

A STUDY OF THE ACTION OF BACTERIA ON MILK PROTEINS.*

GEORGE SPITZER and H. M. WEETER, Purdue University.

It is generally recognized that most bacteria have an action on organic food material which is characteristic for different species and is influenced by their previous environment and the kind and relative proportion of the different foods in the media. As the food and water requirements of higher plant and animal life and of bacteria are similarly related, bacterial metabolism involves the change which the food materials undergo by virtue of bacterial action and is determined by the properties and composition of the end products. With the present chemical methods of analysis it is possible to determine with considerable degree of accuracy the initial composition of the bacterial foods, also the end products. Of what takes place within the organisms little is known. Inferences can only be drawn from the changes in the medium and the nature of the enzymes secreted by the bacteria. When bacteria are grown in a medium containing both proteins and carbohydrates it has been found that the cleavage products are modified, depending upon the source and chemical complexity of the protein and carbohydrates.

B. Coli, when grown in a nitrogenous medium in presence of easily fermentable carbohydrates, fails to produce indol or the production of indol is extremely rare, but when *B. Coli* is grown in a medium containing the same nitrogenous foods in presence of carbohydrates which do not ferment readily indol is produced. The character of the proteins likewise influences the growth and metabolism of bacteria and the cleavage products are not of the same kind and character. The proteins are hydrolized by bacterial enzymes into simpler complexes, such as proteoses, peptones, and possibly peptids and amino acids.

There is a marked difference depending on the source of nitrogen, and a still greater difference depending on the species of bacteria, in the production of cleavage products. According to Taylor (*Ztschr. f. Physiol. Chem.*, Vol. 36), *B. Coli* digests casein mainly into proteoses and peptones with the formation of only a small per cent. of amino acids,

* "Contribution from Purdue University Agricultural Experiment Station, Department of Dairy Husbandry."

while when grown in egg meat mixture according to Rettger (Journal Bio. Chem., Vol., 13), this same bacterium produces profound changes, giving indol, skatol, and amino acids.

Also, the utilization of any of these simpler nitrogenous products of hydrolysis depends upon the life history and the species of the bacteria and of food material other than the nitrogen compounds; that is, carbohydrates, salts, etc. Concerning the utilization of the amino acids, under certain conditions the basic amino acids or diamino acids are used to a greater extent as a source of nitrogen instead of the monoamino acids, and the reverse may happen; the monoamino acids are used more readily and fail to appear in the final products.

From our own work during the past year on bacterial metabolism, unpublished data are at hand showing the utilization of the amino acids. Lots of 500 c. c. of sterile milk were inoculated with pure cultures of *B. proteus*, *B. liquifaciens*, *B. subtilis*, and *B. megatherium*. These lots of inoculated milk were stored at room temperature for six months. The nitrogen distribution was then determined, ammonia, melanin, amino acids, etc.

The following table shows the per cent. of monoamino and diamino acids obtained upon hydrolyzing the milk before inoculation, also the per cent. of the same amino acids after inoculation for six months.

TABLE I.

	Sterile Milk.		At End of Six Months' Incubation.	
	Monoamino Acid N. %	Diamino Acid N. %	Monoamino Acid N. %	Diamino Acid N. %
<i>B. proteus</i>	56.50*	23.66	42.14	5.61
<i>B. liquifaciens</i> ..	56.50	23.66	45.02	5.82
<i>B. subtilis</i>	56.50	23.66	54.14	7.61
<i>B. megatherium</i> ..	56.50	23.66	40.00	7.24

*Per cent. of total nitrogen.

In Table I the relative proportion of the utilization of the two groups of amino acids is shown for the four different bacteria. It will be noted that the diamino acids are used in greater amounts than the monoamino acids.

Table II shows the per cent. of the total monoamino and diamino acid nitrogen utilized by the four bacteria calculated from Table I.

TABLE II.

	Monoamino Acid N. %	Diamino Acid N. %
<i>B. proteus</i>	25.42	76.29
<i>B. liquifaciens</i>	20.32	75.40
<i>B. subtilis</i>	4.17	67.83
<i>B. megatherium</i>	29.15	69.40

In general, this is in agreement with the work of Robinson and Tartar (Journal Bio. Chem., Vol. XXX, page 135). However, this comparison can only be roughly made since their medium consisted of an aqueous soil extract plus a nitrogenous food material; i. e., fibrin, pepton, egg albumen, gliadin, and casein, with a small amount of carbohydrate in the form of mannite and synthetic solution of salts in addition to the salts extracted from the soil.

The pure cultures used by Robinson and Tartar were *B. mycoides*, *B. subtilis*, and *B. vulgaris*. The above facts concerning the utilization of the amino acids by bacteria are in harmony with the work of most investigators on bacterial metabolism. No doubt the utilization of the amino acids is influenced by the character and quantity of proteins and carbohydrates present in the media. We know, if carbohydrates are absent or hydrolyzed into compounds which do not yield the desired food material—namely, the carbon—as readily as the original carbohydrates, bacteria must necessarily derive their carbon supply from the protein or amino acids. There is no quantitative relation connecting the increase of acidity with the loss of carbohydrates by bacterial action on the respective carbohydrates. So some of the carbohydrates must be used in supplying energy to the organisms.

About six years ago, while the senior author was conducting an extensive investigation concerning the keeping qualities of butter when placed in cold storage, the results of the investigation suggested to him the advisability of taking up a systematic study of pure cultures of known bacteria in a medium composed of milk proteins in presence of carbon compounds such as lactose and lactic acid, etc.

By pursuing this method of investigation it will be possible to arrive at more definite information regarding the bacterial action on milk proteins and the character and quantity of the final cleavage products. The

selection of the respective bacteria are those frequently found in milk, cream, and butter. By the selection of these bacteria and using a medium which is naturally present in milk products, we are able, in a great measure, to avoid introducing disturbing factors on the end products, also factors foreign to our previous work concerning the changes produced in stored butter.

Our preliminary study included the following bacteria: *B. proteus vulgaris*, *B. viscosus*, *B. butyricus*, *B. mycoides*, *B. lactis acidii*, *B. mesentericus*, *B. liquifaciens*, *B. fluorescens putidus*, *B. subtilis*, *B. megatherium*, and *B. coli*. The medium was sterilized milk to which the pure cultures were added and kept at room temperature. The pure cultures were previously grown in the same media and transfers were made three times before being used for experiment. At intervals of three days an analysis of the inoculated milk was made. The following products were determined each time the analysis was made: acidity, aldehyde number*, lactore (polariscope), ammonia (Folin's method), and nitrogen compounds not precipitated by phospho tungstic acid. This was continued for five periods or during a period of sixteen days. (First period four days.)

The following table shows the changes in the nitrogenous constituents of the milk and the change in lactose by the different bacteria at the end of the sixteenth day.

TABLE III.

Showing the per cent. of gain of ammonia (NH_3) and amid nitrogen based on total nitrogen and the loss of lactose based on the total lactose.

	Ammonia (NH_3), N. % Gain.	Amid, N. % Gain.	Lactose, % Loss.
<i>B. proteus</i>	5.42	1.63	27.65
<i>B. viscosus</i>	11.01	22.13	50.30
<i>B. butyricus</i>	4.49	6.59	23.04
<i>B. mycoides</i>	10.28	8.38	14.81
<i>B. lactis acidii</i>	2.04	1.88	34.87
<i>B. mesentericus</i>	10.28	12.38	62.92
<i>B. liquifaciens</i>	20.20	25.63	60.00
<i>B. fluorescens putidus</i>	1.46	2.13	17.83
<i>B. subtilis</i>	12.10	22.84	47.10
<i>B. megatherium</i>	7.34	24.64	54.11
<i>B. coli</i>	3.66	3.63	17.63

* The aldehyde number gave no more information concerning protein hydrolysis than did phospho-tungstic acid.

Ammonia, amid nitrogen, lactose, and acidity were estimated in the sterile milk before inoculation for the purpose of comparison. This gave for lactose 4.99 per cent., total nitrogen .56 per cent., and acidity .17 per cent. as lactic acid. Ammonia .89 per cent. and for amid nitrogen 2.87 per cent. based on total nitrogen present in the sterile milk.

The changes in acidity for the different bacteria are shown in Table IV.

TABLE IV.

Showing changes in acidity, expressed in per cent. of lactic acid, during the period of sixteen days.

	Per Cent. Lactic Acid.
<i>B. proteus</i>	.027
<i>B. viscosus</i>	.324
<i>B. butyricus</i>	.189
<i>B. mycoides</i>	.261
<i>B. lactis acidii</i>	1.161
<i>B. mesentericus</i>	.459
<i>B. liquifaciens</i>	.909
<i>B. fluorescens putidus</i>	.045
<i>B. subtilis</i>	.468
<i>B. megatherium</i>	.369
<i>B. coli</i>	.135

Comparing Tables III and IV, it is shown that the acidity of the milk medium is not in proportion to the loss of lactose, nor gain in ammonia. Therefore neither the production of ammonia nor the acidity is an exclusive measure of the activity of the organisms. It has been stated that the production of ammonia is an index of the metabolic activity of the organisms. This must be taken with some qualification inasmuch as proteolysis does not take place by leaps; that is, that the different cleavage products are produced in regular order, as proteoses, peptids, amino acids, etc., but it is more natural and in harmony with enzymic action on proteins and carbohydrates, that as soon as proteolysis begins, a series of simpler compounds are formed and all the cleavage products appear, the proportion depending upon the medium, kind of organisms, and enzymes produced by each specific bacterium. Since it is possible to measure the production amino acids and ammonia at short intervals with a good degree of accuracy, it has given additional evidence to show the mode and rate of the activity of bacterial metabolism and their proteolytic power.

Of the eleven bacteria studied there was a continual change in acid-

ity from the first period until the last, except the lactic acid bacillus which produced its maximum acidity within the first period (four days) which was 1.61 per cent. as lactic acid. No change in acidity occurred after this period, nor was there any increase in ammonia. The amid nitrogen increased slightly at the expiration of four days and there was a gain of amid nitrogen of .0077 per cent. and at the expiration of the sixteenth day there was a gain of .0105 of amid nitrogen, a gain of .5 per cent. on total nitrogen, showing a continual proteolytic action due either to enzymes or auto-proteolytic digestion.

It may be noted that some bacteria utilizing the larger amount of lactose were also quite active in the production of ammonia and amino acids. On the other hand, in Table III the fermentation of lactose was proportionately greater than the production of ammonia and amino acids by *B. proteus*, *B. butyricus*, *B. mesentericus*, *B. fluorescens put.*, and *B. Coli*.

We hope to study further the action of these organisms in pure culture on nitrogen from different sources, the effect of carbohydrates and also the associative action of these cultures on milk proteins.