Microbiology of Foods and Food Processing

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PREFACE

There are many books texts and monographs, which deal with the subject of microbiology and especially with bacteriology. There are also some books which cover the subject of microbiology of foods and others which are specifically concerned with food-borne diseases. Few, if any single publication, consider the subjects of the microbiology of foods, the microbiology of food processing, and the microbiology of food-borne diseases. In this volume we have attempted to tabulate the pertinent information concerning these three subjects since we believe that knowledge in these areas is most valuable to food handlers, especially those who are concerned or will be concerned with food handling outside of the home.

It is believed that this book will be of interest and provide valuable information to those who supervise the various aspects of food manufacturing, the catering of foods, or the preparing and serving of foods in restaurants or institutions. Students of Food Science or Technology and students of Hotel Management or Home Economics will also find that the information in this publication is directly concerned with their field of endeavor. No attempt has been made in this volume to tabulate all of the information available in all of the subjects covered. This would not be possible. For instance, separate volumes have been written on thermal processing, the freezing of foods, food-borne diseases, and other subjects covered in this book. However, we believe that sufficient information is given herein to provide for the understanding of derivations and application of methods, for the prevention of food spoilage, and for the use of procedures which would tend to prevent food-borne diseases.

This volume is different from others in the general field in that it covers a wider area of subject matter without including a large amount of material which is interesting but not absolutely essential to the understanding of the various subjects. It is also different in that it covers a wider variety of subjects than is usually attempted in a book of this kind.

For the use of selected material which appears in this book a special note of appreciation is due to the following: The American Public Health Association, Inc. for the MPN tables; Dr. G. G. Knock, *Journal of Scien-*

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This book is dedicated to better foods and better health.

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CHAPTER 1

Methods Used for the Microbiological Examination of Foods

The methods described in this Chapter are those that would be applicable to the microbiological examination of foods as required for most purposes. It is obvious that all microbiological procedures which might be applied to foods cannot be included. Actually, the methods which have been detailed are mainly bacteriological, since foods are most often spoiled because of bacterial growth, and the common types of food-borne disease are caused by bacteria. However, food spoilage is sometimes caused by yeasts or molds, and molds or viruses may cause some types of food-borne disease. Procedures to determine the presence of yeasts and molds, or their end products, have, therefore, been described.

Sampling of Foods for Microbial Testing

One of the problems associated with the testing of foods for microbial contamination of any kind has been and continues to be the question of how many samples should be examined from a particular lot and also what constitutes a particular lot.

For some types of production past experience has shown that this may be worked out in the plant rather simply by trial and error. For instance, it is the practice in canning plants to set aside 12 cans from each canning line taken 15–20 min after the start of the operation and 12 cans from each line near the end of the day's run (six at each time for No. 10 can sizes). The cans from one-half of each group are incubated at 30°C (86°F), for 10 days and the other half at 50°C (122°F), for 7 days. They are examined daily for swells, and bacteriological examinations of the swelled cans are then normally conducted. At the end of incubation periods all cans are opened and checked for spoilage odors, discoloration, and pH. The spoiled products are examined microscopically (National Canners Association, 1956).[19]

In some plants producing precooked frozen foods in which the product was produced in 400-gal lots by batch processing, it has been found that, if the last carton filled from each batch were examined microscopically and the total number of viable organisms were also determined, a good evaluation of the bacteriological quality could be obtained.

When microbiological sampling of lots of product already produced is to be carried out, as by regulatory agencies, the problem of sampling becomes quite complex. Some suggestions have been made regarding the sampling of such lots. These suggestions are in the following list (Thatcher, 1963):[23]

Canadian Food and Drug Directorate	5 packs per lot or the square root of the number of samples in the lot
England—Imported foods	5 to 10% of the number of packs in the lot
Association of Food and Drug Officials of the U.S. (for precooked frozen foods)	10 packs per lot

It would appear that the AFDOUS system of sampling lots may be more realistic. The examination of only five units per lot would seem insufficient to provide adequate information as to the bacteriological quality. With some lot sizes the square root of the number of units would require a high percentage loss of product and with other lot sizes excessive laboratory work, although the percentage loss decreases as the number of samples in the lot increases. The testing of 5–10% of the units in a lot seems entirely unrealistic both from the standpoint of loss of product and the use of personnel and materials. The "Production Lot" has been defined by AFDOUS as follows: "Refers to a plant's designation as such, usually all of the units of a product of the same size produced under essentially the same condition" (Association of Food and Drug Officials of the United States, 1966).[3] This definition seems to be lacking in specificity and might allow, under some interpretations, days or weeks of production units to be considered as components of the same lot. Under such circumstances the examination of 10 units per lot would not provide for an adequate or representative sampling of production units.

The Plate Count

The equipment and materials required for plate-count techniques include diluent in bottles of 90 or 99 ml, or in tubes of 9 ml, a high-speed blender if

solid food is to be examined, pipets 10, 1, and 0.1 ml, petri dishes which may be glass or plastic, a molten culture medium, and an incubator which will maintain the required temperature ± 1°C (1.8°F). In counting colonies on plate cultures a Quebec-type colony counter is ordinarily employed.

All materials contacting the sample in any form, blender tops, bottles, stoppers, tubes, pipets, diluent, and media, must be free from viable microorganisms when used. It is preferable to precool the diluent to 3°C (37.4°F) to prevent excessive heating during blending and to prevent growth of bacteria during plating. Butterfield's phosphate buffer (Butterfield, 1933)[7] or a 0.1% peptone solution are recommended as diluents. Such solutions minimize the dying off of bacteria during plating. The medium should be poured into plates at 44–46°C (111.2–114.8°F); otherwise, it will be too cold and lump during pouring or too hot and destroy some of the heat-labile bacteria. Also, hot media will cause condensation of moisture on the underside of the top of the petri dish which may drip and facilitate the growth of spreaders. Normally, the media is held in a water bath, regulated at the temperatures stated, until used.

The number of bacteria present in foods is usually so large that in order to obtain discrete colonies on petri dish cultures the food must be cultured after dilution. If solid, the food must be diluted for purposes of testing in any case, since it is not possible to make plate counts on solid food and even the plating of low dilutions (1:10) often causes difficulties in distinguishing food particles from colonies.

The first dilution, usually 1:10, may be prepared by placing 10 ml of the food in 90 ml of diluent (11 ml in 99 ml may also be used) if the food is liquid. If solid or semisolid, a 1:10 dilution of the product should be made by weighing a portion into a sterile blender top, adding the required amount of dilution water and blending at high speed for 2–3 min. Portions of 50 g of food and 450 ml of diluent are often used for this purpose. Serial dilutions are made from the 1:10 dilution by adding 10 or 11 ml of this to 90 or 99 ml of diluent (1:100 dilution), 1 ml to 99 ml of diluent (1:1000 dilution) and so on. Dilutions not agitated by blending should be shaken mechanically or by hand (with the hand moving from the shoulder to a horizontal position 25 times) prior to transfer. Either some knowledge of the number of bacteria in the product is required or several dilutions of the product must be plated in order to prepare cultures in such a manner as to have countable numbers of colonies on petri dish cultures after incubation.

The plate culture is usually prepared in one of two ways. In the regular method 1 ml of various dilutions of product is transferred to each of two separate petri dishes after which approximately 15 ml of the molten agar [illegible] is added to each dish. After gentle mixing of the bacterial suspen-

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sion with the medium, the agar is allowed to solidify. After solidification the plate cultures are inverted to prevent condensation of moisture on the surface of the agar medium during incubation.

In some cases surface counts are made, especially when the predominant flora in the food is strictly aerobic. In such instances the culture medium is first poured into petri dishes and allowed to cool and solidify in such a manner as to prevent condensation of moisture on agar surfaces. These plates are then preincubated to make certain that they have not become contaminated (no colonies on surfaces). In plating, usually 0.1–0.3 ml of the appropriate dilution of the food being analyzed is pipetted onto the surface of the solid culture medium. A sterile bent glass rod (glass hockey stick) which has been previously treated with silicone (to prevent the adherence of bacteria to the rod) is then used to distribute the sample over the entire surface of the medium.

Plate count agar (Difco) or Milk Protein Hydrolysate agar (Difco) have been recommended as culture media for plate counts, but Eugon agar (Difco, BBL) both with or without supplementation with yeast extract, and trypticase agar (a product of BBL) have been found to give higher counts for some foods such as certain kinds of fish. Therefore, it is usual to evaluate the efficiency of various media for a particular food.

Incubation temperatures between 32 and 37°C (89.6 and 98.6°F) have been recommended for plate-count cultures, but 35 ± 1°C (95 ± 1.8°F) is probably most often used. Incubation periods of 48 ± 3 hr are commonly employed (American Public Health Association, Inc., 1960).[1] In making counts on flesh-type foods, the spoilage flora are normally psychrophilic. Higher counts, therefore, can often be obtained by incubating at 20°C (68°F) for approximately 120 hr.

Plate counts should be reported only from plates containing 30–300 colonies. The count is reported as the number of colonies (average of two plates) times the dilution from which the plate cultures were made. Only the first two significant figures are reported, thus, a count of 261 through 264 would be 260, and a count of 265 through 270 would be 270. In cases in which two consecutive dilutions provide plate cultures with between 30 and 300 colonies, they may be averaged according to the count at each dilution provided that the highest count obtained at one dilution is not more than double that of the lower count obtained with the other dilution; otherwise, the lower count is used as such. In those instances in which all dilutions provide plate cultures with more than 300 colonies, the number of colonies should be determined as nearly as is possible and the average number per plate times the dilution reported as an estimate.

Tests for Coliform Bacteria in Foods

In water the quality standards are such that, in effect, the presence of more than one organism of the coliform group per 100 ml is considered as indicating that the water is not potable. In foods, coliform bacteria as a group do not have the same significance as with water. Coliform standards have been set up for some foods, such as raw certified and pasteurized milk (U.S. Department of Health, Education, and Welfare, 1953)[25] and precooked foods used by some military groups (U.S. Army Natick Laboratories, 1965),[24] but these standards are much more lenient than those for water. Many bacteriologists consider that the results of tests for the coliform group in foods have little significance and that the presence of fecal coliforms *(Escherichia coli)* only should be used to evaluate sanitary quality. Even fecal coliform standards for foods are in some instances (U.S. Department of Health, Education, and Welfare, 1953)[25] much more lenient than those for coliforms in water.

Tests for coliforms include presumptive tests, confirmation tests, and completed tests. Recommended methods for the first two tests are commonly applied and agreed upon; however, recommendations for completed tests vary from none to the application of methods which go beyond the regulation completed tests; which is the utilization of the IMViC reactions (Tests to determine production of: indole; acid, as indicated by methyl red; 2,3 butylene glycol, and growth in Koser's citrate broth or utilization of citrate).

Presumptive Tests

Plating Method. In using the plating method as a presumptive test, 1 ml of appropriate decimal dilutions of the water or food is added to petri dishes in duplicate, molten violet red bile agar (Difco, BBL) is added, mixed, and then allowed to harden in the manner described for plate counts. Plate counts are incubated at 35 ± 1°C (95 ± 1.8°F) for 18–24 hr. Positive results are indicated by dark-red colonies with a surrounding zone of precipitated bile at least 0.5 mm in diameter. A dark-field type of Quebec colony counter is recommended for counting colonies. As in plate counting, the number of colonies per plate culture is multiplied by the reciprocal of the corresponding dilution to determine numbers per gram of product.

Plating methods when used as presumptive tests have the disadvantage that low numbers of coliforms cannot always be identified by this procedure

since it is not possible to plate out undiluted solid materials in such a manner as to obtain a count, and small numbers of coliform may be overgrown by large numbers of other types of bacteria.

Most Probable Number (MPN) Method. The medium used for this procedure is lauryl sulfate tryptose broth (L fço). Quantities of 10 ml of broth are placed in each tube, and a small inverted vial is also placed in each tube in such a manner that no air bubbles are present in the vial after sterilization of the medium. If 10-ml portions of a particular dilution or of liquid food or water are to be added to a series of tubes, double-strength medium is used for that particular dilution. No fewer than three tubes may be used per dilution, but five tubes per dilution is recommended. The ordinary procedure is to add 10 ml of a dilution of the material being examined or 10 g of product to the first series of five tubes, 1 ml or 1 g to the next series of five tubes, and 0.1 ml or 0.1 g or the equivalent to the next series of five tubes. Tube cultures are incubated at 35 ± °C (95 ± 1.8°F) for 48 hr and examined for gas formation after 24–48 hr of incubation.

The presumptive MPN is determined by selecting the number given in Table 1–1 (Haskins, 1933)[15] corresponding to the number of positive tubes. For instance, if in 10 ml five were positive, in 1 ml four were positive, and in 0.1 ml three were positive, the number in MPN tables corresponding to five, four, three would be selected. The most probable number of coliforms per gram of sample may then be determined as follows:

$$\frac{\text{Number from tables}}{100} \times \text{ dilution factor of middle series of tubes} = \text{MPN/gram.}$$

A membrane-filter technique may be used as a presumptive test for coliforms, but this is applicable mainly to water.

The presumptive test for coliforms does not constitute absolute identification of these organisms. All positive presumptive tests for coliforms should be confirmed by further testing if the results are to be considered as valid.

Confirmation of Presumptive Tests for Coliforms

If violet red bile agar has been used as a presumptive medium, select six representative colonies and transfer with a sterile inoculating needle from the center of each colony to a separate tube of brilliant green lactose bile broth. The broth should be in 16 × 150-mm tubes with an inverted vial (10 × 75 mm). A volume of 10 ml of broth should be used per tube, and after sterilization no air should be present in the inverted vials. If the MPN technique using lauryl sulfate tryptose broth has been used as a presumptive test, three loopfuls are transferred from each gas-positive tube of the three

TABLE 1-1

Table of Most Probable Numbers (MPN) of Coliform Bacilli per 100 ml of Sample (Using Five Tubes with 10, 1, and 0.1 Volumes[15]

Positive[a] 10 1 0.1	MPN[b]	*Positive*[a] 10 1 0.1	MPN[b]	*Positive*[a] 10 1 0.1	MPN[b]	*Positive*[a] 10 1 0.1	MPN[b]	*Positive*[a] 10 1 0.1	MPN[b]	*Positive*[a] 10 1 0.1	MPN[b]
0 0 0	0	1 0 0	2	2 0 0	4.5	3 0 0	7.8	4 0 0	13	5 0 0	23
0 0 1	1.8	1 0 1	4	2 0 1	6.8	3 0 1	11	4 0 1	17	5 0 1	31
0 0 2	3.6	1 0 2	6	2 0 2	9.1	3 0 2	13	4 0 2	21	5 0 2	43
0 0 3	5.4	1 0 3	8	2 0 3	12	3 0 3	16	4 0 3	25	5 0 3	58
0 0 4	7.2	1 0 4	10	2 0 4	14	3 0 4	20	4 0 4	30	5 0 4	76
0 0 5	9	1 0 5	12	2 0 5	16	3 0 5	23	4 0 5	36	5 0 5	95
0 1 0	1.8	1 1 0	4	2 1 0	6.8	3 1 0	11	4 1 0	17	5 1 0	33
0 1 1	3.6	1 1 1	6.1	2 1 1	9.2	3 1 1	14	4 1 1	21	5 1 1	46
0 1 2	5.5	1 1 2	8.1	2 1 2	12	3 1 2	17	4 1 2	26	5 1 2	64
0 1 3	7.3	1 1 3	10	2 1 3	14	3 1 3	20	4 1 3	31	5 1 3	84
0 1 4	9.1	1 1 4	12	2 1 4	17	3 1 4	23	4 1 4	36	5 1 4	110
0 1 5	11	1 1 5	14	2 1 5	19	3 1 5	27	4 1 5	42	5 1 5	130
0 2 0	3.7	1 2 0	6.1	2 2 0	9.3	3 2 0	14	4 2 0	22	5 2 0	40
0 2 1	5.5	1 2 1	8.2	2 2 1	12	3 2 1	17	4 2 1	26	5 2 1	70
0 2 2	7.4	1 2 2	10	2 2 2	14	3 2 2	20	4 2 2	32	5 2 2	95
0 2 3	9.2	1 2 3	12	2 2 3	17	3 2 3	24	4 2 3	38	5 2 3	120
0 2 4	11	1 2 4	15	2 2 4	19	3 2 4	27	4 2 4	41	5 2 4	150
0 2 5	13	1 2 5	17	2 2 5	22	3 2 5	31	4 2 5	50	5 2 5	180
0 3 0	5.6	1 3 0	8.3	2 3 0	12	3 3 0	17	4 3 0	27	5 3 0	79
0 3 1	7.4	1 3 1	10	2 3 1	14	3 3 1	21	4 3 1	33	5 3 1	110
0 3 2	9.3	1 3 2	13	2 3 2	17	3 3 2	24	4 3 2	39	5 3 2	140
0 3 3	11	1 3 3	15	2 3 3	20	3 3 3	28	4 3 3	45	5 3 3	180
0 3 4	13	1 3 4	17	2 3 4	22	3 3 4	31	4 3 4	52	5 3 4	210
0 3 5	15	1 3 5	19	2 3 5	25	3 3 5	35	4 3 5	59	5 3 5	250
0 4 0	7.5	1 4 0	11	2 4 0	15	3 4 0	21	4 4 0	31	5 4 0	130
0 4 1	9.4	1 4 1	13	2 4 1	17	3 4 1	24	4 4 1	40	5 4 1	170
0 4 2	11	1 4 2	15	2 4 2	20	3 4 2	28	4 4 2	47	5 4 2	220
0 4 3	13	1 4 3	17	2 4 3	23	3 4 3	32	4 4 3	51	5 4 3	250
0 4 4	15	1 4 4	19	2 4 4	25	3 4 4	36	4 4 4	62	5 4 4	350
0 4 5	17	1 4 5	22	2 4 5	28	3 4 5	40	4 4 5	69	5 4 5	430
0 4 6	9.4	1 5 0	13	2 5 0	17	3 5 0	25	4 5 0	41	5 5 0	240
0 5 1	11	1 5 1	15	2 5 1	20	3 5 1	29	4 5 1	48	5 5 1	350
0 5 2	13	1 5 2	17	2 5 2	23	3 5 2	32	4 5 2	56	5 5 2	540
0 5 3	15	1 5 3	19	2 5 3	26	3 5 3	37	4 5 3	64	5 5 3	920
0 5 4	17	1 5 4	22	2 5 4	29	3 5 4	41	4 5 4	72	5 5 4	1600
0 5 5	19	1 5 5	24	2 5 5	32	3 5 5	45	4 5 5	81	5 5 5	2400+

[a] Number of positive tubes with each of three volumes used.
[b] All figures under MPN may be divided by 100 for reporting MPN per milliliter (or per gram).

highest dilution to a separate tube of brilliant green lactose bile broth with an inverted vial.

All inoculated tubes of brilliant green bile broth are now incubated for 48 hr at 35 ± 1°C (95 ± 1.8°F) and examined for gas in the inverted vial or for effervescence from a cloudy medium. Absence of gas or effervescence is considered to indicate a negative test. The number of coliforms per gram may be determined according to the number of colonies from violet red bile agar plates which gave positive results in brilliant green lactose bile broth. The MPN may be calculated from the number of tubes of lauryl sulfate tryptose broth of various dilutions which gave positive results in brilliant green bile broth.

The confirmation test may also be made on prepoured and solidified eosine–methylene blue agar plates (Difco, BBL). These are streaked using a curved inoculating needle with broth from gas-positive lauryl sulfate tryptose fermentation tubes that were used for the presumptive test. This may also be done by transferring a loopful of the tubes of broth to an eosin–methylene blue agar plate and spreading the broth over the surface of the plate with a glass hockey stick or inoculating needle. The plates are incubated for 18–24 hr at 35 ± 1°C (95 ± 1.8°F).

Completed Test for Coliforms

Colonies which are typical on eosin–methylene blue agar or positive Brilliant Green Lactose Bile broth cultures are streaked and stabbed (butt portion) on a slant of nutrient agar and are also transferred to a tube of lauryl sulfate tryptose broth containing an inverted vial. After incubation for 18–24 hr at 35 ± 1°C (95 ± 1.8°F) the slant is examined for the growth on the surface and in the stabbed portion of the slant. A Gram stain using the Hucker modification is also made on the growth from the slant. The lauryl sulfate tryptose broth is observed for growth and gas formation after 18–24 and 48 hr incubation at 35 ± 1°C (95 ± 1.8°F). A spore stain is also made.

Typical colonies on eosin–methylene blue agar are either deep brown or blue–black with a raised portion at the center and usually, but not always, have a metallic sheen, or are pale pink, lavender, or grayish in color and mucoid, with or without streaks of metallic sheen.

The completed test for coliforms, then, includes culture characteristics which indicate that the organisms are gram-negative, nonspore-forming rods which are mesophilic, will grow either aerobically or anaerobically, and ferment lactose in LST broth within 48 hr.

Test for Fecal Coliforms (U.S. Dept. of Health, Education, and Welfare, 1965)[26]

A portion of three loopfuls of each positive culture of lauryl sulfate tryptose is transferred to a tube of EC medium (Difco, BBL) and incubated immediately. If colonies from violet red bile agar or gas-positive brilliant green lactose bile cultures are to be tested, they should be first transferred to lauryl sulfate tryptose broth and incubated until gas is produced or for 48 hr or less. Incubation in EC broth must be carried out in an accurately regulated water bath at 45.5 ± 0.1°C (113 ± 0.2°F) for 24 hr. The presence of turbidity and gas constitutes a positive test.

Incubation temperatures for this test most commonly used are 44.5 or 45.5°C (112.1 or 113.8°F). It has been found that the lower temperature may produce some false-positive results and the higher temperature some false-negative results.

Detection of Salmonellae in Foods

Pre-enrichment and Sample Size

No standard procedures can be given for pre-enrichment in tests for salmonellae in foods. Some groups (Galton et al., 1964; Lewis and Angelotti, 1964)[12,16] have suggested pre-enrichment in nutrient broth for 24 hr and others have suggested preculturing in lactose broth for 24 hr (North, 1961)[20] prior to subculturing in standard liquid enrichment media. For dried or freeze-dried foods pre-enrichment has been suggested in most cases.

The recommended sample size of food which is being examined for salmonellae by enrichment or pre-enrichment varies. Some investigators suggest 20- or 30-g single samples, others suggest that two 25-g samples be used. The Food and Drug Administration examines a total of 400 g per lot.

Since the AFDOUS (Association of Food and Drug Officials of the United States) procedure (A.F.D.O.U.S., 1966)[2] is that which would probably be used by regulatory agencies, the sequence of methods of testing for salmonellae in foods recommended by this group will be described.

Enrichment of Sample

Two 25-g samples of the food are weighed out aseptically into separate 16-oz screw-capped jars. A 225-ml portion of tetrathionate broth is added to

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