PRINCIPLES OF FOOD MICROBIOLOGY*

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A discussion of some of the fundamental principles that are involved in food microbiology. Particular stress is placed on the factors that contribute to initial and inplant contamination and to those affecting the growth of the organisms which can give rise to very large numbers in a relatively short time when conditions are favorable. Tables and figures are used to illustrate the importance of avoiding contamination with actively growing cultures.

If you lived in an environment where the dirt that got into food doubled in amount every 20 minutes, you would be faced with a very difficult problem of sanitation. This sounds unrealistic, but actually it can happen if you regard microorganisms as dirt, and it serves to emphasize the difficult problem faced by those who handle foods. Microorganisms are self-duplicating units, so that a few can grow to make many.

Let us focus our attention for the moment on a packing plant where meat is being cut on wooden blocks as is often common practice. The wood becomes saturated with meat juices, and these are difficult to remove by the most thorough washing. At the end of the day the blocks are washed and look clean, but still there are meat juices down in the grain of the wood. Due to continued use the surfaces of the wooden blocks are not smooth. They are irregular so that after rinsing, small puddles of water are left on the blocks. If this water is allowed to remain, the few microorganisms, which are present after adequate cleaning, will begin to multiply so that by next morning each puddle will be an actively growing culture teaming with healthy vigorous organisms. In fact the population in this water could reach values as high as one billion bacteria per milliliter. The next morning when the operations again are started, the first pieces of meat which are cut on this block will absorb this water and thus become contaminated very heavily with an actively growing culture of organisms. Let us assume that the population in this water is 1 billion organisms per milliliter and let us assume further that the amount of meat which comes in contact with this water has a volume one thousand times greater than the water itself. This means that each gram of the meat will become contaminated with 1/1000 of 1 billion or 1 million organisms. This in itself is a heavy contamination. However, it is unlikely that this number of organisms would cause any immediate spoilage. In food that begins to become off-flavored or spoiled, the population is probably at least 10 million organisms per gram. This means that in our sample of meat the population would have to increase 10-fold before incipient spoilage became evident. How long would this take? If the food is contaminated with an actively growing culture, we may assume that the population can double every 30 minutes. Thus our 1 million per gram would become 2 million per gram at the end of 30 minutes, 4 million at the end of 1 hour, 8 million at the end of 2 hours. The keeping time of this meat would be just a few hours.

Now you may question the above arguments on the grounds that if the block had been properly cleaned, and if only clean water formed the puddles, there would not be enough food material to allow the bacteria to grow to the extent indicated. Let me, therefore, cite you an experiment that will demonstrate that bacteria can grow in places where there is a very small amount of food. Take a tube of ordinary broth such as the bacteriologist uses for culturing bacteria. This broth is made by dissolving enough meat extract in water to make a 1 percent solution, so it is to begin with a relatively weak solution. Let us now take a loopful of this broth (a loopful contains approximately 0.04 ml) and transfer this loopful to a test tube containing 10 ml of pure water. We will mix this thoroughly and then remove a loopful from this tube and transfer it to another tube containing 10 ml of pure water. You may now rightly assume that a very small amount

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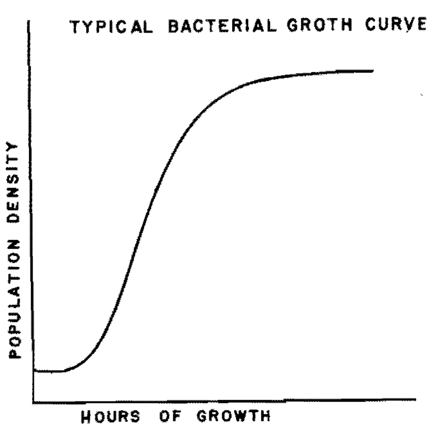
*Presented at 39th Annual Meeting of the *International Association of Milk and Food Sanitarians, Inc.*, Minneapolis, Minn., Sept. 18-20, 1952.

of the original food material present in the broth had been transferred to the second tube of water and yet there is enough food material in this second tube to support a population of 3 million bacteria per ml. I question whether any one could wash a block of wood that has been saturated with meat juices well enough so that the clean water left in the puddles would not contain sufficient food material to support a tremendous population of bacteria.

What makes this type of contamination particularly dangerous is that the food is contaminated with organisms that are in the active stage of growth. Perhaps this can best be illustrated by calling your attention to the growth curve of bacteria. Let us imagine that we introduce some organisms into a flask of broth and then at 15-minute intervals remove samples and count the number of organisms that are present. If we do this over an extended period of time, we will get data which, when plotted on graph paper where the vertical axis represents the number of organisms per ml and the horizontal axis represents the time, will produce an S-shaped curve such as is illustrated in figure 1.



It is to be noted that in the early stages of this curve the population increases at an accelerated rate. In bacteriology we refer to the life span of an organism as the "generation time". This is the time which elapses between the formation of a new daughter cell and its subsequent division. In the early stages of the growth curve this generation time is very long, may in fact be several hours. But as growth begins it becomes shorter and shorter until it reaches a limit of 20 to 30 minutes. In our previous illustration we were contaminating the fresh meat with organisms that were in the mid stage of their growth curve, and therefore, had a very short generation time. Under these conditions food can spoil very quickly. On the other hand, if the food is contaminated with organisms which are not in the active growth stage, but rather in the dormant stage, the generation time will be long and thus spoilage may not take place for a considerable period of time.

Let us assume that the same food is contaminated to the same extent as previously illustrated in the case of meat, but with organisms in the dormant stage. The initial population we will assume to be 1 million per gram of food, but let us say the generation time is now 4 hours instead of 30 minutes. In our previous illustration we found that we would be able to detect incipient spoilage in a very few hours. In this event we should not be able to detect spoilage until after the elapse of a day or more. The situation can be improved even more by assuming that the initial contamination is much less. If instead of having 1 million organisms per gram we introduce 100,000 and the organisms are in a dormant stage, so that the generation time is long, the keeping time could be materially extended.

My topic for to-day was to deal with the fundamentals of food microbiology. A few of these have been illustrated by the examples I have just given you. In order to more sharply delineate this topic, let us enumerate the factors that are of fundamental importance. This is my concept of them:

(1) CONTAMINATION
- (a) Initial – in food
- (b) In plant

(2) SUBSEQUENT GROWTH
- (a) Vigor of organisms
- (b) Elapsed time
- (c) Temperature
- (d) Nutrients
- (e) Retarders
- (f) Moisture content

CONTAMINATION

It is practically impossible to obtain any kind of food material that is not already contaminated with microorganisms. Thus, no matter how carefully we might draw milk from cows, we will find that some organisms have been there at the very beginning. No matter how carefully we slaughter animals, we shall find that the flesh is contaminated with a few organisms. The same thing will hold for almost any kind of food that might be mentioned; even though it is procured under the most sanitary and most carefully controlled conditions, it will contain a number of bacteria. Thus the initial contamination is real and always present even though it may be of minor importance from a quantitative standpoint.

More important perhaps is the contamination the food receives during handling, or what we call "in-plant" contamination. The extent of this can vary greatly, depending upon the sanitary conditions of the plant. By maintaining scrupulous care of equipment, and avoiding the holding of the food in unfavorable environments, the amount of this contamination can also be kept to a minimum. That, of course, is always the thing to strive for.

The keeping time of the food will undoubtedly bear some relation to the extent of this contamination. If food is procured relatively free from bacteria, and if it is handled in a plant that is scrupulously clean, the in-plant contamination can be held down low enough so that the number of organisms should not exceed a few thousand or at the most a few hundred thousand per gram. However, if the plant operations are accompanied by poor-housekeeping, the in-plant contamination can become considerable and may result in the introduction of from 1 to 3 million organisms per gram of food. It is questionable whether conditions can be bad enough to account for larger numbers. It is however important to remember this fact. Whether the initial contamination is low or high the organisms that are introduced as contaminates do not in themselves cause spoilage. This is done by the off-spring.

SUBSEQUENT GROWTH

Whether contamination is low or high, spoilage results from the *growth* of the organisms that are in the food.

This emphasizes the importance of growth and the factors that control it. First of all, as indicated previously, the rate of growth will depend upon the vigor of the organisms introduced. If these come from actively growing cultures, they will continue to multiply at a high rate, so that relatively few cells can give rise to large populations and early organoleptic changes. Thus in the illustration which was mentioned earlier the contamination was 1 million per gram but within 2 hours this increased to 16 million.

The nature of the organisms and their position in their growth curve are probably more important than the actual number introduced. If one introduces a few organisms that are in an active growth phase, they can multiply fast enough to overtake a much higher population made up of organisms that are obtained from a dormant phase in their growth cycle. This serves to emphasize the importance of maintaining proper plant sanitation and avoiding the accumulation of areas or environments where organisms can grow in equipment previous to its use for the handling of food.

The second important factor in governing growth and the resulting damage done by organisms is the time of storage. Food, with a light contamination, stored for a long period of time can attain as high a population as other samples that may have been contaminated very heavily and stored for a rather short period of time. We must not lose sight of the fact that microorganisms are self-duplicating units that keep on multiplying, so if we give them enough time, they can produce unwanted populations even though the initial numbers may have been small and even though their rate of growth may be slow.

The third important factor governing growth is the temperature. Temperature affects the rate of growth of bacteria approximately the same as it does the rate of ordinary chemical reactions. The rate will generally double for each 10°C rise in temperature. This, of course, does not go on indefinitely because if the temperature is raised too high, it becomes lethal. This does not sound like a very marked effect. But let us look at it more closely. Let us examine the problem in the reverse direction by seeing how a reduction in temperature decreases the growth rate. Assume that we have an organism at an optimum temperature of 40°C and that at that temperature the generation time is 20 minutes. If we reduce the temperature to 30°C, the generation time will have been lengthened to 40 minutes, at 20°C it will be 80 minutes, and at 10°C 160 minutes. Over a period of 24 hours a single cell with a generation time of 20 minutes can reach an ultimate population of 4,800 billion billion—a figure represented by 48 followed by 20 zeros. With a generation time of 160 minutes the population after 24 hours will be only 500. You can see from this that the difference is very great indeed.

In this connection tables 1 and 2 will be helpful. The first one shows the ultimate population that can be attained from a single cell after various numbers of generations. It is assumed in this, of course, that all of the cells survive and that each one grows at the same rate as all other sister cells in the population. The second table shows the number of generations that can be attained in 24 hours for various generation times.

TABLE 1 — POPULATIONS ATTAINED FROM A SINGLE CELL WITH VARYING GENERATIONS

No. of generation	*No. of individuals*
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024
20	1000 x 1000 = 1,000,000
30	1000 x 1,000,000 = 1,000,000,000
40	1000 billion
50	1 million billion
60	1 billion billion

TABLE 2 —NUMBER OF GENERATIONS ATTAINABLE IN 24 HOURS WITH VARIOUS GENERATION TIMES

Generation time	*No. of generations*
20	72
30	48
40	36
50	30
60	24
80	18
100	14
120	9

There is probably no other factor that is more effective in lowering the rate of growth than lowering the temperature. If it is a product that should not be frozen, then the temperature should be brought as close to the freezing point as possible. If we follow the reasoning used above, it is apparent that refrigeration will not completely eliminate spoilage but will merely increase the length of time food will remain wholesome. However, give it sufficient time and it can spoil also in the refrigerator. If one wishes to stop the growth of the organisms completely, then one must either sterilize the product so as to kill all organisms or store the product at temperatures below freezing. If this is done and the temperature is low enough so that the water in the food is all frozen, then growth can be completely stopped. This will probably require temperatures from 10° to 15°C below freezing.

It is self-evident that nutrients can influence the growth of microorganisms. Adequate nutrients in sufficient concentration will permit more extensive and more rapid growth than a deficiency in the quantity or quality. Usually, however, this is not a matter which can be controlled by the food microbiologist. He usually deals with natural products, and such natural materials or extracts of them nearly always contain the ingredients that are ideal nutrients for bacteria. It must be assumed that in any food product there are sufficient good quality nutrients to permit the most rapid growth.

It has long been a common practice in many food industries to retard the growth of microorganisms by the use of inhibitors. Many of these have been used since antiquity certainly long before the science of bacteriology was developed. Thus, we find that in packing industries common salt is depended upon to prevent bacterial growth in many meat products. Various organic acids have been used very effectively either by adding the organic acid directly, as for example the addition of vinegar to pickles, or by the formation of organic acid through bacterial fermentations as for example in sauerkraut. In the meat-packing industry, nitrate and nitrite also exert some inhibitory action on the growth of bacteria. Sodium benzoate has been used in some tomato products. More recently there has been considerable interest in the use of antibiotics to inhibit the growth of bacteria in food. Although to-day a usable and practical antibiotic has not been found, there still is hope that one may be found in the future.

While the number of compounds that can be used as inhibitors in food is somewhat limited, there are the few mentioned above which are fairly effective. Whenever these can be used without altering the quality and flavor of the food, they can be depended upon to retard the growth of bacteria and thus enhance the keeping quality of the food. All of these will be most effective if they are added to food products which have relatively few organisms to begin with. Most of these inhibitors are relatively ineffective if added to products that have large numbers of actively growing cells.

The moisture content of food materials has an extremely important bearing on the rate and extent of growth of bacteria. These organisms must get their nutrients in the dissolved form and consequently they cannot grow unless there is an abundance of water present. In many food products the moisture content is high enough to be ideal for growth, but it is often possible to remove some of that moisture without damaging the food. In fact this is nature's way of preserving foods. Seeds and grains owe their keeping quality to the lack of moisture. The quality of water that has to be present depends on the type of material involved. In a non-absorbent material such as sand, bacteria can grow even though the moisture content is as low as 10 percent. However, in a product such as meat, bacterial growth can be stopped when the moisture content is around 35 percent. Likewise bacteria do not grow in bread with a moisture content below 30 percent. Fundamentally it is not the percentage of water that is important, rather it is the equilibrium vapor pressure. Most bacteria do not grow in products when the equilibrium vapor pressure is below 94 percent of what it should be for pure water. The percentage of moisture necessary to produce this equilibrium vapor pressure varies with the product and in particular with the amount of soluble materials that are present.

From the foregoing it is evident that there are a number of fundamental factors that need to be taken into consideration when one wishes to prevent or limit the extent of damage that microorganisms can bring about in food products. The fundamental principles are self-evident but the interpretation of them requires an understanding of the factors that control contamination and subsequent growth of the organisms involved.

THE EFFECTS OF HEAT ON BACTERIA

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