STUDIES ON THE PROTEOLYTIC BACTERIA OF MILK

IV. ACTION OF PROTEOLYTIC MILK BACTERIA ON AMINO ACIDS AND OTHER SIMPLE NITROGENOUS COMPOUNDS

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Although it was the purpose of the present work to group the proteolytic organisms chiefly according to their action on milk and important milk constituents, a study was also made of the use of simple nitrogenous compounds by these organisms in the hope that a better understanding of their nitrogen metabolism might be attained. The organisms were inoculated into synthetic media with various simple nitrogenous compounds as a sole source of nitrogen.

According to Benton (1919) organisms may have individual requirements in amino acid: some may have more catholicity of taste in amino acids than others. Koser and Rettger (1919), however, found that with the organisms they used the various amino acids were similar in their ability to support growth, with the possible exception of tryptophan which was found to be superior to the other amino acids. These workers found that with the exception of the cholera vibrio all the organisms studied could use diammonium phosphate as well as they could use any of the amino acids. Urea was found to be inferior to the amino acids as an immediately available source of nitrogen. Combinations of amino acids or of amino acids and other simple nitrogen-containing compounds were found to be little better than any of the single amino acids.

Gordon and McLeod (1926) worked on the inhibitory action of various amino acids on bacterial growth in peptone broth. They used chiefly delicate pathogenic bacteria, for they found that

B. coli and some of the staphylococci were not much influenced. These workers divided the amino acids into three groups: (1) indifferent amino acids, which included arginine, glutamic acid, l-histidine, l-leucine, d-lysine, tyrosine, and valine; (2) favoring amino acids which included taurine, aspartic acid and alanine; and (3) inhibitory amino acids which included: cystine, glycine, phenylalanine and tryptophan. Tryptophan was found to be the most toxic. The amino acids were used in concentrations of from 0.1 to 1 per cent.

Hirsch (1918) discusses the possible courses of decomposition of the various amino acids by microörganisms and records the end-products found by different workers, and Dox (1917) lists possible decomposition products from amino acids due to the action of yeasts and bacteria. References cited by Hirsch and by Koser and Rettger (1919) are not included in the literature reviewed below.

Most reports on the action of organisms on amino acids discuss the action of one or two species of bacteria on only one or two amino acids. Bierema (1909) worked with pure and mixed cultures of soil bacteria and studied their growth on leucine, aspartic acid, tyrosine and asparagine with glycerol or different carbohydrates as sources of carbon. He also tried the following as the sole source of nitrogen: urea, uric acid, hippuric acid, guanidin, formamide, acetamide, and various ammonium salts of inorganic and organic acids. Frouin and Ledebt (1911) showed the effect of monoamino acids on the production of volatile acids by different bacteria. Miyaji (1925) found that his acetic bacteria could decompose l-tyrosine, l-leucine, d-glutamic acid, glycine, dl-phenylalanine and l-histidine. Herzfeld and Klinger (1915) and Sasaki and Otsuka (1921) discussed the decomposition of tryptophan. Raistrick and Clark (1921) studied the action of *B. pyocyaneus*, *B. fluorescens*, *B. prodigiosus* and *B. proteus-vulgaris* on tryptophan and tyrosine. The splitting of tyrosine by *B. proteus-vulgaris* and *B. subtilis* was studied by Tsudji (1917, 1918), by *B. proteus* by Otsuka (1921), and by *B. lactis-aerogenes* by Hirai (1918). The decomposition of tryptophan and of amino-benzoic acid by *B. pyocyaneus* with the

formation of ammonium carbonate was reported by Supiniewski (1924). *B. coli-communis* was used by Hanke and Koessler (1919, 1922) in a study of the decomposition of histidine; and members of the colon-typhoid group were used by Raistrick (1917, 1919). Mayer and Schaeffer (1919) concluded that arginine with its guanidin nucleus and histidine with its imidazole nucleus are essential for the growth of *B. tuberculosis* and that the idea of indispensable amino acids might apply to bacteria as well as to animals. Long (1919) studied the nitrogen metabolism of *B. tuberculosis* in a glycerol-phosphate medium. He found that the organism grew well with urethane, glycine, alanine, asparagine, the acid amides, ammonia, methyl and ethyl amine, or the ammonium salts of the fatty, ketone and hydroxy-acids which correspond to the three amino acids. The splitting of alanine and histidine by *B. tuberculosis* was reported by Campbell (1925). Arai (1921) discussed the decomposition of l-leucine by *B. proteus* and *B. subtilis*. *B. coli*, *B. subtilis* and *B. prodigiosus* are able to split glucosamine according to Takao (1923); and *Bact. tenuis* was found by Abderhalden and Fodor (1913) to be able to split d-glucosamine. Kondo (1923) found that *B. coli* and *Proteus vulgaris* formed hydrogen sulphide and ethyl sulphide from cystine, and that *Proteus vulgaris* also formed mercaptans under certain conditions. Gordon (1924) reported that organisms like those of the colon-typhoid group and the anaerobes, which form hydrogen sulphide, can tolerate large quantities of it. The growth of anaerobes is probably improved by cystine, according to this writer, but delicate organisms do not split cystine and are highly sensitive to it.

In practically all the work reviewed above a source of carbon was provided in the form of a fermentable carbohydrate or similar compound. Thus the nitrogenous compound needed to serve only as a source of nitrogen. Ths use of asparagine, histidine, and alanine as a sole source of carbon and nitrogen for *B. fluorescens-liquefaciens* was reported by Blanchetière (1916, 1917, 1920).

The fermentation of salts of organic acids with ammonia as the only nitrogen source was used by Ayers, Rupp and Johnson (1919) in their work with the alkali-forming bacteria of milk and by Koser (1923) in work with organisms of the colon-aerogenes group.

METHODS

In preliminary work the basic medium of Koser and Rettger (1919) was tried. This medium contains distilled ammonia-free water, 1000 cc.; NaCl, 5 grams; $MgSO_4$, 0.2 gram; $CaCl_2$, 0.1 gram; KH_2PO_4, 1 gram; K_2HPO_4, 1 gram; and glycerol, 30 grams or glucose, 10 grams. To this medium is added 0.1 per cent of the nitrogenous compound to be tested. It was found that this medium gave rather erratic results and that some organisms were consistently negative in growth although they were positive in the modified medium described below. Sodium chloride in the amount of 0.5 per cent seemed especially inhibitive to some organisms. Therefore only 0.02 per cent of potassium chloride was substituted for sodium chloride in the modified medium; and the quantity of soluble salts was maintained by an increase in the quantity of phosphate buffer salts. The basic medium finally adopted after considerable experimentation contained:

K_2HPO_4	3.1 grams
KH_2PO_4	0.8 gram
KCl	0.2 gram
$MgSO_4 \cdot 7H_2O$	0.2 gram
Distilled water to 1000 cc.	

To this basic medium were added glucose or glycerol when desired and the nitrogenous compound in small quantities which varied with the solubility, toxicity, and nitrogen content of the compound. All nitrogen compounds were C. P. Kahlbaum. The pH was adjusted to 7.0 or 7.2. When ammonia was to be the sole source of nitrogen the two phosphate salts in the medium were replaced by 2.5 grams of sodium ammonium phosphate, and 1 per cent of glucose was added. The urea medium contained 1 gram of urea per liter and 0.2 per cent of glucose, and the medium was sterilized by filtration through a Berkefeld filter. All tubes of urea medium were incubated at 30°C. for several days to test for sterility. The amino acids were used with and without sugar. When sugar was added 1 per cent glucose was used. Glycerol was tried but did not satisfy so many organisms nor give so large changes in pH as the glucose. The following amounts of

amino acid were used per liter of medium: sodium aspartate, 3.6 gram; glutamic acid, 3 grams; tyrosine, 1 gram; leucine, 0.5 gram; α alanine, 3 grams; glycine, 3 grams; and tryptophan, 0.03 gram. Asparagine was used at the rate of 3 grams per liter. In the series of amino acids without sugar a medium with 3 grams of ammonium succinate per liter was used. In preliminary experiments potassium nitrate, potassium nitrite, potassium sulphocyanate, and hippuric acid were also tried as sources of nitrogen but were not found useful.

Light inoculations from fresh agar cultures were used, and incubations were for ten days at 30°C. Then growth, pH, and ammonia were noted. Ammonia was roughly estimated as described in paper II of this series except in the media which originally contained ammonia. The results of these experiments are shown in tables 1, 2 and 3. No growth is indicated in the tables by a minus mark and growth by "slight" or a single plus mark with no further distinction as to the amount of turbidity produced. This was done because it was observed that many of the organisms which barely clouded the medium and might be called negative if turbidity were the criterion for growth were nevertheless fairly active as shown by a marked change in pH or increase in ammonia.

DATA

The following organisms did not grow on any of the nitrogenous compounds tried and are therefore not included in the tables: *M. citreus*, *M. casei*, *M. subflavescens*, *Staph. albus*, *Str. liquefaciens*, *Str. bovis*, *Achromobacter liquefaciens* and *Flavobacterium tremelloides*. The cultures of *M. perflavus*, *M. varians*, *M. luteus*, *M. ureae* and *M. freudenreichii* grew only in the urea medium and are found only in table 1.

The action of the organisms on urea is shown in table 1. For the sake of comparison the ability of the same organisms to use ammonia and asparagine as a sole source of nitrogen is indicated in the same table. A number of organisms which can use ammonia and asparagine as nitrogen sources but can not use urea are not included in the table. All cultures of *B. vulgatus*, *B. cereus*, *B.*

mesentericus, *B. cohaerens*, *B. megatherium*, and *B. macerans* fall in this category, whereas only one of the cultures called *B. albolactus* breaks down urea. This culture also differs from the other *B. albolactus* cultures in other characteristics. It is interesting to note that with the media used cultures of *M.*

TABLE 1

Urea, ammonia and asparagine as sole source of nitrogen in a medium with glucose

Incubation for ten days at 30°C.

ORGANISM	NUMBER OF CULTURE	NH_3 AS N SOURCE; GROWTH	ASPARAGINE AS N SOURCE; GROWTH	UREA AS N SOURCE		
				Growth	pH	NH_3
Control		−	−	−	7.0	−
M. perflavus	11	−	−	+	7.6	++
M. varians	9	−	−	+	8.2	++
M. percitreus	5	+	+	+	8.0	++
M. luteus	5	−	−	+	6.5	−
M. freudenreichii	9	−	−	+	8.3	++
M. ureae	2	−	−	+	8.4	++
P 147	1	+	+	+	8.3	+++
P 269	1	+	+	+	7.1	++
Serratia ruber	2	+	+	+	8.3	++
Serratia indica	1	+	+	+	7.1	Sl.
P 268 (Serratia)	1	+	+	+	8.3	++
Achromobacter coadunatum	7	+	+	+	6.0	−
Achromobacter delictatulum	1	+	+	+	5.7	Sl.
Proteus vulgaris	1	+	+	+	5.9	−
P 107 (Escherichia)	1	+	+	+	8.0	+++
B. subtilis	2	+	+	+	7.2	+
B. simplex	2	+	+	+	7.8	++
B. tumescens	1	+	+	+	7.4	++
B. ruminatus	1	+	+	+	8.1	++
P 67 (Pseudomonas)	1	+	+	+	8.2	++

perflavus, *M. varians*, *M. luteus*, *M. freudenreichii* and *M. ureae* were able to use urea but not ammonia or asparagine as a source of nitrogen, although the amide group is present in asparagine as well as in urea and ammonia is probably the next step in the degradation of urea.

Most of the organisms which can use urea produce an alkaline

reaction in the medium which is probably due to the liberation of ammonia. It will be observed however that certain organisms like *M. luteus* and *Achromobacter coadunatum* produce no ammonia and cause an acid reaction in the medium. These organisms apparently break down only enough urea to satisfy their nitrogen needs.

The action of the organisms on media which contained glucose and ammonia, asparagine, or one of the amino acids is shown in table 2. Data on the tryptophan medium are omitted from the table because none of the organisms grew to any extent in this medium. The "blank" medium contained no added source of nitrogen. Some few organisms which were apparently able to obtain ammonia from the incubator air (Braun and Goldschmidt, 1927) or enough nitrogen from the light inoculum were able to grow to some extent. This occurred with only four single cultures of organisms. The results in table 2 are in agreement with the conclusions of Koser and Rettger (1919) that if an organism can use ammonia as its only source of nitrogen when a suitable carbon source is furnished, it can also use any of the simpler amino acids as a nitrogen source. As found by Gordon and McLeod (1926), however, certain amino acids may be toxic to some organisms.

A few organisms which were unable to grow in any of the ammonia- or amino-acid media grew to some extent with asparagine as the source of nitrogen. This growth took place in the presence or absence of glucose.

It is apparent that an amino-acid medium which contains a fermentable sugar is not a good differential medium. If, on the other hand, the sugar were omitted and the amino acid were forced to serve as the sole source of both nitrogen and carbon for the organism, some differentiation between organisms might be expected. In table 3 is shown the action of the proteolytic organisms on media in which single amino acids are the only source of both nitrogen and carbon. The results with the amino-acid media without sugar were surprisingly consistent for a simple synthetic medium. Only with cultures of *B. albolactus* were there differences within the species. As will be shown in a fol-

TABLE 2

Ammonia, asparagine and amino acids as sole source of nitrogen in a medium with glucose

Incubation for ten days at 30°C.

ORGANISM	NH_3 AS N SOURCE		BLANK NO. N			GLYCINE			GLUTAMIC ACID			TYROSINE			ASPARAGINE			ASPARTIC ACID			ALANINE			LEUCINE		
	Growth	pH	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3
Control	−	7.0	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−
M. percitreus	+	5.0	−	7.0	−	+	5.9	−	+	5.9	−	+	6.6	−	+	6.2	+	+	5.9	−	+	6.4	−	+	6.8	−
M. cereus	−	7.0	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	Sl.	6.8	Sl.	−	7.0	−	−	7.0	−	−	7.0	−
P 147	+	4.6	+	5.0	−	+	4.6	−	+	4.7	−	+	4.5	−	+	4.5	++	+	6.8	+	+	5.8	++	+	4.6	−
Flavobacterium synxanthum	−	7.0	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	+	7.0	+	−	7.0	−	−	7.0	−	−	7.0	−
Flavobacterium lactis	−	7.0	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	+	7.0	Sl.	−	7.0	−	−	7.0	−	−	7.0	−
Serratia ruber	−	7.0	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	+	7.2	+	−	7.0	−	−	7.0	−	−	7.0	−
Serratia indica	+	4.6	+	4.6	−	+	5.1	−	+	6.5	−	+	4.5	−	+	6.8	++	+	8.6	++	+	6.5	+	+	4.6	Sl.
Achromobacter coadunatum	+	5.2	−	7.0	−	+	5.2	−	+	5.3	−	+	5.0	Sl.	+	5.0	+	+	5.4	−	+	5.6	−	+	5.0	−
Achromobacter delictatulum	+	4.6	+	6.6	−	+	5.0	Sl.	+	5.9	−	+	4.5	−	+	6.3	++	+	7.8	+	+	6.1	++	+	4.4	−
Alcaligines bookeri	−	7.0	−	7.0	−	−	7.0	−	−	7.0	−	−	7.0	−	+	7.1	Sl.	−	7.0	−	−	7.0	−	−	7.0	−
Proteus vulgaris	+	5.4	−	7.0	−	+	5.8	−	+	5.4	−	+	5.6	−	+	5.7	++	+	6.0	++	+	5.6	−	+	5.1	−
P 107	+	6.3	+	4.8	−	+	7.0	+	+	5.0	−	+	5.1	−	+	6.8	+	+	7.5	++	+	6.0	++	+	4.5	−
B. albolactus	+*	6.0	−	7.0	−	+†	6.4	−	+	5.5	−	+†	6.6	Sl.	+	6.2	+	+	5.7	−	+†	6.6	−	+†	6.0	−
B. cereus, Strain "A"	+	5.8	−	7.0	−	+	6.7	−	+	5.5	−	+	6.5	−	+	6.0	+	+	5.7	−	+	6.4	−	+†	6.5	−
B. cereus, Strain "B"	+	6.5	−	7.0	−	+	6.7	−	+	5.5	−	+	6.6	−	+	6.5	+	+	5.7	Sl.	+	6.7	−	−	7.0	−
B. vulgatus	+	6.4	−	7.0	−	+	6.2	−	+	5.5	−	+	6.7	−	+	6.0	++	+	6.1	−	+	6.7	−	+†	6.8	−
B. subtilis	+	5.8	−	7.0	−	+	5.9	+	+	5.9	−	+	6.4	−	+	6.4	++	+	8.2	++	+	7.4	++	+	6.7	−
B. simplex	+	5.8	−	7.0	−	+	6.4	−	+	6.2	−	+	6.8	−	+	6.3	−	+	6.1	−	+	5.8	−	+	6.2	−
B. mesentericus	+	6.0	−	7.0	−	+	6.3	−	+	5.9	−	Sl.	6.7	−	+	6.3	++	+	7.3	+	+	5.8	−	+	6.8	−

B. cohaerens	+	5.4	−	7.0	−	+	5.2	+	+	6.7	+	+	6.8	−	+	6.4	++	+	6.0	+	+	6.1	++	+	7.0	−
B. tumescens	+	5.8	−	7.0	−	+	5.8	−	+	6.4	−	+	6.6	−	+	6.8	+	+	6.4	−	+	6.6	−	−	7.0	−
B. megatherium	+	5.4	−	7.0	−	+	6.8	−	+	5.4	−	+	6.1	−	+	6.0	++	+	6.2	−	+	6.3	−	−	7.0	−
B. ruminatus	+	5.4	−	7.0	−													+	6.2	+	+	5.8	+			
B. macerans	+	6.0	−	7.0	−	+	5.9	Sl.	+	6.4	−	Sl.	6.8	−	+	6.5	+	+	7.1	+	+	6.4	Sl.	+	5.9	−
P 67	+	4.6	+	5.2	−	+	6.3	+	+	5.0	−	+	4.8	−	+	6.4	+	+	7.6	++	+	4.7	++	+	4.8	−

* Of 28 cultures, 21 were positive in medium and 7 negative.

† Not all positive.

TABLE 3

Ammonium succinate, asparagine, and amino acids as a sole source of nitrogen and carbon

Incubation for ten days at 30°C.

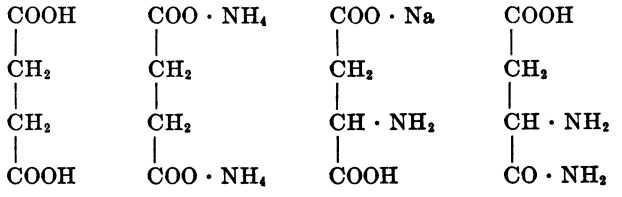
ORGANISM	NUMBER OF ORGANISMS	GROWTH IN AMMONIUM PHOSPHATE WITH GLUCOSE	AMMONIUM SUCCINATE		SODIUM ASPARTATE			ASPARAGINE			GLYCINE			α-ALANINE			GLUTAMIC ACID			TYROSINE*		
			Growth	pH	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3	Growth	pH	NH_3
Control		−	−	7.0	−	7.2	−	−	7.2	−	−	7.2	−	−	7.2	−	−	7.2	−	−	7.2	−
M. percitreus	5	+	+	7.4	+	7.4	−	+	7.5	+	−	7.2	−	−	7.2	−	+	7.6	+	+Y	7.2	Sl.
M. cereus	3	−	−	7.0	−	7.2	−	+	7.2	+	−	7.2	−	−	7.2	−	−	7.2	−	−	7.2	−
P 147	1	+	+	8.2	+	8.5	+	+	8.4	++	Sl.	7.2	+	+	8.2	++	+	8.3	+	+	7.2	+
P 269	1	+	+	8.1	+	8.5	+	+	8.3	++	−	7.2	−	+	8.0	++	+	8.5	+	+Y	7.2	+
Flavobacterium synxanthum	13	−	−	7.0	Sl.	7.3	Sl.	Sl.	7.3	+	−	7.2	−	−	7.2	−	+	7.6	Sl.	−	7.2	−
Flavobacterium lactis	1	−	−	7.0	−	7.2	−	−	7.2	−	−	7.2	−	−	7.2	−	+	7.4	Sl.	+Y	7.2	−
Serratia ruber	2	−	+	7.3	+	7.6	+	+	7.4	++	−	7.2	−	−	7.2	−	+	7.4	+	−	7.2	−
Serratia indica	1	+	+	8.2	+	8.5	++	+	8.1	++	−	7.2	−	+	8.1	++	+	8.6	+	+	7.5	+
P 268 (Serratia)	1	+	+	8.2	+	8.6	++	+	8.2	++	+	7.5	+	+	8.2	++	+	8.5	+	+B	7.3	−
Achromobacter coadunatum	7	+	+	8.0	+	8.5	+	+	8.1	++	−	7.2	−	+	8.2	++	+	8.2	+	+	7.2	+
Achromobacter delictatulum	1	+	+	8.1	+	8.3	+	+	8.0	++	−	7.2	−	+	8.1	++	+	8.5	+	+	7.2	+
Alcaligines bookeri	2	−	−	7.0	−	7.2	−	−	7.2	−	−	7.2	−	−	7.2	−	Sl.	7.3	−	S	7.2	−
Proteus vulgaris	1	+	+	7.6	+	8.6	+	+	8.3	+	−	7.2	−	+	7.8	+	+	8.4	+	+	7.2	+
P 107	1	+	+	7.6	+	8.4	++	+	7.8	++	−	7.2	−	+	7.9	++	+	8.4	++	−	7.2	−
B. cereus, Strain "A"	8	+	−	7.0	+	7.5	Sl.	+	7.8	+	−	7.2	−	−	7.2	−	+	7.6	+	S	7.2	−
B. cereus, Strain "B"	3	+	−	7.0	−	7.2	−	−	7.2	−	−	7.2	−	−	7.2	−	+	7.6	+	S	7.2	−
B. vulgatus	6	+	−	7.0	+	7.7	+	+	7.9	++	−	7.2	−	−	7.2	−	+	7.6	+	S	7.2	−
B. subtilis	2	+	−	7.0	+	8.4	+	+	8.1	++	−	7.2	−	+	7.8	+	+	7.6	Sl.	−	7.2	−

B. simplex	2	+	−	7.0	+	8.4	+	+	+	8.2	+	+	−	7.2	−	+	7.5	+	+	8.0	+	−	7.2	−
B. mesentericus	1	+	−	7.0	+	7.8	+	+	+	7.6	+	+	−	7.2	−	−	7.2	−	+	7.2	−	−	7.2	−
B. cohaerens	2	+	−	7.0	+	8.2	+	+	+	8.1	+	+	−	7.2	−	+	7.4	+	+	7.6	Sl.	−	7.2	−
B. tumescens	1	+	−	7.0	+	8.2	+	+	+	7.4	+	−	−	7.2	−	−	7.2	−	+	7.6	+	−	7.2	−
B. megatherium	1	+	−	7.0	+	7.5	−	+	−	7.2	−	−	−	7.2	−	−	7.2	−	+	7.4	−	−	7.2	−
B. ruminatus	1	+	−	7.0	+	8.6	++	+	+	8.3	++	−	−	7.2	−	+	7.6	+	+	8.2	+	−	7.2	−
B. albolactus, "A"	12	+	−	7.0	+	7.5	Sl.	+	+	7.7	++	−	−	7.2	−	−	7.2	−	+	7.7	+	S	7.2	−
B. albolactus, "B"	8	+	−	7.0	+	7.9	+	−	−	7.2	−	−	−	7.2	−	−	7.2	−	+	7.9	+	S	7.2	−
B. albolactus, "C"	6	−	−	7.0	+	7.7	+	−	−	7.2	−	−	−	7.2	−	−	7.2	−	−	7.2	−	S	7.2	−
B. albolactus, "D"	1	+	−	7.0	−	7.2	−	−	−	7.2	−	−	−	7.2	−	−	7.2	−	+	7.5	Sl.	−	7.2	−
B. albolactus, "E"	1	+	−	7.0	−	7.2	−	−	−	7.2	−	−	−	7.2	−	−	7.2	−	−	7.2	−	−	7.2	−

* In tyrosine medium, Y = yellow coloration of medium, B = brown, S = salmon color.

lowing paper the cultures which have been grouped together as *B. albolactus*, according to the descriptions of that organism usually given, can be further subdivided on differences of some of their characteristics.

Included in table 3 is an ammonium-succinate medium which is to be compared with the closely related compounds, sodium aspartate and asparagine. These three compounds may all be considered derivatives of succinic acid:



Succinic acid | Ammonium succinate | Sodium aspartate | Asparagine

Most of the ammonium succinate was probably present as monoammonium succinate after sterilization in the autoclave. Most of the organisms which could use asparagine could also use the aspartate. The only exception was *M. cereus* which produced a rather dubious growth in the asparagine media, both with and without sugar, and should probably be eliminated from tables 2 and 3. *B. megatherium* and 14 cultures of *B. albolactus* were able to use sodium aspartate and did not grow on asparagine. The larger amount of ammonia formed from the asparagine is to be expected because of the amide group. Blanchetière (1917) found that *B. fluorescens-liquefaciens* first attacked the amide group of asparagine and might leave the amino group practically untouched if sugar were present. Long (1919) found the same to be true with *B. tuberculosis*. An examination of table 3 shows that of the organisms which can use ammonia as a nitrogen source in the presence of sugar all the nonspore-forming organisms, both cocci and rods, are able to use ammonium succinate as a sole source of nitrogen and carbon, whereas none of the spore-forming rods are able to do so.

Of the amino acids, other than aspartic acid, the dicarboxylic

glutamic acid served as the best combined source of nitrogen and carbon. All the organisms except *B. mesentericus* and certain *B. albolactus* cultures were able to use it. None of the cultures except P 147 and P 268 grew in the glycine medium and these cultures grew in every medium, including blanks. The α-alanine medium served as a good differential medium to divide the organisms. None of the organisms appeared to grow to any great extent in the tyrosine medium, although increases in ammonia or changes in the color of the medium were noted with some organisms. In no case was there a marked change in the pH of the tyrosine medium.

The action on these media of the organisms grouped under the name *B. albolactus* deserves special mention. These organisms, which agree with the descriptions of *B. albolactus* usually given, can be split into five distinct subdivisions on the basis of their growth on the amino acids without sugar. Subdivision "A" is positive in sodium aspartate, asparagine and glutamic acid and produces a color change in tyrosine; subdivision "B" is positive in sodium aspartate and glutamic acid and produces a color change in tyrosine; subdivision "C" is positive only in sodium aspartate and colors the tyrosine medium; the organism in subdivision "D" grows only in glutamic acid; and the organism in subdivision "E" grows in none of the media.

It is evident that the organisms may be divided into a number of different groups on the basis of their growth and action on urea, ammonia, asparagine and amino acid media. This will be discussed in more detail in a following paper on classification.

SUMMARY

The 229 cultures of proteolytic organisms from milk were inoculated into synthetic media which contained various simple nitrogenous compounds as a sole source of nitrogen, and growth, increase in ammonia and change in pH were noted. The nitrogenous compounds included sodium ammonium phosphate, ammonium succinate, urea, asparagine and the following amino acids: glycine, alanine, leucine, aspartic acid, glutamic acid, tryptophan and tyrosine.

Some of the organisms which can use urea as a sole source of nitrogen cause an alkaline reaction due to the liberation of ammonia, whereas others cause an acid reaction and apparently liberate no free ammonia.

Organisms which can use ammonia as a sole source of nitrogen can apparently use any of the simpler amino acids if the medium contains a fermentable sugar as a source of carbon.

In media which contain no sugar or similar carbon compound and in which the amino acid has to serve as a source of both carbon and nitrogen, results are obtained which may be useful in grouping the organisms.

The proteolytic bacteria can be differentiated into groups on the basis of their growth and action on sugar media which contain ammonia or urea as their only source of nitrogen and on media which contain ammonium succinate, asparagine or various amino acids as a sole source of both carbon and nitrogen.

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