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The Formation of Certain Transaminases and its Control during the Growth of *Streptococcus thermophilus*

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In this investigation the transaminase activities of 27 different *Streptococcus thermophilus* strains and the effect of end product repression on the formation of the transaminases were studied. Variations were observed in the transaminase activities of the different strains. In general, aspartate was the most effective amino group donor. Isoleucine, leucine, valine, phenylalanine, tryptophan, tyrosine, and methionine were effective amino group donors in addition to aspartate, but arginine, asparagine, cysteine, cystine, glycine, histidine, and lysine were either weak amino donors or did not take part in the reaction. The abilities of serine and threonine to act as amino group donors varied markedly in the different strains.

As a part of a research program dealing with enzymatic activity during the active growth periods of bacteria, we have now established that variations exist between different strains of *Strepotococcus thermophilus* in the formation of transaminases promoting the reaction:

L-Amino acid + α-ketoglutaric acid $\rightleftharpoons$ keto acid + L-glutamic acid.

In addition it was studied whether the formation of transaminases is repressed in the same way in lactic acid bacteria as Raunio[1] has recently found in this laboratory to be the case in *Escherichia coli* cells.

EXPERIMENTAL

Growth organisms and their cultivation. For this investigation 27 different *Streptococcus thermophilus* strains were chosen (Table 1). In the experiments on enzyme formation, only the strain KQ was employed because the activities of enzymes have been more closely studied with this strain than with the others in this laboratory.

The strains were transferred at intervals of 3–4 weeks to a new TSHGA medium with the following composition: 2 g of Bacto tryptone, 0.5 g each of lactose, glucose, sucrose, and yeast extract, 0.25 g of gelatine and 70 mg of ascorbic acid in 100 ml of water (pH 6.7–6.8, adjusted with NaOH). After each transfer the tubes were incubated 10–15 h at 37° and then preserved at + 2°.

Generally the TSHGA medium was used also as the inoculum medium. In some of the experiments on end-product repression, however, an ScQ medium with the following composition was employed: 5.0 g each of sucrose and lactose, 5.2 g of β-glycerophosphate, 3.4 g of sodiumacetate, 0.2 g of ascorbic acid, 0.267 g of NH_4Cl, 0.146 g of L-glutamic acid, 0.06 g of L-cysteine, 0.307 g of $MgSO_4 \cdot 7H_2O$, 0.746 g of KCl, 0.03 g of $CaCl_2$, 1 mg each of thiamine, nicotinic acid, pyridoxal phosphate, riboflavin, and *p*-aminobenzoic acid, 0.5 mg of calcium pantothenate, and 1 μg of biotin in 1 litre of once distilled water. The medium was adjusted to pH 6.8 with sodium hydroxide and sterilized 10 min at 105°.

The growth medium was either the TSHGA or the ScQ medium, of which 200-ml aliquots were added to vessels of 500 ml capacity. Before the inoculation the cells had been incubated in the inoculum medium overnight (10—14 h) at 42°, centrifuged 10—15 min at 4000—5000 *g* and washed twice with 0.9 % sodium chloride. During the growth the medium was gently stirred. The samples were generally withdrawn at intervals of 15 min, their turbidities were measured with a Klett-Summerson colorimeter using filter 62, and centrifuged after cooling 5—7 min at 1500 *g*. The cells were then washed once with cold 0.9 % sodium chloride solution.

Table 1. Transaminase activities of 27 different strains of *Streptococcus thermophilus*. The transaminase activity the enzyme preparations as controls before the reactions were deducted from those obtained after the reactions.

Amino donor	*Str. thermophilus* strains													
	10	10R	11R	12	40	50	60	70	71	72	74	75	76	77
Ala	0	2.0	3.4	0	2.5	0	3.2	0	3.2	1.0	1.2	1.4	1.2	2.0
	3.8	1.1	2.0	1.0	3.8	1.4	4.3	2.7	3.5	1.8	1.2	2.5	1.0	0
Arg	0	0	0	0	0	1.8	1.1	0	2.7	1.2	2.0	0	0	3.4
Asp	42	83	58	88	38	6.0	24	11.8	70	93	10.0	103	120	115
	91	120	128	125	60	10.5	16.0	18.8	75	50	34	64	57	92
Asp—NH_2	0	0	1.3	0	0	0	0	0	0	0	1.2	0	0	0
CySH	2.3	3.4	2.7	3.2	2.2	1.0	0	2.0	3.0	3.3	3.0	2.0	0	5.2
	0	4.0		0	0	0	0	1.0	4.0	3.5	0	3.4	1.1	0
CyS^-	0	2.5	5.3	0	2.5	0	1.2	4.0	3.5	2.3	7.2	0	0	4.3
	0	3.2		2.0	0	1.0	0	0	4.7	4.2		4.6		
Gly	2.3	3.0	2.5	1.2	1.0	1.0	3.0	2.2	1.5	0	1.6	2.4	3.2	7.2
	3.0	1.0	2.1	2.5	3.2	3.8	1.5	2.8	2.0	1.2	1.2	1.4	0	0
His	0	0	0	0	0	0	0	0	0	0	2.0	0	0	0
Ile	20	13.4	10.2	14.0	11.0	0	8.8	1.5	3.5	17.8	6.3	23	4.8	20
	20	10.2	17.7	7.8	2.1	4.6	6.0	2.3	9.1	9.4	5.9	9.1	6.2	12.2
Leu	27	29	17.5	24	18.5	2.3	19.7	4.0	6.8	31	10.5	45	9.6	34
	27	13.9	12.3	14.7	2.7	8.4	7.2	3.2	15.1	16.8	11.3	20	10.0	17.0
Lys	0	0	0	0	0	1.7	0	0	0	0	3.6	0	0	1.7
Met	7.5	12.5	10.4	6.5	9.5	0	9.6	2.7	6.5	13.5	5.0	17.5	0	11.7
	11.5	11.5	9.0	12.6	1.7	3.7	4.0	1.7	21	18.0	6.2	18.2	6.7	8.8
Phe	6.2	10.3	7.2	8.3	5.8	3.7	7.7	0	5.7	6.7	26	16.3	2.0	17.7
	10.0	5.4	7.9	5.1	2.7	2.2	4.2	1.0	6.1	5.1	8.1	19.2	4.5	3.8
Ser	2.4	1.4	1.7	0	0	0	6.0	1.0	0	1.5	4.2	1.9	0	4.2
	3.6	2.0	1.5	1.2	0	6.7	1.0	4.5	4.6	0	2.6	0	2.1	2.8
Thr	1.2	1.9	0	0	0	0	3.7	0	0	4.6	3.6	2.2	2.7	1.5
	3.6	3.4	2.0	1.4	0	3.6	7.4	1.5	4.2	1.8	3.1	1.5	1.8	0
Try	5.7	8.3	6.8	9.5	4.8	2.8	6.7	0	4.5	4.5	22	14.1	1.5	13.2
	8.4	7.6	5.8	6.7	2.7	2.6	3.0	2.1	4.5	4.2	5.6	25	3.0	9.2
Tyr	4.0	5.3	4.5	6.7	3.0	2.7	4.0	0	3.0	2.0	15.7	12.2	0	10.7
	6.0	5.0	4.6	4.2	1.9	2.2	2.3	1.5	3.0	2.5	4.1	22	2.2	4.4
Val	18.7	11.2	8.8	10.3	9.3	0	10.5	0	3.2	17.2	6.8	17.9	3.5	13.0
	17.2	8.6	5.3	7.8	2.0	2.3	3.0	2.3	9.1	7.8	5.9	8.2	5.0	10.0

Enzyme preparation. The washed cells were cooled to −35° (mostly overnight) and thawed at 37° for 2−3 min. After this 0.5 ml of 0.05 M Tris-HCl buffer, pH 8.0, was pipetted over the cell mass and the tubes were cooled to −35° during about 5 min and thawed as above. The milky extract was used for chromatographic enzyme activity determinations.

Determination of transaminase activity. For the activity determinations 50 µl of enzyme preparation and 50 µl of substrate solution containing 10 µmoles of an L-amino acid, 20 µmoles of α-ketoglutaric acid and 5 µg of pyridoxal phosphate in 0.05 M Tris-HCl buffer, pH 8.0, were pipetted into 60 × 7 mm test tubes. The reaction mixture was incubated at 37° and the reaction was stopped after 90 min. The reaction was stopped with 25 µl of 5 N sulphuric acid and the precipitated protein was centrifuged. A 25-µl volume of the supernatant was pipetted onto Whatman No. 1 paper and the substrate amino acid and L-glutamic acid were separated chromatographically using the solvents butanol:glacial acetic acid:water 120:30:50 (leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, and methionine), ethanol:water-ammonia 180:10:10 (serine, threonine, alanine, and glycine), pyridine:glacial acetic acid:water 100:70:30 (aspartic acid), and phenol:water 4:1 (histidine, lysine, arginine, and asparagine). After the run the papers

given in mU/mg of dry weight of cells. When calculating the enzyme activities Klett readings obtained with ll dry weights were calculated using the dry weight-turbidity curve determined for strain KQ.

Str. thermophilus strains													*Lactobacillus arabinosus*
78	90	100	101	110	Th 1187	Th 7952	Th	Ths	H.th.str.	Kungsb.	Ylil.	KG	
0	0	0	0	2.8	1.8	2.0	0	0	1.2	2.5	2.0	0	4.8
4.2			0	5.6	2.4	4.8	1.6	0	5.4	5.6	1.4		3.0
0	12.5	0	2.4	1.8	0	3.6	7.0	2.4	3.3	7.2	0	4.8	
20	102	124	8.1	18.2	107	48	55	84	54	19.6	81	32	80
37	11.5	37	77	42	35	30	65	27	27	30	55		57
0	1.0	0	0	3.6	0	0	0	0	0	2.0	0		
0	0	0	5.4	12.8	4.7	1.5	2.3	3.1	2.5	2.0	5.8	0	2.5
2.3		2.1		2.7	4.5	3.2	0	2.2	1.4	1.1	4.0	3.8	0
0	3.7	0	4.6	9.1	0	0	3.2	2.8	0	3.8	8.7	0	3.5
2.1		0		4.8	1.5	0		1.1	1.0	1.0	3.2	1.5	0
0	0	0	10.0	2.0	0	1.2	3.4	0	2.0	1.7	1.3	1.1	1.1
1.4	0	2.7	0	1.6	0	5.2	0		3.2	4.3	1.5		1.6
0	2.7	2.4	0	0	0	0	4.5	0	0	1.2	0	0	
9.8	17.1	4.4	2.9	20	7.3	6.7	22	28	7.3	11.0	22	4.5	61
25	2.1	12.5	9.6	4.8	14.9	8.8	4.7	7.5	10.4	8.5	7.4	5.8	26
13.0	2.4	6.6	5.8	27	8.8	7.2	40	45	8.0	17.0	34	6.0	91
37	1.8	23	15.0	8.0	19.7	13.4	8.3	9.6	13.5	12.5	12.0	8.0	37
1.6	0	1.9	0	0	0	0	1.6	0	0	1.6	5.2	1.8	
0	5.6	0	1.9	12.0	6.6	4.0	14.3	26	5.0	3.1	18.6	9.9	40
28	4.4	12.5	8.2	4.8	13.4	15.8	5.4	8.2	11.7	16.0	16.0	10.0	21
0	9.7	0	0	24	3.8	0	13.3	20	3.7	13.1	13.1	8.8	52
20	4.8	7.3		7.8	10.0	16.5	1.7	5.0	7.2	23	13.2	10.5	15.2
0	1.6	10.5	10.2	2.5	2.2	0	0	1.2	0	2.8	2.4	1.0	1.8
8.4	0	0	1.2	4.8	2.5	4.6	1.0		3.0	3.7	0		1.2
0	4.2	12.7	10.5	1.5	0	0	0	1.3	1.3	2.7	1.0	0	2.5
11.0	0	5.7	3.8	3.8	2.4	3.7	1.2		4.2	5.8	2.2		2.2
2.9	12.4	0	0	22.	0	0	11.3	18.1	2.8	12.5	10.5	6.1	37
21	4.0	7.3	10.0	6.0	7.5	10.0	0	5.0	5.0	20	17.2	8.5	14.0
0	8.4	0	0	12.8	0	0	7.5	13.6	1.5	8.2	7.3	4.1	22
18	1.8	5.7	4.8	5.2	4.8	7.4	0	4.3	3.6	15.2	14.9	5.7	76
7.2	13.4	3.0	4.6	17.0	6.4	4.0	17.3	18.1	5.0	8.0	18.4	4.2	54
21	1.8	9.9	9.4	3.8	12.7	5.8	4.8	6.4	7.7	7.0	7.2	5.0	26

were dried either at room temperature or at 40–50°. When dry, they were sprayed with 0.2 % ninhydrine in acetone containing 5 % pyridine. The colour was allowed to develop overnight at room temperature, after which the glutamic acid spots, all equal in size, were extracted with 2.5 ml of methanol and the intensity of the colour was measured with the Klett-Summerson colorimeter employing filter 54. In the control test 50 μl of water was substituted for the substrate solution. The reading in this test (on average 30–40 Klett units) was subtracted from the values obtained in the actual analysis.

The activity was calculated as enzyme units, mU/mg of dry weight or mU/ml of growth medium (mU = μmoles of glutamic acid formed/min at 37°). Before the dry weight was determined, the cells had been washed twice with 0.9 % saline and once with distilled water and then dried at 105° overnight. After the dry weights of cells had been plotted against the Klett readings obtained for cell suspensions, the dry weight of cells could be determined from the colorimeter readings. By this procedure it was found, for example, that the Klett reading 100 (filter 62) corresponded to a dry weight of 0.37 mg of cells per ml.

RESULTS

Transamination reactions. Previously it has been found in this laboratory that many *Streptococcus thermophilus* strains require growth media with a more complicated amino acid composition than does strain KQ, which requires only the amino acids L-glutamic acid and L-cysteine for growth.[2] In the present investigation, in order to determine a possible relationship between transaminase activities and the amino acid requirements, 27 different strains were

Table 2. Formation of transaminases during the growth of the *Str. thermophilus* strain Ths in media ScQ (part A) and TSHGA (part B). The enzyme activity is expressed in mU/ml of growth medium. Growth was measured with a Klett-Summerson colorimeter employing filter 62.

A Transaminase	Growth time, min									
	0	15	30	45	60	75	90	105	120	220
Ile	0.68	1.44	3.32	5.52	5.88	6.12	6.00	6.17	6.00	5.04
Leu	1.20	2.64	5.97	8.20	9.20	10.23	10.31	10.68	9.65	8.61
Val	0.80	1.60	3.36	5.08	5.60	5.84	5.92	5.60	5.70	4.77
Phe	0.40	0.56	1.08	1.84	3.12	4.32	5.00	5.37	4.28	2.56
Try	0.52	0.64	1.36	1.92	3.04	4.08	5.40	5.32	3.92	2.64
Tyr	0.40	0.64	0.80	1.32	2.48	3.36	3.92	4.40	3.04	1.94
Asp	3.60	6.16	12.2	16.6	16.2	19.6	18.8	16.4	15.4	7.40
Growth	53	50	50	51	53	56	60	65	70	95

B Transaminase	Growth time, min									
	0	10	30	40	50	60	70	80	90	100
Ile	0.72	1.28	1.44	2.00	2.44	2.60	2.36	2.00	2.36	2.24
Leu	0.96	1.88	2.56	4.32	4.92	4.96	5.04	4.80	5.20	5.48
Val	0.40	0.72	1.32	1.68	2.08	2.20	2.08	1.95	2.08	2.08
Phe	0.40	0.76	1.20	1.72	2.20	2.28	2.16	2.38	2.38	2.12
Try	0.32	0.52	1.04	1.56	1.96	2.32	204	2.24	2.36	2.16
Tyr	0.24	0.40	0.68	1.32	1.60	1.92	1.80	1.85	2.00	1.92
Asp	1.72	7.00	12.9	16.2	17.0	17.4	17.2	16.8	16.8	17.2
Growth	53	53	51	51	56	59	63	66	70	77

tested for their ability to promote the transamination reactions in which α-ketoglutaric acid functions as the acceptor of the amino group and the protein amino acids as donors. Table 1 shows the results of two parallel tests. For the determinations of enzyme ativity the strains were grown in the TSHGA medium and the samples from which the frozen and thawed preparations were made were withdrawn at the beginning of the exponential phase when the enzyme activity is at a maximum (Table 2). The results presented in Table 1 show that, in general, the transaminase activity was high when aspartic acid, isoleucine, leucine, valine, methionine, phenylalanine, tryptophan, or tyrosine functioned as donor of the amino acid group to α-ketoglutaric acid, and weak or absent when alanine, arginine, asparagine, cysteine, cystine, glycine, histidine, or lysine were the donors. When the amino donors were serine and threonine, marked variations were observed between different strains and the enzyme activity was mostly weak. Table 1 shows also many marked differences between the parallel determinations, a fact that may have been due to varying degrees of inactivation of the transaminases during the treatment of the preparations. *Lactobacillus arabinosus* was also included among the tested organisms. The values obtained with this organism corresponded to previous values.[3]

Formation of transaminases. Seven amino donors were chosen on the basis of the values given in Table 1. Transaminase activities were determined during

Table 3. Formation of transaminases during growth of strain KQ in media ScQ (A) and TSHGA (B). Explanations as in Table 2.

A	Transaminase	Growth time, min									
		0	15	35	50	65	80	100	120	150	180
	Ile	1.24	2.20	3.28	3.24	3.40	3.40	3.20	2.76	2.28	2.00
	Leu	1.92	2.68	5.48	5.80	5.68	5.28	4.76	2.64	3.16	
	Val	1.36	2.00	3.00	3.20	3.40	3.60	3.20	3.20	3.40	3.20
	Phe	0.92	1.48	2.64	2.88	2.80	2.44	2.32	2.00	1.60	1.36
	Try	1.04	1.60	3.28	3.32	3.04	2.80	2.72	2.48	1.96	1.72
	Tyr	0.68	1.08	2.68	2.52	2.00	2.12	2.08	1.72	1.48	1.12
	Asp	3.60	4.40	11.2	15.8	18.4	19.6	18.8	18.0	17.6	19.2
	Growth	127	116	116	117	123	134	147	163	186	192

B	Transaminase	Growth time, min									
		0	10	20	30	40	50	60	70	80	90
	Ile	0.64	0.80	0.80	1.00	1.28		2.24	2.08	2.20	1.92
	Leu	0.96	0.96	1.28	2.00	2.76	3.68	3.40	3.76	3.56	
	Val	0.40	0.56	0.80	1.16	1.48	2.60	2.60	2.40	2.64	2.32
	Phe	0.36	0.68	0.68	0.92	1.56	2.48	2.56	2.72	2.48	2.24
	Try	0.40	0.72	0.76	1.00	1.40	2.24	2.40	2.56	2.28	2.12
	Tyr	0.32	0.52	0.60	0.76	0.92	1.64	1.80	1.64	1.48	1.36
	Asp	0.80	1.40	3.08	4.16	6.60	9.80	9.92	9.20	5.60	4.24
	Growth	116	107	113	120	133	148	163	175	186	196

the growth of strains Ths and KQ. The seven amino donors were isoleucine, leucine, valine, phenylalanine, tryptophan, tyrosine, and aspartic acid. Table 2 shows the formation of the transaminases in strain Ths in the growth media ScQ and TSHGA. As can be seen from the table, the maximum activity values (mU/ml of growth medium) were obtained after incubation for 75—105 min in medium ScQ, or when the cells were in the acceleration phase of growth. With isoleucine and leucine the increase in activity was about 9-fold, with valine 7-fold, with phenylalanine 13-fold, with tryptophan 11-fold, with tyrosine 10-fold, and with aspartic acid about 5-fold. It should also be noted that once the enzyme concentration had reached a maximum, it remained at the same level for a comparatively long time before it began to decrease. In addition, it can be seen from Table 2 that the formation of all the enzymes started at the very beginning of cell growth. The rate of formation of enzymes for the reactions in which phenylalanine, tryptophan or tyrosine functioned as amino donors was lowest. The increase in enzyme activity was greater in medium ScQ than in the TSHGA medium (with isoleucine about 4-fold, with leucine and valine 5-fold, with phenylalanine 6-fold, with tryptophan 7-fold, with tyrosine 8-fold, and with aspartic acid about 10-fold). The enzyme formation was maximal between 40 and 80 min after the inoculation, and thus earlier than in medium ScQ. However, just as in medium ScQ, the enzyme content remained at the same level for a comparatively long time after the maximum had been reached.

Table 3 shows values obtained with strain KQ in the ScQ and TSHGA media. These values correspond to those in Table 2 obtained with the strain Ths. In medium ScQ the rate of formation of enzymes was about 3-fold when the substrates were isoleucine, leucine, valine, phenylalanine, and tryptophan, about 4-fold with tyrosine, and about 5-fold with aspartic acid. The enzyme level was usually a maximum 65—80 min after the inoculation. In the TSHGA medium the corresponding activity values were 3-fold for isoleucine, 4-fold for leucine, 6-fold for valine, 8-fold for phenylalanine, 6-fold for tryptophan and tyrosine, and about 12-fold for aspartic acid, and the formation of enzymes was maximal about 40—80 min after the beginning of growth. Also with strain KQ the enzyme formation began immediately after the inoculation

Table 4. Effects of oxalacetic acid (OA) and L-aspartic acid (Asp) on the formation of aspartate transaminase during the growth of strain KQ. The activity is expressed as mU/ml of growth medium and the growth as Klett-Summerson readings obtained using filter 62.

Addition		Growth time, min								
		0	15	30	45	60	75	90	120	180
—	Growth	67	59	60	60	61	63	65	68	78
	Activity	1.24	2.32	2.44	2.30	2.96	4.20	4.56	4.40	4.32
OA	Growth	1.24	59	60	60	60	63	65	69	82
	Activity	1.24	2.12	2.52	2.60	3.24	4.16	4.40	4.36	4.52
Asp	Growth	1.24	59	59	58	59	60	61	65	72
	Activity	1.24	2.20	2.20	2.48	2.80	3.56	4.20	4.52	3.80

Table 5. Effect of oxalacetic acid (OA) and L-aspartic acid (Asp) on the formation of aspartate transaminase during the growth of strain KQ when the cells used as inoculum had grown for 40 min in a medium containing no nitrogen sources. The activity is expressed as in Table 4.

Addition		0	10	20	30	40	50	70	90
—	Growth	135	129	129	129	128	129	130	132
	Activity	4.44	4.52	5.92	9.56	12.24	13.48	14.12	13.88
OA	Growth		129	127	130	129	130	132	133
	Activity		4.36	5.24	8.40	11.40	14.36	14.32	13.84
Asp	Growth		127	128	127	126	126	127	128
	Activity		4.00	5.40	7.52	11.76	12.24	13.88	13.88

and the rate of formation was lowest in the case of those enzymes which promoted amino group transfer by aromatic amino acids.

Repression of the formation of transaminases. The results presented in Tables 2 and 3 indicate that the formation of transaminases may be repressed; the enzyme formation was smaller in medium TSHGA than in medium ScQ, the latter of which contained no other amino acids than L-glutamic acid and

Table 6. Effect of aromatic amino acids on the formation of the phenylalanine, tryptophan and tyrosine transaminase during growth of strain KQ. Enzyme activity: mU/ml. The experiment is described in the text.

Additions to growth media	Substrates	Growth time, min							
		0	15	30	45	60	75	90	110
1	Growth	148	137	137	138	145	147	152	162
—	Phe	0.70	1.20	1.48		1.92	3.16	3.04	
	Try	0.60	1.20	1.28	1.84	1.76	2.80	2.80	
	Tyr	0.40	0.60	1.00		1.16	1.52	1.60	
2	Growth	148	139	138	138	145	147	150	161
Phe	Phe	0.70	1.16	1.20	1.70	1.86	3.08	3.16	
	Try	0.60	1.04	1.12	1.60	1.60	2.80	2.72	
	Tyr	0.40	0.70	0.80	1.04	1.20	1.60	1.56	
3	Growth	148	138	138	138	144	147	150	162
Try	Phe	0.70	1.36	1.38	1.36	1.72	2.88	3.00	
	Try	0.60	1.26	1.20	1.30	1.90	2.60	2.78	
	Tyr	0.40	0.60	0.90	0.84	1.28	1.44	1.40	
4	Growth	148	138	137	144	147	150	162	
Tyr	Phe	0.70	1.14	1.36	1.32	1.90	2.70	2.86	
	Try	0.60	0.96	1.26	1.44	1.70	2.70	2.56	
	Tyr	0.40	0.72	0.90	0.96	1.28	1.42	1.40	
5	Growth	148	137	137	138	143	145	148	160
	Tyr	0.40	0.72	0.90	0.96	1.28	1.42	1.40	
5	Growth	148	137	137	138	143	145	148	160
Phe	Phe	0.70	1.00	1.20	1.16	1.66	2.60	2.56	
Try	Try	0.60	0.96	1.10	1.22	1.60	2.50	2.40	
Tyr	Tyr	0.40	0.46	0.66	0.80		1.36	1.40	

Table 7. Effect of phenylalanine, tryptophan and tyrosine on the formation of the enzyme system transaminating these amino acids during the growth of strain KQ in medium ScQ. The cells had grown in a medium containing no nitrogen sources before the inoculation. Enzyme activity: mU/ml.

Additions to growth media	Sub-strates	Growth time, min							
		0	15	30	45	60	75	105	165
1	Growth	85	59	56	61	66	70	84	113
—	Phe	0.80	0.96	0.92	1.60	1.52	1.60	3.08	3.12
	Try	0.72	1.00	1.00	1.40	1.44	1.52	2.60	2.68
	Tyr	0.40	0.48	0.50		0.72	0.80	1.48	1.52
2	Growth	85	59	55	62	65	69	78	105
Phe	Phe	0.80	1.00	1.20	1.64	1.56	1.64	3.00	3.20
	Try	0.72	0.92	1.08	1.44	1.32	1.56	2.80	1.72
	Tyr	0.40	0.52	0.52	0.76	0.72	0.76	1.52	1.48
3	Growth	85	58	57	64	69	73	86	121
Try	Phe	0.80	1.00	1.08	1.64	1.72	1.92	3.12	3.04
	Try	0.72	0.84	0.96	1.60	1.60	1.69	2.80	2.80
	Tyr	0.40	0.52	0.52	0.84	0.80		1.48	1.52
4	Growth	85	55	56	60	66	73	85	119
Tyr	Phe	0.80	1.04	1.08	1.56		1.76	2.72	2.92
	Try	0.72	1.00	1.00	1.48	1.60	1.60	2.60	1.72
	Tyr	0.40	0.52	0.48	0.76	0.80	0.80	1.40	1.40
5	Growth	85	56	56	61	67	75	85	116
Phe	Phe	0.80		1.12	1.60	1.56	1.56	2.60	2.60
Try	Try	0.72	1.04	1.12	1.44	1.40	1.44	2.48	2.32
Tyr	Tyr	0.40	0.56		0.80	0.76	0.76	1.32	1.28

L-cysteine. On the other hand, the fact that transaminases were formed in medium TSHGA indicates that the repression cannot be very strong.

The repression of aspartate transaminase was studied by adding oxalacetic acid or L-aspartic acid to medium ScQ at the beginning of the growth of strain KQ in a quantity sufficient to make the solution 5 mM. The KQ cells used as inoculum in the experiment had been precultivated 10 h in medium TSHGA. Table 4 shows that neither oxalacetic acid nor L-aspartic acid repressed the formation of aspartate transaminase. In both cases the enzyme level increased about 4-fold. As the test organism has a large amino acid pool, a series of tests was made under conditions that would lessen the amino acid pool. For this purpose, the strain KQ was precultivated as mentioned above, and the cells were transferred to a ScQ medium containing no nitrogen sources. Only after they had been in this medium for 40—60 min the cells were used to inoculate three different ScQ media, one containing oxalacetic acid, one L-aspartic acid, and the third without either. Table 5 shows, however, that it was not possible to repress aspartate transaminase even in this way.

The repression of the formation of transaminases catalyzing the transamination of aromatic amino acids was studied also with strain KQ. The results are shown in Table 6. In this experiment the inoculum cells were grown in medium TSHGA for 8 h and then in medium ScQ. The cells were divided equally into five vessels, one of which was used without amino acid additions as a control vessel (No. 1). To the other vessels were added phenylalanine (No. 2), L-tryp-

Table 8. Effect of isoleucine, leucine and valine on the formation of the enzyme system deaminating these amino acids during the growth of strain KQ.

Additions to growth media	Substrates	Growth time, min									
		0	15	30	45	60	75	90	105	140	180
1	Growth	125	116	118	119	123	125	130	134	152	177
—	Ile	0.80	1.04	1.60	1.80	2.40	2.62	2.88	2.50	2.76	2.38
	Leu	1.14	1.56	2.60	3.16	6.50	6.30	7.00	6.80	6.62	5.12
	Val	0.80		1.40	2.38	3.16	3.28	3.36	3.10	3.10	2.82
2	Growth	125	116	119	121	126	135	143	154	195	218
Ile	Ile	0.80	1.26	1.70	2.00	2.60	2.44	2.54	2.86	2.92	2.10
Leu	Leu	1.14	1.86	2.40	4.04	6.50	6.90	6.70	6.80	6.60	5.30
Val	Val	0.80	1.30	1.74	2.44	2.90	3.00	2.80	3.04	2.90	2.20
3	Growth	125	116	117	120	124	128	136	141	154	168
Ile	Ile	0.80	1.14	1.28	2.22	2.50	2.46	2.62		2.36	2.02
Val	Leu	1.14	1.60	3.00	3.84	6.54	6.76	6.60		6.00	5.30
	Val	0.80	1.32	1.80	2.04	2.18	3.16	2.90		2.76	1.82
4	Growth	125	116	118	120	124	130	136	147	160	177
Ile	Ile	0.80	1.22	1.40	1.96	2.10	2.48	2.60	2.63	2.30	1.80
Leu	Leu	1.14	1.66	3.02	3.14	5.10	5.60	7.00	6.90	5.90	5.80
	Val	0.80	1.30	1.80	2.00	2.06	2.90	2.70	3.40	3.26	2.04
5	Growth	125	116	117	120	121	125	130	137	149	164
Ileu	Ile	0.80	1.10	1.50	1.86	1.96	2.10	1.44	2.62	2.36	2.36
	Leu	1.14	1.40	3.30		5.60	6.10	6.50	6.90	5.90	5.40
	Val	0.80	1.16	1.80	1.74	2.06	2.06	2.60	2.74	2.80	2.44

tophan (No. 3), L-tyrosine (No. 4), and all these amino acids together (No. 5). After the additions the concentration of each amino acid in the media was 2 mM. As can be seen from Table 6, phenylalanine and tryptophan had no effect.

When the cells were grown without nitrogen sources in the media in the same way as in the experiments to which the data in Table 5 refer, the results presented in Table 7 were obtained. These results show that only tyrosine alone or in the combination phenylalanine + tryptophan + tyrosine, caused a slight repression (5—10 %) of the enzyme system transaminating amino acids.

The repression of the formation of enzymes catalyzing the transamination reactions in which isoleucine, leucine and valine function as substrates was found to be slight when studied with strain KQ in medium ScQ (Table 8). Still it can be concluded that the effect of these three amino acids on the formation of the transaminase system is a repression and that the repression is equal for all three corresponding transaminases. In this experiment the concentration of each amino acid was 2 mM. The following additions were made to growth vessels: No. 1, the control, no addition; No. 2, L-isoleucine, L-leucine and L-valine; No. 3, L-isoleucine and L-valine; No. 4, L-isoleucine and L-leucine, and No. 5, L-isoleucine. The cells had been precultivated 10 h in medium TSHGA. By growing them without nitrogen sources as described above in connection with the experiment reported in Table 5, a somewhat more marked repression was observed (Table 9). The combination of three amino acids

Table 9. Effect of isoleucine, leucine and valine on the formation of the enzyme system deaminating these amino acids during the growth of strain KQ in medium ScQ. The cells used as inoculum had grown without nitrogen sources as described in connection with Table 5. Enzyme activity: mU/ml.

Additions to growth media	Sub-strates	Growth time, min							
		0	15	30	45	60	90	120	150
1	Growth	150	148	147	147	148	150	153	155
—	Ile	2.96	2.62	3.74	4.30	4.60	4.32	4.54	4.36
	Leu	4.90	4.86	6.04	7.60	8.32	8.52	8.54	8.54
	Val	2.80	2.56	4.04	4.80	4.82	5.06	5.24	
2	Growth	150	152	149	147	148	152	159	172
Ile	Ile	2.96	2.48	3.58		3.68	3.92	4.16	4.10
Leu	Leu	4.90	4.72	6.08	6.72	6.88	6.92	7.56	7.62
Val	Val	2.80	2.24	3.68	4.32	4.32	4.20	4.40	
3	Growth	150	152	150	147	147	148	153	158
Ile	Ile	4.90	2.70	3.84	4.08	4.08		4.00	4.26
Val	Leu	2.80	4.90	6.84	7.68	7.68		7.90	8.16
	Val	2.80	2.60	4.04	4.40	4.48		4.70	
4	Growth	150	151	150	147	148	150	154	162
Ileu	Ileu	2.96	2.80	3.80	3.70	3.92	4.08	4.32	4.24
Leu	Leu	4.90	4.90	6.30	7.10	7.50	7.44	7.82	8.08
	Val	2.80	2.80	3.84	4.36	4.48	4.48	4.74	
5	Growth	150	151	149	148	148	149	153	161
Ile	Ile	2.96	2.70	3.62	4.10	4.32	4.16	4.38	4.10
	Leu	4.90	5.10	5.90	7.00	7.82	7.96	7.90	8.28
	Val	2.80	2.56	3.74	4.40	4.62	4.62	4.76	
6	Growth	150	151	148	148	137	148	150	153
α-Keto-	Ile	2.96	2.80	3.24		4.08	4.24	4.60	
isovale-	Leu	4.90	5.22	5.50	7.64	7.80	8.02	8.28	
ric acid	Val	2.80	2.90	3.64	4.20	4.88	4.96	5.22	

gave an about 10—15 % repression, two amino acids a 5—10 % repression, and one amino acid hardly repressed the enzyme formation at all. Ketovaline and α-ketoisovalerate did not affect the formation of transaminases.

DISCUSSION

One of the main results of this investigation is the observation that the activities of enzymes promoting transamination reactions are, generally speaking, similar to those in *Escherichia coli*, the transaminases of which have recently been clarified by Raunio [1] in this laboratory. The amounts of transaminases formed were about the same as in *E. coli*. Still it was observed that the maximal levels of transaminases mostly occurred in the acceleration or at the beginning of the exponential phase of growth in the *Str. thermophilus* strains; the maximal enzyme activities thus appeared earlier in the growth cycle than in the case of *E. coli*.[1]

The transaminases A and B and aspartic acid transaminase were very active in all investigated *Str. thermophilus* strains. Also the transamination reactions of serine and threonine were remarkably strong in certain strains.

This fact may be useful in attempts to purify and investigate these transaminases in detail. Certain differences were observed between parallel tests. This may have been due to the fact that some transaminases are inactivated during the preparation of cell extracts.[4,5] It is interesting that asparagine was hardly deaminated at all. On the other hand, aspartic acid was deaminated very strongly, a fact indicating that the activity of arginase is evidently very low in *Str. thermophilus* strains.

The results give reason to suppose that transaminase A (which deaminates isoleucine, leucine, and valine) and transaminase B (which deaminates phenylalanine, tryptophan, and tyrosine) were repressed in the investigated strains. However, the repression was not observed clearly even in the experiments in which the inoculum cells were grown in media without nitrogen sources. Evidently, the reason for this was the high amino acid pool in *Str. thermophilus* strains (*cf.* Holden [6]). In general, all the observations made indicate that the transaminases here are repressed largely according to the system found by Raunio [1] in *E. coli*.

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