SPRAY DRIED MILK OF THE SAKHA PLANT

II. IDENTIFICATION OF PREDOMINATING MICROORGANISMS

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ABSTRACT

The bacterial groups isolated at 30 C and 37 C from spray-dried milk proved to be streptococci, micrococci, microbacteria, and spore-formers in decreasing order. These bacteria were identified to species.

Initiation of the dry milk industry in U.A.R. has emphasized the importance of not only counting the microorganisms but also of determining their types. Enumeration of organisms belonging to the different groups was undertaken by Taha et al. *(21)*, while the complete identification is the subject of this report. The possibility of reconstituted milk being held for some time before use, and the implication of milk-containing foods in food poisoning of the enterotoxin type made it important that the types of surviving organisms which grow readily after reconstitution should be known.

Mattick et al. *(12)* found that spray dried milk contained streptococci, micrococci, and spore-formers in decreasing order. According to Thiel and Pont *(22)* spray-dried milk contained almost exclusively streptococci and only a few micrococci. Many fresh powders, especially spray-dried skim-milk, gave positive coliform tests with 2 g samples, whereas 0.1 g samples were almost uniformly negative. Richter *(18)* reported the absence of coliforms in 109 out of 155 samples of spray-dried milk. Cihova and Saxl *(5)* stated that enterococci (*Streptococcus faecium*) were the major component of the total microflora of 126 samples of dried milk. They added that *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, Bacillus cereus*, and *Bacillus megatherium* occurred most frequently among the sporulating flora.

MATERIALS AND METHODS

Bacterial identification

After recording the total viable counts at 30 C and 37 C for 35 nonfat spray-dried milk samples examined *(21)*, prevalent colonies were isolated, purified, and identified to species by the following schemes: *(a)* streptococci and microbacteria according to Bergey's Manual *(4)*; differentiation between *Streptococcus faecalis* and *S. faecium* according to Barnes *(3)*; *(b)* micrococci according to the scheme of Abd-el-Malek and Gibson *(2)*; *(c)* aerobic spore-formers according to Smith, Gordon, and Clark *(20)*; *(d)* coliforms as outlined by the Coliform Sub-Committee *(16)*.

RESULTS AND DISCUSSION

Predominance of bacterial groups (Table 1)

For the detailed study of the bacterial flora of spray-dried milk produced at the Sakha plant, 598 cultures were isolated. Of these 296 were taken from plates incubated at 30 C and 302 from plates incubated at 37 C. Results showed that the bacterial flora was comprised of streptococci, micrococci, microbacteria, and aerobic spore-forming bacilli in decreasing order. Out of the 296 cultures isolated at 30 C, 199 (67.2%) were found to be streptococci, 58 (19.6%) micrococci, 22 (7.4%) microbacteria, 10 (3.4 %) spore-forming bacilli, and 7 (2.4%) miscellaneous which were identified as sarcinae. Of the 302 cultures isolated from plates incubated at 37 C, 220 (72.9%) proved to be streptococci, 39 (12.9%) micrococci, 27 (8.9%) microbacteria, and 16 (5.3%) spore-forming bacilli.

The predominance of streptococci in spray-dried milk powder agrees with the findings of such investigators as Mattick et al. *(12)*, Thiel and Pont *(22)*, and Cihova and Saxl *(5)*. This could be due to the high pre-heating temperature (90 C) used at the Sakha plant. Also, Hiscox *(10)* and Mattick et al. *(12)*

TABLE 1. PERCENTAGE DISTRIBUTION OF THE BACTERIAL GROUPS ENCOUNTERED IN SPRAY DRIED MILK POWDER WHEN PLATES WERE INCUBATED AT 30 C AND 37 C

	30 C		37 C	
Microorganisms	No. of isolates	%	No. of isolates	%
Streptococci	199	67.2	220	72.9
Micrococci	58	19.6	39	12.9
Microbacteria	22	7.4	27	8.9
Spore-forming bacilli	10	3.4	16	5.3
Miscellaneous	7	2.4	—	—
Total	296	100.0	302	100.0

TABLE 2. PERCENTAGE DISTRIBUTION OF STREPTOCOCCI ISOLATED FROM SPRAY DRIED MILK WHEN PLATES WERE INCUBATED AT 30 C AND 37 C

Streptococci	30 C		37 C	
	No.	%	No.	%
Enterococcus group	119	59.8	142	64.8
S. faecium	(106)	(89.1)	(142)	(100.0)
S. faecalis	(13)	(10.9)	(–)	(–)
Viridans group	73	36.7	65	29.7
S. bovis	(47)	(64.4)	(14)	(21.5)
S. thermophilus	(26)	(35.6)	(51)	(78.5)
Lactic group				
S. cremoris	7	3.5	12	5.5
Total isolates	199	100.0	219	100.0

TABLE 3. PERCENTAGE DISTRIBUTION OF MICROCOCCI ISOLATED FROM SPRAY DRIED MILK WHEN PLATES WERE INCUBATED AT 30 C AND 37 C

	30 C		37 C	
	No.	%	No.	%
Dairy micrococci	51	87.9	30	76.92
M. luteus	(38)	(74.5)	(30)	(100)
M. varians	(13)	(25.5)	(–)	(–)
Staphylococcus sub-group D	4	6.9	6	15.4
Intermediate group	3	5.2	3	7.7
Total	58	100.0	39	100.0

attributed the predominance of streptococci at both 30 C and 37 C to the high pre-heating temperature used. They found that at 87.7 C and 93.3 C, streptococci tended to predominate over other types of organisms.

Micrococci were second in order to streptococci, as also reported by Hiscox *(10)* and Mattick et al. *(12)*.

Microbacteria, the next most prevalent group, were round in comparatively lower numbers. Similar results were obtained by Hiscox *(10)* and Mattick et al. *(12)*. The presence of microbacteria in relatively low numbers could also be attributed to the high pre-heating temperature (90 C) used at the Sakha plant, which is apparently near the limit of their heat resistance and consequently few survived the heating process *(10)*. The presence of microbacteria on plates incubated at 37 C had been previously shown by Hiscox *(10)*, Nashif and Nelson *(15)*, Thomas and Thomas *(23)*, Sabbour *(19)*, Robinson *(17)*, and Jayne-Williams and Skerman *(11)*. The latter authors concluded that the inability of microbacteria to grow at 37 C was not suitable for differential purposes. In this respect a taxonomic study of the genus *Microbacterium (Microbacterium lacticum, Microbacterium flavum,* and *Microbacterium liquefaciens)* carried out by Robinson *(17)*, verified that these bacteria grow in the range 30 C and 37 C, and also certain strains grow well at 39 C.

Aerobic spore-forming bacilli were less frequently encountered and were isolated in small numbers in 16.7% and 8.5% of the samples at 30 C and 37 C, respectively. These low figures possibly resulted from testing the samples within a few hours of their manufacture, since Crossley and Johnson *(6)* found that on prolonged storage, spore-forming bacilli tended to predominate.

Identification of microorganisms

Streptococci (Table 2). At 30 C, 199 cultures were isolated. The enterococcus group predominated and 119 cultures (59.8%) were identified as *Streptococcus faecalis* and *S. faecium (3)*. The latter was represented by 106 cultures (89.1%) against 13 cultures (10.9%) of the former. The viridans group comprised 73 cultures (36.7%) of which 47 (64.4%) were identified as *Streptococcus bovis* and 26 (35.6%) as *Streptococcus thermophilus*. The lactic group was far less common only comprising 7 cultures (3.5%) which were identified as *Streptococcus cremoris*.

At 37 C, 219 cultures of streptococci were isolated. Of these, 142 cultures (64.8%) were enterococci identified as *S. faecium*. The viridans group comprised 65 cultures (29.7%) of which 51 (78.5%) were identified as *S. thermophilus* and the remaining 14 (21.5%) as *S. bovis*. The lactic group was far less frequently encountered, comprising 12 cultures (5.5%), identified as *S. cremoris*.

It is clear that the streptococci isolated at 30 C and 37 C belonged to the enterococcus, viridans, and lactic groups in descending order. The enterococci predominated, and *S. faecium* was predominant over *S. faecalis*. Similar results were obtained by Hashimoto *(7)* and Hashimoto et al. *(8)* who attributed this phenomenon to the comparative thermostability of *S. faecium* over *S. faecalis*.

Streptococcus thermophilus and *S. bovis*, representing the viridans group, were isolated in fewer numbers than those of the enterococcus group. This is in accordance with the work of Naguib *(14)*. The presence of *S. thermophilus* in dried milk was reported by Crossley and Johnson *(6)* and Higginbottom *(9)*.

Far less frequently encountered was *S. cremoris* representing the lactic group. Similar results were reported by Naguib *(14)*.

Micrococci (Table 3). Results showed the predominance of the dairy micrococcus group over the other two groups, namely the staphylococcus and the intermediate groups.

At 30 C, out of the 58 isolates, 51 cultures (87.9%) were identified as dairy micrococci of which 38 (74.5%) were found to be *Micrococcus luteus* and 13 (25.5%) *Micrococcus varians*. *Staphylococcus* subgroup D was represented by 4 cultures (6.9%), and the intermediate group by 3 cultures (5.2%). This clearly shows the predominance of *M. luteus* over the other species encountered.

At 37 C, 39 cultures of micrococci were isolated. The dairy micrococci comprised 30 cultures (76.9%) and were identified as *M. luteus*. Six cultures (15.4%) were identified as *Staphylococcus* subgroup D and 3 cultures (7.7%) as intermediate. The presence of micrococci in milk powder was also shown by Mattick et al. *(12)*, Hiscox *(10)*, Thiel and Pont *(22)*, and Naguib *(14)*.

Microbacteria. Twenty two cultures were isolated at 30 C and 37 cultures at 37 C and were identified as *M. flavum*.

Spore-forming bacilli. Ten cultures were isolated at 30 C, of which 6 (60%) were *Bacillus brevis* and 4 (40%) *B. pumilus*. At 37 C, 16 cultures were isolated. Out of these 10 (62.5%) were *B. pumilus* and the remaining 6 (37.5%) were identified as *B. brevis*. The presence of *B. pumilus* in dried milk was also shown by Naguib *(14)* and Cihova and Saxl *(5)*.

Coliforms. All the 180 cultures of the coli-aerogenes group isolated during this investigation were identified as *Escherichia coli* type 1. The presence of coliforms in dried milk was also shown by Thiel and Pont *(22)* and Richter *(18)*.

In view of the fact that all strains isolated proved to be of the non-thermoduric type (resistance to 63 C for 30 min) their presence in the dried milk is an indication of post-heating contamination *(1, 12, 13)* resulting from improper cleaning and sterilization of post-heating equipment *(1)*.

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REPORT OF THE EDITOR JOURNAL OF MILK AND FOOD TECHNOLOGY 1970-1971

Review of Volume Thirty-Three

Publication of the December, 1970 issue of the *Journal of Milk and Food Technology* completed volume 33. Volume 33 established new records for the Journal. It was the largest volume (688 pages) ever published and represented a 10% increase in size over volume 32 and a 34% increase over volume 30 published in 1967. The single largest issue (68 pages) ever published appeared in volume 33; in fact, four issues of this volume each contained 68 pages. More papers of all kinds (104) and more research papers (66) were published than appeared in previous volumes. The number of research papers published in 1970 represents an increase of approximately 40% over 1969 and 120% over 1967. The number of technical general interest papers in volume 33 also was greater than in recent years and occupied approximately 14% of the total pages in the journal. Twenty non-technical general interest papers appeared in volume 33. This number compares well with that of recent years. Publication of a greater number of research papers means that readers are better informed on current developments in the food and dairy fields. Details on the composition of volume 33 and a comparison to other volumes appear in Table 1.

As in the recent past, a wide variety of topics were considered by papers which appeared in this volume of the journal. Rather than to enumerate all the subjects, it perhaps is more meaningful to indicate that approximately 41% of the papers dealt with subjects related to dairy foods, 42% discussed topics dealing with other foods, and 17% were concerned with environmental or other matters.

Present Status of Volume Thirty-Four

The first six issues of volume 34 contained 36 research papers, 11 technical general interest papers, and 4 non-technical general interest papers. This compares with 25, 7, and 8 papers in the same catagories for the first six issues of volume 33. In addition, the March and June issues each contained 14 pages of 3-A or E-3-A standards.

On July 15, 1971 there was a backlog of 42 papers awaiting publication. This included 20 research papers, 16 technical general interest papers, and 6 non-technical general interest papers. In addition, on July 15 there were 12 research papers, 1 technical general interest paper, and 1 non-technical general interest paper being reviewed or revised. The backlog is somewhat less than in 1970 at this time because somewhat fewer pages in volume 34 than in volume 33 have been devoted to standards and 36 additional pages over those in volume 33 have thus far appeared in volume 34.

Review Papers

Volume 33 contained review papers on a number of timely topics including: detection of microorganisms in foods, perfringens food poisoning, the salmonella problem, egg processing technology, textured vegetable proteins, nitrates in plants and waters, activation of bacterial spores, microorganisms in raw plant foods, and environmental carcinogens.

Thus far, review papers on the following subjects have appeared in volume 34: microbiology of poultry products, changing patterns in food production and processing, solid waste disposal, bacteriological testing of milk, and fecal contamination of fruits and vegetables. In addition, review papers awaiting publication deal with the following topics: 50 years of progress in the cheese industry, guidelines for production of non-carcinogenic food additives, *Vibrio parahaemolyticus*, staphylococcus food poisoning, sodium in foods and beverages, and perfringens enterotoxin.

A number of scientists have expressed either an interest in or willingness to prepare review papers on the following topics: lead in food and the environment, the propionic acid bacteria, ecology of the lactic acid bacteria, process cheese, mercury in food and the environment, Swiss cheese flavor, machine milking and udder health, effects of abnormal milk on processing, *Bacillus cereus* food poisoning, thermal properties of foods and heat processing, and heat resistant molds of importance in foods. It must be remembered, however, that people who agreed to prepare reviews are all busy with their daily tasks. Hence, some time may elapse before all of the reviews become available for publication.

Editorial Board

The Editorial Board has been expanded to include 41 scientists in university, industry, and government laboratories. The following persons were added to the Editorial Board in 1970 or early in 1971: J. A. Alford, F. L. Bryan, W. J. Dyer, H. R. Groninger, N. F. Insalata, R. T. Marshall, S. A. Matz, E. M. Mikolajcik, R. L. Olson, Z. J. Ordal, J. W. Pence, H. J.

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