Role of Amino Acids on the Growth and Lipase Production of *Streptococcus faecalis*

Lactic acid bacteria have been reported to elaborate lipases[1–4]. Incorporation of amino acids in the basal medium has been found to enhance lipase production[5–7]. The present study was conducted to investigate the effect of amino acids incorporation on the growth of *Streptococcus faecalis* and synthesis of lipase.

Materials and methods. A strain of *S. faecalis* (272) was isolated from stored Khoa samples. The culture was propagated in a basal medium consisting of 2% peptone (Oxoid), 0.3% yeast extract (Oxoid), 1% glucose (B.D.H), 0.5% NaCl (B.D.H), 10% tomato juice, with pH adjusted to 7.5.

Lipase activity was determined by the method of Oi et al.[8] with some modifications with regard to substrate and buffer. The reaction mixture contained 5.0 ml butter oil emulsion, 5.0 ml of 0.2 *M* *Tris*-HCl buffer (pH 7.5), 2 ml of 0.2 *M* NaCl solution, 2 ml of glass distilled water and 1 ml of enzyme solution. In the control set, the enzyme was boiled for 20 min. The pH of the reaction mixture in the control set was first noted and then the pH of the experimental set was brought to the same level by the addition of 0.01 *N* alkali. The amount of 0.01 *N* alkali required to bring the pH of the enzyme solution to that of control corresponded to the lipase activity in terms of μmoles of free fatty acids.

The requirement of essential amino acids for growth and lipase production by *S. faecalis* was determined by deleting each of the amino acids from the complete synthetic medium[8]. *S. faecalis* was inoculated into 19 sets of media and incubated at 30 °C for 24 h. The extracellular enzyme obtained, was then adjusted to pH 7.5 at 4 °C by the addition of 0.01 *N* alkali. Lipase activity was then estimated in each case[9].

Results and discussion. The omission of amino acids like arginine, glutamic acid, histidine, isoleucine, leucine, methionine, threonine, tryptophane and valine from the synthetic medium resulted in a marked decline in growth (0.40 to 0.43, O.D.) as well as lipase production (0.2 to 0.7 μmoles) (Table). Amino acids such as alanine, glycine, lysine and serine appeared to stimulate lipase production, whereas aspartic acid, cystine, phenylalanine, proline and tyrosine were non-essential for both growth and lipase production. In contrast, Snell and Guirard[10] reported that methionine, valine, histidine and isoleucine were not essential for the growth of *S. faecalis*. Our results as to the essential nature of 9 amino acids are comparable with those of Greenhut et al.[11], where 4 essential amino acids were not found to be essential for the growth of *Streptococcus faecalis* (272).

Amino acids requirement of *S. faecalis* in relation to growth and lipase production

Amino acids omitted	O.D. (660 nm)	Lipase activity[a]
None	0.70	4.6
Alanine	0.60	3.0
Aspartic acid	0.71	4.5
Arginine	0.42	0.20
Cystine	0.70	4.2
Glutamic acid	0.43	0.30
Glycine	0.64	3.7
Histidine	0.42	0.4
Isoleucine	0.43	0.7
Leucine	0.41	0.7
Lysine	0.64	3.5
Methionine	0.43	0.75
Phenylalanine	0.69	4.60
Proline	0.69	4.7
Serine	0.60	2.5
Threonine	0.43	0.5
Tyrosine	0.70	4.5
Tryptophane	0.42	0.30
Valine	0.40	0.25

[a] μmoles of free fatty acids released/ml of broth.

Summary. A study was conducted on the requirement of amino acids for the growth of *S. faecalis* and its lipase production. Arginine, glutamic acid, histidine, leucine, isoleucine, methionine, threonine, tryptophane and valine were found to be essential, with alanine, glycine, lysine and serine as stimulatory and aspartic acid, cystine, phenylalanine, proline and tyrosine as non-essential for both growth and lipase synthesis.

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Studies on the Effect of Allicin (Diallyl Disulphide-Oxide) on Alloxan Diabetes

The author has reported in a previous paper[1] on the beneficial effects of allicin (C_3H_5-S-$\overset{\overset{O}{\uparrow}}{S}$-$C_3H_5$) on alloxan diabetes with special reference to its hypoglycemic action. Such an effect was found only in diabetic animals which responded to tolbutamide, Viz. mild alloxan diabetes. The effects of both these drugs were found to be dependent on the insulin reserves of the animals. In the present study the author has investigated the dose-effect relation of allicin and also its effect on short-term treatment, as compared to the standard drug tolbutamide on the fasting

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Table I. Dose effect relation of allicin as compared to a standard dose of tolbutamide (0.25 g/kg) in alloxan diabetic rabbits

Drug administered	Dose (g/kg)	Blood sugar (mg/100 ml)		Hypoglycemic potency as blood sugar fall (%)
		0 h	2 h	
Allicin	0.05	265.0 ± 7.0	240.0 ± 6.0[a]	9.4
	0.10	270.0 ± 6.0	225.0 ± 8.0[b]	16.7
	0.25	255.0 ± 8.0	204.0 ± 7.0[c]	20.0
Tolbutamide	0.25	265.0 ± 10.0	200.0 ± 7.0[d]	24.5
Control	–	250.0 ± 5.0	245.0 ± 6.0	2.0

25 ml distilled water was administered to all the groups. Results are expressed as mean values of 6 rabbits ± SE.
Student's t-test; [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.005$; [d] $P < 0.001$. These values are significantly lower than the initial values.

blood sugar and glucose nitrogen ratio (G:N) of alloxan diabetic rabbits.

Materials and methods. The blood sugar lowering effect of allicin in the dosage range of 0.05 g to 0.25 g/kg was investigated on fasting alloxan diabetic rabbits. Alloxan diabetes was produced in male albino rabbits as described previously[2]. Allicin was prepared from fresh garlic cloves according to the method of CAVALLITO and BAILEY[3]. Tolbutamide was supplied by Hoechst Pharmaceuticals Ltd., Bombay. Animals were fed the normal laboratory diet (Hindustan Lever rabbit feed) and they were used after 2 weeks when their blood sugar levels stabilized. Before each experiment the aimals were fasted for 18 h. Rabbits with blood sugar range of 200–300 mg/100 ml were used for the present study, and the dose-effect relation of allicin was determined following a procedure described previously[4]. One group served as control, and in two other groups the hypoglycemic effects of a standard dose of tolbutamide (0.25 g/kg) and varying doses of allicin were studied for a period of 2 h. As the maximum effects of these drugs were observed at the 2nd h in the previous study[1], the hypoglycemic action of each at this interval was taken for comparison at present. Blood sugar was estimated by the method of ASATOOR and KING[5] using the low alkaline copper reagent of SOMOGYI[6]. The effect of allicin on the glucose/nitrogen ratio of alloxan diabetic rabbits after short-term treatment was compared with that produced by the same dose of tolbutamide. Initially the fasting blood sugar levels and the G:N ratios of 3 groups of alloxan diabetic rabbits were studied separately. The G:N ratio was studied by estimating glucose and nitrogen in the urine of rabbits fed only casein[7,8]. Allicin and tolbutamide at a dosage of 100 mg/kg/day were then administered orally to separate groups of diabetic rabbits for a period of 7 days. The 3rd group was kept as control. After 1 week of treatment, the fasting blood sugar and G:N ratio in urine were again estimated as before in all the groups. The effects of allicin were expressed as percentage effects on blood sugar and G:N ratio, and were compared with those of tolbutamide.

Results. The results obtained on the dose-effect relation of allicin are given in Table I. It indicates that 50 mg/kg is an effective dose of allicin to produce a significant blood sugar fall in alloxan diabetic rabbits ($p < 0.05$). There is an increase in hypoglycemic activity with respect to the dose of allicin. In terms of biological potency against tolbutamide, allicin at a dosage of 0.25 g/kg is 80% active. The effects of allicin and tolbutamide on fasting blood sugar and G:N ratio of alloxan diabetic rabbits after a short-term treatment are shown in Table II. Both the drugs produced significant reductions in the fasting blood sugar levels and G:N ratio. The hypoglycemic actions of allicin and tolbutamide are highly

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Table II. Effects of allicin and tolbutamide on fasting blood sugar and G:N ratio of alloxan diabetic rabbits on short term treatment

Drugs administered (0.1 g/kg/day)	Blood sugar (mg/100 ml)		Blood sugar fall and G:N ratio decrease (%)
	1st day	8th day	
Tolbutamide	270.0 ± 10.0	205.0 ± 7.0[c]	24.1
Allicin	260.0 ± 6.0	220.0 ± 8.0[b]	15.4
Control	250.0 ± 7.0	240.0 ± 6.0	4.0
G:N ratio of the above animals on casein feeding			
Tolbutamide	3.3 ± 0.2	2.2 ± 0.15[b]	33.3
Allicin	3.2 ± 0.1	2.7 ± 0.1[a]	15.6
Control	3.0 ± 0.2	2.9 ± 0.1	3.3

Results are expressed as mean values of 6 rabbits ± SE.
Student's t-test; [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. These values are significantly lower than the initial values.

significant ($p < 0.01$, $p < 0.001$ respectively). However, allicin is only 64% as active in hypoglycemic action and 47% as active in reducing the G:N ratio, compared with the effects of tolbutamide. Rediction in the G:N ratio is a measure of the improvement in the capacity of the diabetic animal to utilize the glucose derived from protein.

Discussion. The improvement in the diabetic condition of the animals brought about by tolbutamide and allicin may be dependent on the insulin reserves of the animals, reported previously[1]. The inability of these drugs to produce a greater fall in the blood sugar of diabetic animals may be due to several unknown factors which control the hyperglycemic condition of alloxan diabetes. The action of oral hypoglycemic drugs is much dependent on endogenous and exogenous sources of insulin[9]. The control of hyperglycemia in alloxan diabetes is possible by oral drugs provided the blood sugar levels are near normal[10]. Ketosis and hyperlipaemia may be other factors which prevent the lowering of blood sugar beyond a particular range. However the hypoglycemic action of allicin at dosages of 0.05 g to 0.25 g/kg is significant ($p < 0.05$–0.005), whilst that of tolbutamide at a standard dose of 0.25 g/kg is even more marked ($p < 0.001$). An improvement is definitely observed in the diabetic condition of the animals as evident from the reduction in G:N ratio on a short-term treatment with the drugs. Studies employing higher dosages on long-term treatment of diabetic animals are warranted. In a previous study[11] the effects of allicin at a dosage of 100 mg/kg/day on long-term feeding to normal rats were examined. There was a significant reduction in lipid constituents of blood and liver on allicin treatment. In this respect allicin might have an advantage over tolbutamide which has been shown to produce hyperlipaemia under certain conditions[12,13]. Very recently also reports supporting the beneficial uses of garlic and onion in the treatment of diabetes were made by some physicians[14]. All these findings justify further studies on the therapeutic effects of these vegetables which are rich in allicin type compounds[15–17]. The blood fibrinolytic effect of garlic has been attributed to its oil[18], and a preliminary study[19], conducted by the author at the Royal Victoria Infirmary of Newcastle-upon-Tyne, revealed that allicin is one of the sulphur compounds with blood fibrinolytic action.

Summary. On oral administration to alloxan diabetic rabbits, allicin produces an increase in its hypoglycemic action with relation to dose. A short-term treatment with allicin, as well as with tolbutamide, significantly reduced the blood sugar levels and glucose nitrogen ratio of the above animals.

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Effects of Tryptophan and Other Amino Acids on Glucose Uptake and Carbon Dioxide Output by the Insulin-Stimulated Adipose Tissue

The i.v. infusion of amino acid mixtures to healthy subjects produces an initial increase in blood glucose followed by a decrease below control levels[1,2]. These effects are explained by the stimulating action of amino acids on glucagon[3,4] and insulin[1,2] secretion. However, the possible contribution to the glycemia changes of a direct action of amino acids on glucose utilization by peripheral tissues has scarcely been investigated. Such a direct action has been suggested as a way in which some amino acids might prevent epinephrine hyperglycemia, both in normal and in alloxan diabetic rats[5]. The present paper deals with the effects of 7 amino acids on glucose utilization by the rat adipose tissue in vitro.

Materials and methods. Male Wistar rats (90–120 g), fed ad libitum, were killed by decapitation. 2 portions of epididymal fat pad (about 200 mg), one from each side, were removed, weighed and placed into 2 Warburg vessels containing 2 ml of incubation medium. From each animal, one portion was incubated in medium without amino acids (control) and the other one in the same medium containing the appropriate amino acid (experimental). Total gas exchange of the tissue was determined as described by Ball et al.[6]; the incubation medium in this case was Krebs-Ringer-bicarbonate saline[7], pH 7.4, with 10 m*M* glucose, and 5% CO_2–95% O_2 as gas phase. When O_2 uptake was determined, the saline solution was Krebs-Ringer-phosphate[7], pH 7.4, with 100% O_2 as gas phase. Insulin (Nordisk Insulinlaboratorium, Copenhagen) was added to the medium either before putting into it the tissue or after a period of incubation at 37 °C, to give a final concentration of 10^3–10^5 μU/ml. Manometric readings were carried out during 60–120 min at 37 °C. Glucose and lactate were determined by a glucose-oxidase and a lactate-dehydrogenase method, using analytical kits from Boehringer, Mannheim. Amino acids (L-isomers) were purchased from Sigma, London. The Student paired *t*-test was applied for comparations between control and experimental values.

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