AMINOACID DEAMINATION BY THERMOPHILIC BACTERIA—PART II

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The thermophilic bacteria are fairly widely distributed in soil, etc., and play a role in thermogenic fermentations. It would be interesting to investigate the aminoacid deaminating properties of these bacteria. The present study is devoted to the deaminating properties of *Bacillus thermophilus*, *B. aerothermophilus* and *B. thermoacidurans*. In Part I of this paper it has been shown that these bacteria oxidatively deaminate a number of aminoacids in an alkaline pH. Results of further investigations on this subject are recorded in this paper.

Methods of Analysis and Experimental Methods

These were in general similar to those described in Part I of this paper.

Role of carbohydrates in deamination by thermophilic bacteria

Stephenson and Gale (1937), Gale and Stephenson (1938) and Gale (1938) have found that *Bacteria coli* cells grown in nutrient broth containing glucose and chalk could not deaminate any of the aminoacids tested; whereas when growth medium was plain nutrient broth the cells could deaminate a large number of aminoacids. According to Lutwak-Mann (1936) *Bacteria coli* cells grown in tryptic broth containing 0·5% glucose could not deaminate or dephosphorylate adenosine triphosphate. Kendall (1922) found that presence of carbohydrates in growing cultures of bacteria prevents the formation of ammonia but it is not clear whether the formation of the deaminating enzyme system is checked or whether the apparent lack of production of free ammonia is due to the utilisation of free ammonia in any side reactions in the growing culture. The effect of carbohydrates on deamination by the thermophilic bacteria has been studied here in its two aspects, *i.e.*, firstly the effect of carbohydrates on the formation of the deaminating enzyme system and secondly their influence on the course of deamination when cells grown in plain nutrient broth were mixed with the aminoacid solutions.

The bacteria were cultivated simultaneously in the following three media:

(i) Nutrient broth.

(ii) Nutrient broth containing 2% dextrose and 1% calcium carbonate.

(iii) Nutrient broth containing 2% lævulose and 1% calcium carbonate.

Growth was more profuse in the media containing dextrose or lævulose as compared to the media without these carbohydrates. Further experimental procedure was as usual. The aminoacid used was *dl*-aspartic acid.

TABLE I

Organism	Growth medium	Mgm. dry wt. of the suspension per 5 ml.	Mgm. ammoniacal nitrogen liberated by 5 ml. of the suspension	Mgm. ammoniacal nitrogen liberated per 10 mgm. dry wt. of cells
B. thermophilus ..	Nutrient broth	12·01	0·522	0·435
	,,	10·89	..	..
	Nutrient dextrose broth	10·78	0·483	0·448
	,,	11·83	0·491	0·415
	Nutrient lævulose broth	13·05	0·557	0·427
	,,	10·81	0·487	0·451
B. aerothermophilus	Nutrient broth	11·09	0·518	0·467
	,,	10·88	..	..
	Nutrient dextrose broth	12·72	0·579	0·448
	,,	12·65	0·576	0·455
	Nutrient lævulose broth	10·35	0·489	0·472
	,,	13·17	0·630	0·478
B. thermoacidurans	Nutrient broth	13·63	0·612	0·449
	,,	10·75	..	..
	Nutrient dextrose broth	12·52	0·545	0·435
	,,	10·63	0·480	0·452
	Nutrient lævulose broth	11·78	0·534	0·453
	,,	11·71	0·535	0·457

It is therefore obvious that the deaminating ability of these bacterial cells per mgm. dry weight is not at all depressed by the presence of dextrose or lævulose in the medium in which they were cultivated, in fact there is often an increase. It may be pointed out further that in the presence of the carbohydrates a far larger crop of the cells was obtained per 100 c.c. of the culture than when no carbohydrate was present in the medium. If ability to deaminate is compared on the basis of unit volume of culture or unit quantity nitrogen compounds present in the medium then the effect of dextrose and lævulose would seem to be to increase the deaminating ability of the cells considerably.

For studying the effect of carbohydrates on the course of deamination by cells grown in plain nutrient broth, dextrose or lævulose was added in a 2% concentration to a M/40 mixture of *dl*-aspartic acid and McIlvaine's

citrate-phosphate buffer. In the control experiments the addition of carbohydrates was omitted. Blank experiments were set up simultaneously both with and without dextrose and lævulose but omitting the aminoacid.

TABLE II

Organism	Remarks	Mgm. of ammonical nitrogen liberated per 5 ml. of cell suspension
B. thermophilus	No carbohydrate	0·575
	Do.	0·567
	2% Dextrose	0·513
	Do.	0·485
	2% Lævulose	0·623
	Do.	0·509
B. aerothermophilus	No carbohydrate	0·532
	Do.	0·539
	2% Dextrose	0·607
	Do.	0·522
	2% Lævulose	0·562
	Do.	0·478
B. thermoacidurans	No carbohydrate	0·505
	Do.	0·513
	2% Dextrose	0·575
	Do.	0·495
	2% Lævulose	0·483
	Do.	0·500

The effect of carbohydrates on the actual course of deamination is therefore irregular and inconsiderable, but there is no suppression of deamination.

Autodeamination of bacterial cells

It was noticed that cell suspensions of the organisms under study produced small but measurable amounts of ammonia even in the absence of any added aminoacids. To study this phenomenon in greater detail washed cell suspensions were prepared as usual. 10 ml. were added to 25 ml. of McIlvaine's citrate-phosphate buffer of appropriate pH and an aliquot portion was withdrawn immediately after shaking for analysis. The remainder of the mixture was incubated for thirty hours at 40° C. and analysed again for free ammonia. The data is summarised in Table III.

Autodeamination therefore takes place with all the three bacteria. The optimum pH is in the alkaline region; being 8·3 to 8·5 for *B. thermophilus* and *B. aerothermophilus* and about 8·0 for *B. thermoacidurans*.

TABLE III

Organism	pH	Mgm. of ammoniacal nitrogen liberated per 5 ml. of the suspension
B. thermophilus	5·5	0·013
	6·2	0·027
	6·9	0·041
	7·6	0·063
	8·3	0·093
	9·0	0·075
	9·7	0·037
B. aerothermophilus	5·5	0·017
	6·2	0·037
	6·9	0·059
	7·6	0·087
	8·3	0·112
	9·0	0·101
	9·7	0·065
B. thermoacidurans	5·5	0·025
	6·2	0·043
	6·9	0·061
	7·6	0·095
	8·3	0·107
	9·0	0·083
	9·7	0·053

Course of autodeamination at optimum pH

The previous experiments were repeated at optimum pH and with larger quantities of cell suspensions and buffer solutions. Samples were withdrawn at various intervals for analysis as shown below. The flasks were stoppered immediately after withdrawing a sample so as to prevent the loss of ammonia. The results are summarised in Table IV.

TABLE IV

Time after mixing Hours	Mgm. of ammoniacal nitrogen contained in by 5 ml. of suspension of washed cells		
	B. thermophilus	*B. aerothermophilus*	*B. thermoacidurans*
3 ..	0·000	0·000	0·000
6 ..	0·003	0·002	0·000
9 ..	0·010	0·007	0·000
13 ..	0·023	..	0·021
18 ..	0·046	0·041	0·051
24 ..	..	0·083	..
30 ..	0·109	0·141	0·121
36 ..	0·138	..	0·157
42 ..	0·147	0·203	..
48 ..	0·153	0·218	0·187

Very little autodeamination therefore takes place during the first few hours probably because very little aminoacids have been liberated through autolysis. As soon as appreciable autolysis has taken place deamination starts and proceeds more and more rapidly like an autocatalytic process till after thirty-six to forty-eight hours the rate decreases once again. Curves for rate of autodeamination have the S-shape typical of autocatalytic processes.

Effect of carbohydrates on autodeamination

5 ml. portions of the washed suspensions were mixed with 15 ml. of 2·5% dextrose or lævulose solutions prepared in buffer of pH equal to the optimum pH of autodeamination for each bacteria. In the control experiments no sugar was added to the buffer. Increase of ammonia was determined after incubation at 40° C. for twenty-four hours.

TABLE V

Organism	Carbohydrate	Increase in ammoniacal nitrogen, mgm./5 ml. of suspension
B. thermophilus	Nil	0·201
	Dextrose	0·088
	Lævulose	0·118
B. aerothermophilus	Nil	0·192
	Dextrose	0·108
	Lævulose	0·143
B. thermoacidurans	Nil	0·113
	Dextrose	0·064
	Lævulose	0·072

Thus both dextrose and lævulose suppress autodeamination.

Effect of storing suspensions on their deaminating ability

100 ml. of a washed suspension was prepared in each case and duplicate deamination experiments were carried out with aliquot portions from each suspension after every twenty-four hours for seven days. Each sample was withdrawn after shaking the suspensions well. The deaminating ability was tested against *dl*-aspartic acid at optimum pH.

Thus the deaminating capacity of cell suspensions of *Bacillus thermophilus* increases for three to four days and then decreases whereas that of *B. aerothermophilus* and *B. thermoacidurans* cell suspensions starts decreasing gradually from the very beginning.

TABLE VI

Age of Suspension Hours	Mgm. of ammoniacal nitrogen liberated by 5 ml. of the suspension		
	B. thermophilus	*B. aerothermophilus*	*B. thermoacidurans*
0 ..	0·563	0·586	0·621
24 ..	0·972	0·557	0·622
48 ..	1·432	..	0·601
72 ..	1·322	0·438	..
96 ..	..	..	0·465
120 ..	1·033	0·322	..
144 ..	..	..	..
168 ..	0·686	0·187	0·192

Effect of storing suspensions under hydrogen atmosphere

The experimental details regarding removal of oxygen and creation of as nearly perfect an hydrogen atmosphere as possible were the same as in case of deamination experiments under hydrogen, described in Part I of this paper. Each time a sample of the suspension was withdrawn for examination, the remainder of the suspension was re-evacuated and bubbled through with hydrogen. The aminoacid employed was M/40 *dl*-aspartic acid at optimum pH.

TABLE VII

Age of suspension. Hours	Mgm. of ammoniacal nitrogen liberated per 5 ml. suspension		
	B. thermophilus	*B. aerothermophilus*	*B. thermoacidurans*
0 ..	0·593	0·563	0·603
24 ..	1·457	0·561	0·611
48 ..	1·486	0·568	0·600
72 ..	..	..	..
96 ..	1·472	..	..
120 ..	1·441	0·542	0·587

Thus the decrease in deaminating ability noted in the previous experiment is not conspicuous when the storage is done under a reducing atmosphere. The decrease of activity of suspensions of *Bacillus aerothermophilus* and *B. thermoacidurans* is not very significant. The initial increase in activity of *B. thermophilus* suspensions is evident in this experiment also, but unlike storage under atmospheric conditions the subsequent decline in activity is not very noticeable here. Thus the destruction of deaminating power is an oxidative process.

Storing of suspensions and diffusion of the deaminating complex from the bacterial cells into the surrounding liquid

Suspensions were stored under hydrogen. After varying lengths of time samples were withdrawn and one-third of the sample was divided into two portions for estimation of the deaminating power of the whole suspension in duplicate. The remainder of the sample was centrifuged at 3,000 r.p.m. for ten minutes to give a sharp separation of a clear liquid and a sediment of cells. The clear liquid was carefully decanted off and its deaminating capacity determined. The sediment of cells was washed once in the centrifuge with a little distilled water and resuspended in distilled water so as to give the same volume as the volume of the suspension from which the cells were derived. The deaminating capacity of this suspension was also determined. In all cases the aminoacid employed was M/20 *dl*-aspartic acid at optimum pH.

TABLE VIII

Organism	Age of suspension	Mgm. ammoniacal nitrogen liberated by 5 ml. of whole suspension or its equivalent of cells or cell-free suspension		
		Whole suspension	Cell-free juice	Cells only
B. thermophilus ..	Fresh	0·621	0·008	0·622
	48 hours old	1·417	1·473	0·609
	96 ,,	1·625	1·886	0·600
B. aerothermophilus ..	Fresh	0·600	0·022	0·608
	48 hours old	0·589	0·134	0·601
	98 ,,	0·569	0·382	0·593
B. thermoacidurans ..	Fresh	0·584	0·043	0·571
	48 hours old	0·588	0·182	0·575
	98 ,,	0·573	0·349	0·569

The enzyme or enzyme complex responsible for deamination therefore diffuses out into the surrounding cell-free liquid during storage. A curious result is that when a forty-eight hour or ninety-six hour old suspension is separated into its cell portion and the cell-free juice, then the sum of the amount of ammonia liberated by these two fractions is much greater than that liberated by the original whole suspension or by the original whole suspension divided into two portions. In contrast to fresh whole suspensions whose deaminating properties are destroyed by narcotics and in contrast to all whole suspensions or cell portions thereof whose deaminating properties are suppressed by narcotics, such reagents have practically no effect on the deaminating power of the cell-free diffusion factor obtained by centrifuging

suspensions which have been stored for two to four days under hydrogen This will be obvious from Table IX.

Effect of narcotics on the deaminating power of cell-free diffusion factor

The effect of chloroform and toluene was examined.

TABLE IX

Organism	Treatment	Mgm. of ammoniacal nitrogen liberated per 5 ml. of whole suspension or the equivalent quantity of cells or cell-free juice			
		Fresh suspension	48 hours' old suspension	Cells from 48 hours' old suspension	Cell-free juice from 48 hours' old suspension
B. thermophilus ..	No treatment	0·578	1·431	1·401	0·867
	Chloroform	0·010	0·212	0·096	0·871
	Toluene	0·017	0·302	0·117	0·847
B. aerothermophilus	No treatment	0·744	0·737	0·712	0·363
	Chloroform	0·013	0·103	0·086	0·341
	Toluene	0·031	0·166	0·098	0·372
B. thermoacidurans	No treatment	0·663	0·671	0·643	0·296
	Chloroform	0·003	0·132	0·066	0·290
	Toluene	0·011	0·168	0·087	0·301

Relative thermostability of the endocellular and exocellular deaminating systems

The thermostability of the endocellular and exocellular deaminating enzyme systems was determined separately and the results summarised in the following table show that the exocellular enzyme is relatively more thermostable than the endocellular enzyme with all three bacteria. In all these experiments the portions of cell suspension or the cell-free centrifugates were kept at the stated temperature for five minutes and their deaminating power was then studied against *dl*-aspartic acid at optimum pH.

TABLE X

Temperature of heating °C.	Mgm of ammoniacal nitrogen liberated by cells or cell-free liquid equivalent to 5 ml. of suspension					
	B. thermophilus		*B. aerothermophilus*		*B. thermoacidurans*	
	Endocellular	Exocellular	Endocellular	Exocellular	Endocellular	Exocellular
40 ..	0·386	0·342	0·415	0·311	0·473	0·314
45 ..	0·389	0·346	0·423	0·318	0·479	0·304
50 ..	0·363	0·334	0·411	0·304	0·461	0·309
55 ..	0·311	0·341	0·414	0·322	0·471	0·318
60 ..	0·215	0·322	0·497	0·317	0·465	0·311
65 ..	0·068	0·295	0·343	0·309	0·423	0·306
70 ..	..	0·161	0·041	0·301	0·036	0·295
75 ..	..	0·008	..	0·213	..	0·201

Discussion and Summary

The thermophilic bacteria *Bacillus thermophilus, B. aerothermophilus* and *B. thermoacidurans* oxidatively deaminate aminoacids. Cells grown in carbohydrate broth are as efficient deaminating agents as those grown in plain broth. In fact as the growth is much more profuse in presence of carbohydrates like dextrose and lævulose the total quantity of deaminating enzyme system formed in a given volume of the growth medium is greater in presence of the carbohydrates than in their absence. Presence of carbohydrates during actual deamination in mixtures of washed cell suspensions and aminoacid solutions does not have any significant effect. In their behaviour towards carbohydrates therefore the thermophilic bacteria and their deaminating enzymes behave in a manner different from that of other bacteria studied by previous investigators. Apart from deaminating added aminoacids the cells can deaminate the aminoacids produced by their own autolysis. The time curve of this process possesses the S-shape typical of autocatalytic processes. In the beginning when only small amounts of aminoacids have been produced the rate of ammonia formation is small; as autolysis results in the formation of more and more aminoacids the rate of ammonia production also increases rapidly till after thirty-six or forty-eight hours the rate decreases once again. When dextrose or lævulose is added to the cell suspensions undergoing autolysis and autodeamination then the amount of ammonia formed is decreased; in this respect therefore autodeamination differs from deamination of added aminoacids.

The deaminating capacity of cell suspensions of *Bacillus aerothermophilus* and *B. thermoacidurans* gradually decreases on storage, whereas that of *B. thermophilus* cell suspensions increases at first and then decreases. This decrease of deaminating ability is considerably reduced if the cell suspensions are stored in the absence of oxygen. Destruction of the deaminating enzyme system is therefore an oxidative process. Cell-free centrifugates obtained from stored cell suspensions go on increasing in deaminating ability with the increase in age of the suspension. The residual cells are still capable of deamination. When a two or three days old suspension is seperated into its cell portion and the cell-free liquid then the sum of the amount of ammonia liberated separately by these two portions is greater than that produced by the original whole suspension or by the original whole suspension divided into two parts. Chloroform and toluene destroy the deaminating activity of fresh cell suspensions, that of stored suspensions is only partially suppressed, whereas cell-free centrifugates of stored suspensions are not affected by chloroform or toluene. The thermostability of the deaminating enzyme in the cell-free liquid is greater than that of the

deaminating enzyme in the cells. It would therefore appear that with all the three bacteria under study there exist two distinct deaminating enzymes. One of these is endocellular and is intimately connected with the life-process of the cells and is therefore rendered ineffective when the cells are treated with toluene or chloroform, or are warmed to their thermal death point. The second deaminating enzyme system is exocellular, and is formed when cell suspensions are stored at room temperature especially under hydrogen. This exocellular factor is not affected by toluene or chloroform and is relatively more thermostable than the endocellular deaminating enzyme system. The endocellular enzyme is gradually destroyed when the suspensions are stored under atmospheric conditions. The exocellular deaminating enzyme system is much more stable against oxidative destruction than the endocellular deaminating enzyme. When both are present together, as for example in whole suspensions which are two or three or more days' old, then they partially and reversibly neutralise each other since the amount of ammonia produced by the two factors separately is much greater than when they are both present together.

BIBLIOGRAPHY

1. Gale and Stephenson .. *Biochem. J.*, 1938, **32**, 392.
2. Gale .. *Ibid.*, 1938, **32**, 1583.
3. Kendall .. *J. Inf. Dis.*, 1922, **30**, 211, etc.
4. Lutwak-Mann .. *Biochem. J.*, 1936, **30**, 1405.
5. Stephenson and Gale .. *Ibid.*, 1937, **31**, 1316.