in the cans or leakage through seams which causes the entry into the cans of a wide variety of organisms of all types from air, water etc. The problem is of special interest to India particularly from the point of view of its export-oriented prawn canning industry. Though some aspects of this problem have already received attention in India, the different organisms involved in defective cans have not been so far studied. The present study therefore is aimed at providing a complete picture of bacterial flora usually associated with spoilage of prawns.

MATERIALS AND METHODS

Canned prawns collected from different factories were incubated at 37°C for one to two weeks. Cans showing visible signs

of spoilage through swelling were analysed without incubation. Before being opened, the cans were carefully examined for physical defects, particularly of the seams. After examination, the cans were cleaned with soap and water and rinsed with alcohol to remove the grease, if present. The site of opening was then sterilized by flaming or by treatment with alcohol. The heating was carefully distributed in such a way as to avoid overheating of the contents. In the case of badly swollen cans alcohol was used for sterilization. The point of a sterile opener was then inserted into the sterilized area and an opening was cut sufficiently large so as to enable a portion of the brine content to be withdrawn with a sterile pipette. Tubes containing 10 ml of thioglycollate broth and cooked meat medium were inoculated with 1 ml each of the brine. A small quantity of sterile liquid paraffin was added in the cooked meat medium after inoculation so as to form a layer over it. The inoculated tubes were then incubated at 37°C. The tubes were examined for visible growth after 24 and 48 hrs. Tubes with positive growth were analysed microscopically and the strains were isolated, purified and transferred on to Nutrient Agar (NA) slants. These NA slant cultures were used for further morphological and biochemical studies.

The Gram reaction was studied with a 24 hour old culture on NA slants. Motility was observed under the microscope with a young culture in Distilled Water Tryptone (DWT) media. Nitrate reduction and indole production were tested by growing the strains in a modified medium (DWT) having the composition, Tryptone (Difco), 1%; Sodium Chloride, 0.5%; Beef Extract (Difco), 0.3% and KNO_3, 0.2%; adjusted to pH 7.2. A portion of the culture was used for indole test and the other portion for nitrate reduction. With 24 hour old NA slant cultures catalase production was tested by pouring 1 ml of a 3% hydrogen peroxide solution over the surface of the growth. Hydrogen sulphide production was tested in Peptone-cystine-sulphate medium using lead acetate paper strips. The production of acetylmethyl carbinol was tested in glucose-phosphate-peptone media by the method of Vaughn *et al* (modification of Barrit test). The ability of the strains to utilize citrate as a sole source of carbon, to liquefy gelatin and to hydrolyse starch was also studied. Hydrolysis of starch was detected by a simplified method of Iyer, *et al* (1964). Fermentation of the sugars, glucose, lactose, sucrose, mannitol and maltose was studied and production of acid and gas observed. Sodium chloride tolerance and growth at 56°C were also determined in Nutrient Broth (NB). In all the media distilled water was used.

Results and discussion

Fifty pure strains were isolated from 46 cans showing visible spoilage by bulging, of which 22 were Gram positive spore formers (Table 1) belonging to the *Bacillus* type. The species-wise classification of the Gram positive spore formers was made according to the scheme provided in Bergey's Manual (1957). Other types isolated included 7 Gram positive non-spore forming rods, 8 Gram positive *cocci*, 7 Gram negative *cocci* and 6 Gram negative rods.

From cans which did not show bulging but which showed the presence of bacteria in them (as evidenced by positive growth in thioglycollate broth) 1436 pure cultures and 34 mixed cultures were isolated. Their classification according to Gram reaction and morphology is given in Table II.

The Gram positive spore formers were studied in detail and classified into different species as shown in Table III.

TABLE I SPECIES-WISE CLASSIFICATION OF GRAM POSITIVE SPORE FORMERS ISOLATED FROM SPOILED CANS

Species	*No. isolated*
Bacillus firmus	8
Bacillus megaterium	3
Bacillus licheniformis	2
Bacillus brevis	2
Bacillus pulvifaciens	1
Bacillus sphaericus	1
Bacillus badius	1
Bacillus larvae	1
Bacillus pantothenticus	1
Bacillus pumilus	1
Unidentified	1

TABLE II MICRO ORGANISMS ISOLATED FROM DEFECTIVE CANS

Type of micro organisms	No. isolated
Gram positive spore formers	815
Gram positive *cocci*	249
Gram negative rods	222
Gram positive rods (non spore forming)	79
Gram negative *cocci*	46
Gram negative & positive *coccoids*	25
Mixed cultures	34

More than 30 per cent of the spore formers showed growth at 56°C and about 80 per cent of them produced hydrogen sulphide. Tolerance to sodium chloride varied from 5 to 10%.

About 80 per cent of the strains isolated from cans were Gram positive spore formers of the *Bacillus* type. No anaerobe could be isolated. From the tables it can be seen that Gram positive *cocci* and Gram negative rods are next in the frequency of occurrence to Gram positive spore formers. Gram positive non-spore forming rods, Gram negative *cocci* and *coccoids* are rarely observed. In the case of mixed cultures, generally one strain

TABLE III

Species	No. isolated
Bacillus pantothenticus	144
Bacillus firmus	108
Bacillus brevis	96
Bacillus pumilus	86
Bacillus subtilis	50
Bacillus larvae	48
Bacillus cereus	39
Bacillus megaterium	30
Bacillus pulvifaciens	28
Bacillus licheniformis	26
Bacillus circulans	21
Bacillus pasteuri	16
Bacillus badius	15
Bacillus anthracis	12
Bacillus lentus	9
Bacillus sphaericus	5
Bacillus coagulans	3
Bacillus macerans	2
Bacillus alvei	1

was found to be a Gram negative rod. The other strain was either Gram positive spore former or Gram positive *cocci*.

Canned prawns are processed at high temperature in steam under pressure. This procedure has the effect of eliminating all bacteria except those having spores of exceptionally high resistance to heat. The presence of only spore forming organisms growing at 37°C and / or 55°C is generally indicative of underprocessing. Occurrence of spore forming as well as non-sporeforming organisms in cultures might be due to the cans bypassing the retort room without receiving adequate heat processing. The presence of a mixed flora of rods and cocci on microscopic examination and the isolation of these organisms on subculture is indicative of leakage. Spoilage due to leakage may be caused by excessive contamination of the cooling water or damage to the cans through rough handling.

The results indicate that in the bacteriologically defective cans, (which average about 0.3% of the total production) spore formers predominate though present in some cases with a mixed flora. Analysis of cooling water from canning factories where defects were observed also showed presence of spore formers. These observations point out that the cause of contamination of the cans could have been leakage through seams during the cooling process.

The results also show that only a few species of the *Bacillus* type, viz., *Bacillus pantothenticus, B. firmus, B. brevis, B. pumilus*, are frequently met with in the cans. From Tables I and III it is observed that certain common types of organisms occur in already spoiled cans and those which were not spoiled at the time of examination. This is an indication of their role in the spoilage of canned prawns.

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