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Regulation of aspartokinase in *Lactobacillus plantarum*

O.O. Adebawo, J.L. Ruiz-Barba[1], P.J. Warner[2] and G.B.O. Oguntimein[3]
Department of Biochemistry, Obafemi Awolowo College of Health Sciences, Ogun State University, Ago-Iwoye, Nigeria, [1]Department of Food Biotechnology, Instituto de la Grasa (CSIC), Sevilla, Spain, [2]Biotechnology Centre, Cranfield University, Cranfield, Bedford, UK, and [3]Department of Food Technology, Faculty of Technology, University of Ibadan, Nigeria

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O.O. ADEBAWO, J.L. RUIZ-BARBA, P.J. WARNER AND G.B.O. OGUNTIMEIN. 1997. As a rational approach to the genetic development of a stable lysine overproducing strain of *Lactobacillus plantarum* for the fermentation of 'ogi', a Nigerian fermented cereal porridge, regulation of lysine biosynthesis in this species was investigated. Spontaneous lysine overproducing mutants of *Lact. plantarum* were obtained and their aspartokinase activities compared with those of wild-type strains under different conditions. Results showed that aspartokinase activity of *Lact. plantarum* cell extracts was not inhibited by either lysine, threonine, methionine or combinations of lysine and threonine. Instead, methionine enhanced aspartokinase activity *in vitro*. Results indicated that lysine biosynthesis in *Lact. plantarum* could be regulated by lysine via the control of aspartokinase production in a way different to that described for other bacteria.

INTRODUCTION

Lactobacillus plantarum is a major food fermenting micro-organism in many parts of the world (Buckenhüskes 1993). In Nigeria, it is involved in the fermentation of cassava into indigenous main course meals such as 'lafun' and 'fufu' (Oyewole and Odunfa 1990) as well as of cereal grains like maize, sorghum and millet into 'ogi' (Akinrele 1970; Odunfa and Adeyele 1984). Ogi is a smooth porridge which is consumed as a breakfast cereal. It is also popular as a convalescence food, but most importantly, it is traditionally used as a component of the diet of newly weaned infants in most parts of West Africa. Since cereal grains generally lack adequate concentrations of certain essential amino acids, most notably lysine (Flodin 1993), ogi requires supplementation with either the deficient amino acids or a protein source to improve nutritive value of the product. Therefore, the development of starter cultures of *Lact. plantarum* which are able to overproduce lysine would provide a cheap natural route to the complementation of the nutritional value of ogi.

However, the lysine biosynthetic pathway in *Lact. plantarum* has not been studied in detail. In other bacteria, it is regulated primarily by aspartokinase, the first enzyme in the highly branched pathway that converts aspartate to lysine, methionine, threonine and isoleucine. These amino acids are collectively referred to as the aspartate family amino acids. In addition to these amino acids, dihydrodipicolinate (a component of the bacterial spore) and meso-diaminopimelate (a component of the bacterial cell wall) are also derived from this pathway (Patte 1983). Reports have shown that there is no universal mechanism of regulation for aspartokinases produced by different micro-organisms.

Overproduction of lysine in bacteria results from an altered feedback control of aspartokinase (Shiio and Miyajima 1969). Mutants of bacteria which overproduce lysine have been selected by resistance to *S*-(2-amino-ethyl)-L-cysteine (AEC), a sulphur analogue of lysine (Sano and Shiio 1970; Cremer *et al.* 1991). The resistance to AEC has been linked to mutation in the aspartokinase gene conferring feedback resistance to the enzyme (Thierbach *et al.* 1990; Kalinowski *et al.* 1991; Lu *et al.* 1991). Therefore, as a first step in the improvement of *Lact. plantarum* strains for lysine overproduction, mutants resistant to AEC were produced which overproduced lysine, and the mechanism by which their aspartokinase modulates the lysine biosynthetic pathway was investigated.

Correspondence to: Dr J. L. Ruiz-Barba, Food Biotechnology Department, Instituto de la Grasa (CSIC), Avda. Padre García Tejero 4, Aptdo. 1078, 41012-Sevilla, Spain.

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MATERIALS AND METHODS

Culture media

MRS (De Man *et al.* 1960) broth or agar were used for the propagation of lactobacilli. Bacto Lysine Assay Medium (LAM) (Difco) was used for selection of lysine overproducing mutants. For studies on the influence of amino acids of the aspartate family in the aspartokinase activity, a defined synthetic medium (DSM) was formulated, which was a modification of those described by Ledesma *et al.* (1977), Morishita *et al.* (1981) and McFeeters and Kuang-Hua Chen (1986). This medium was devoid of aspartate family amino acids except for isoleucine. It was prepared as follows: the 100-fold concentrated stock solutions I to V (Table 1) were diluted in distilled water (400 ml); to the resulting solution, sodium acetate (20 g), trisodium citrate (2 g), D-glucose (25 g), sodium chloride (0·5 g) and Tween 80 (1·0 ml) were added and the volume was made up to 1 l with distilled water. Sterilization was carried out by autoclaving (121°C, 10 min). *Corynebacterium glutamicum* ATCC 13032 was grown in CGIII medium (Cremer *et al.* 1988).

Preparation of 'ogi'

Ogi was prepared by the traditional method. Maize (500 g) was soaked in 1·5 l of glass-distilled water for 3 d at 30°C. The steep water was decanted and the grains were rinsed in fresh distilled water. The grains were then wet milled in a blender and wet sieved. The obtained fines were allowed to settle and ferment further for about 24 h (souring stage). Samples of steep liquor (10 ml) were taken at 24 h intervals under aseptic conditions, diluted in saline and plated out on MRS agar. The plates were incubated (37°C, 48 h) to isolate lactobacilli.

Characterization of lactobacilli isolates

Lactobacilli were taxonomically identified to species level according to criteria given in *Bergey's Manual of Systematic Bacteriology* (Kandler and Weiss 1986). Sugar utilization tests were carried out using the API 50CH test kit (API System SA, Montalieu-Vercieu, France) following the manufacturer's instructions.

Selection of lysine producing strains of *Lact. plantarum*

Lactobacillus plantarum strains isolated from ogi fermentation which were able to grow in LAM were selected for this study. From these strains, spontaneous mutants showing resistance to AEC (Sigma) were isolated by sequential selection on LAM agar containing increasing concentrations (0·5, 1·0, 2·5 and 5·0 mg ml^{-1}) of this chemical. The wild-type strains as well as the resistant mutants obtained at every AEC concentration were tested for lysine production by the microbiological assay method described in the *Difco Manual for Microbiology Culture Media* (Anon. 1985) using *Pediococcus cerevisiae* ATCC 8042 as the assay micro-organism.

Table 1 Composition of the stock solutions used to make a defined synthetic medium (DSM) for the cultivation of *Lactobacillus plantarum* strains isolated from ogi fermentations*

Component	100 × concentration ($g\ l^{-1}$)
Stock solution I†	
L-Arginine	24·2
L-Cysteine	5·0
L-Glutamic acid	30·0
L-Isoleucine	25·0
L-Leucine	25·0
L-Phenylalanine	10·0
L-Tryptophan	4·0
L-Tyrosine	10·0
L-Valine	25·0
Stock solution II	
Pyridoxine	0·2
Nicotinic acid	0·1
Ca-D-pantothenate	0·1
Riboflavin‡	0·1
Thiamine	0·1
Vitamin B_{12}	$1·0 \times 10^{-4}$
Biotin‡	$1·0 \times 10^{-3}$
p-Aminobenzoic acid	$5·0 \times 10^{-4}$
Folic acid‡	$1·0 \times 10^{-3}$
Adenine	0·5
Stock solution III	
Guanine	0·5
Uracil	0·5
Stock solution IV	
NH_4Cl	200
$MgSO_4.7H_2O$	15·0
$MnSO_4.4H_2O$	2·0
$FeSO_4.7H_2O$	1·0
Stock solution V	
K_2HPO_4	456

*The 100 × stock solutions were dispensed into small polypropylene screw-capped tubes (10 ml per tube) and stored frozen at −20°C until use.
†The amino acids were dissolved in glass-distilled water containing HCl (0·2 mol l^{-1}) in the order listed to ensure that they dissolved completely.
‡One or two drops of ethanol were added to help these components to dissolve.

Assay of aspartokinase activity

Lactobacillus plantarum was grown in DSM. Cell-free protein extracts were obtained as described by Cremer *et al.* (1991) and aspartokinase activity was assayed by the method of Black and Wright (1955). For studies on the inhibition of the enzyme, L-lysine, L-threonine, L-methionine (Sigma) and combinations of L-lysine and L-threonine were added to final concentrations of 60 mmol l^{-1} each to the assay system. *Corynebacterium glutamicum* ATCC 13032 containing the plasmid pJC300 carrying the *LysC* gene (Cremer *et al.* 1991) which codes for the wild-type *Coryne. glutamicum* aspartokinase was used as control. This strain was kindly supplied by Dr L. Eggeling, from the Institut für Biotechnologie der Kernforschungsanlage at Jülich, Germany.

Effect of aspartate-family amino acids on *Lact. plantarum* growth and aspartokinase activity

Washed cells of *Lact. plantarum* grown on DSM were inoculated into DSM containing either L-lysine, L-threonine, L-methionine or L-homoserine (Sigma), singly or in combination, at a concentration of 1 mg ml^{-1} each. L-Lysine was also used at 0·5 mg ml^{-1}. The cultures were incubated at 37°C and growth monitored by measuring $O.D._{600}$ at 6 h intervals for 48 h. As a control, bacteria were also grown in DSM. When cells were to be used for the aspartokinase activity assay, they were grown in DSM and DSM plus either L-lysine (1 mg ml^{-1}), L-threonine (1 mg ml^{-1}) or combination of L-lysine and L-threonine. Cells were harvested when $O.D._{600}$ reached 0·8–1·0 units and processed as described above.

RESULTS

Isolation and characterization of lactobacilli

A total of 24 strains identified as *Lactobacillus* spp. were isolated at both the steeping and souring stages of ogi fermentation. These strains were further characterized as *Lact. plantarum* (13 strains), *Lact. brevis* (6 strains), *Lact. pentosus* (4 strains) and *Lact. cellobiosus* (1 strain).

Selection of *Lact. plantarum* mutants resistant to AEC

A total of six *Lact. plantarum* strains were found to grow in LAM. From four of these strains (*Lact. plantarum* OG 025, OG 046, OG 251, OG 261), a total of 48 spontaneous mutants were obtained which grew in LAM containing 1·0, 2·5 or 5·0 mg ml^{-1} AEC, respectively (Fig. 1).

L-Lysine secretion by the AEC-resistant mutants

The 48 *Lact. plantarum* AEC-resistant mutants were examined for L-lysine secretion as well as their four original wild-type strains, which were used as controls. In all cases they were found to secrete L-lysine into the culture medium (Fig. 1). L-Lysine secretion by the wild-type strains ranged from 16·5 to 23·0 μg ml^{-1}, while the AEC-resistant mutants ranged from 15·0 to 76·0 μg ml^{-1} (Fig. 1). Eight of the mutants secreted L-lysine in the medium at concentrations over 60 μg ml^{-1}, five of which were obtained from the selection medium containing 2·5 mg ml^{-1} of AEC.

Aspartokinase activity of *Lact. plantarum* strains

The specific activity of aspartokinase in two of the wild-type strains, *Lact. plantarum* OG 025 and OG 261, and four AEC-resistant mutants derived from them (*Lact. plantarum* OG 025-1, OG 025-5, OG 261-5 and OG 261-9), and selected on the basis of maximum L-lysine production, was determined. The results indicated that there was no significant difference between levels of aspartokinase specific activity when wild-type bacteria and mutants were compared for the same strain (Fig. 2), even though the mutants produced higher amounts of L-lysine.

When the assay was carried out in the presence of L-lysine, L-threonine, L-methionine or L-lysine plus L-threonine, the results revealed that none of these amino acids inhibited aspartokinase activity in any of the *Lact. plantarum* strains tested (Fig. 2). Rather, their aspartokinase activity increased significantly in the presence of L-methionine. These results contrast those obtained with *Coryne. glutamicum*, for which feedback inhibition was observed when L-lysine plus L-threonine were present in the assay system (Fig. 2).

Effect of aspartate-family amino acids on *Lact. plantarum* growth and aspartokinase activity

The wild-type *Lact. plantarum* strain OG 025 was selected to study the effects of aspartate family amino acids on its growth. This micro-organism was unable to grow in DSM containing L-lysine at concentrations of 0·5 or 1·0 mg ml^{-1} (Fig. 3a). Conversely, growth was stimulated in the presence of L-homoserine, L-methionine and L-threonine when compared with a control culture on DSM (Fig. 3a). When the DSM contained both L-lysine and L-homoserine or L-lysine and L-threonine (1·0 mg ml^{-1} each) growth was also stimulated (Fig. 3b). Maximal growth was obtained when L-lysine, L-methionine and L-threonine were added together at a concentration of 1·0 mg ml^{-1} each to the DSM. However,

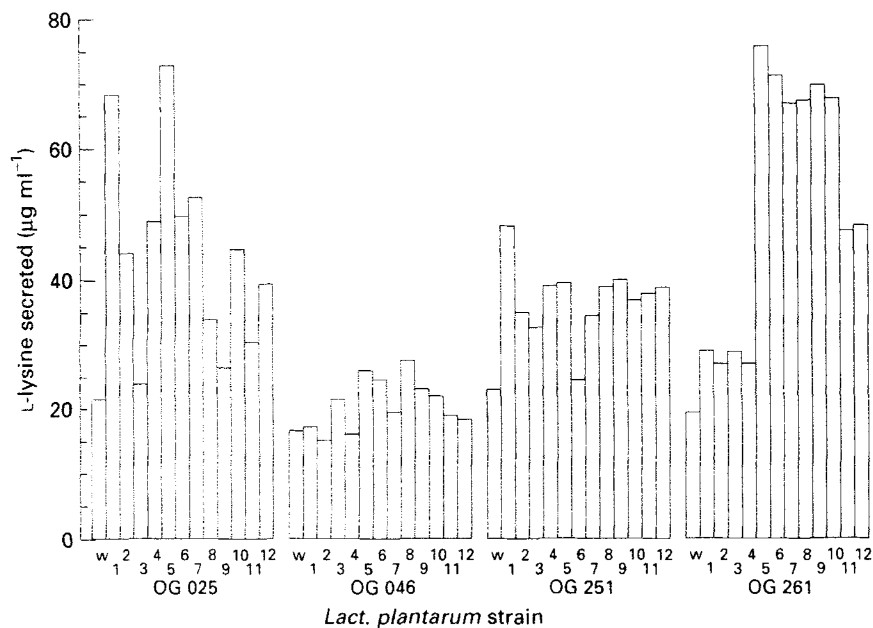

Fig. 1 L-Lysine secreted into the culture medium, Bacto Lysine Assay Medium (LAM), by *Lactobacillus plantarum* strains isolated from ogi fermentations. The mutant strains derived from each of the four original wild-type strains (OG 025, OG 046, OG 251 and OG 261) are indicated by the numbers 1 to 12; w = wild-type strain. Mutant strains 1 to 4 in each series are resistant to 1·0 mg ml^{-1} of AEC; mutant strains 5 to 8 are resistant to 2·5 mg ml^{-1}; mutant strains 9 to 12 are resistant to 5·0 mg ml^{-1}

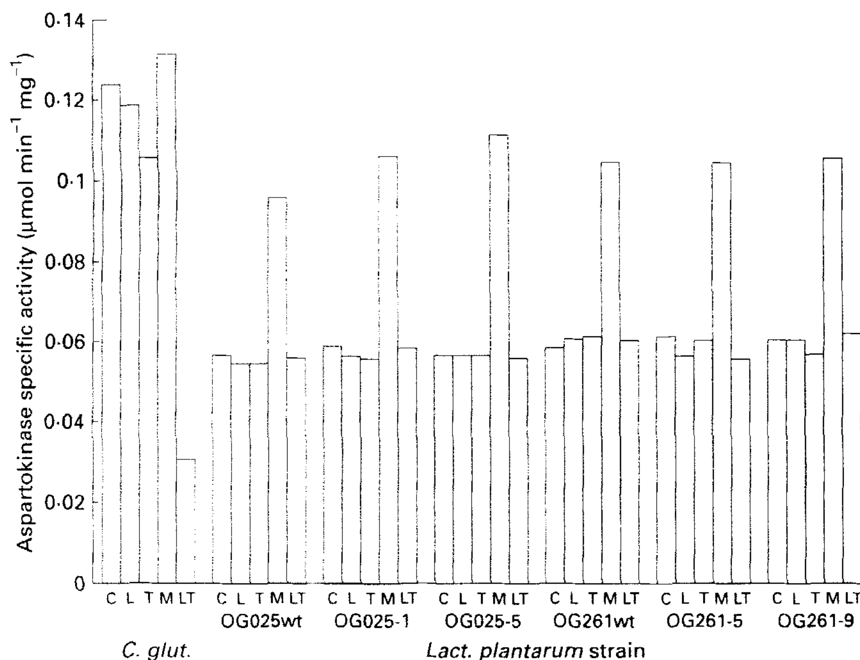

Fig. 2 Effect of aspartate-family amino acids on the aspartokinase specific activity of *Lactobacillus plantarum* strains isolated from ogi fermentations. *Corynebacterium glutamicum* ATCC 13032 (*C. glut.* in the figure) was used as a control; wt = wild-type strain. Determinations were done in the absence, control assay (C), and in the presence of 60 mmol l^{-1} of either L-lysine (L), L-threonine (T), L-methionine (M) or L-lysine plus L-threonine (LT)

addition of L-lysine and L-methionine (1·0 mg ml^{-1} each) to DSM showed no difference with the culture containing only L-lysine (Fig. 3a, b).

The wild-type *Lact. plantarum* strain OG 025 and its derivative AEC-resistant mutant, L-lysine overproducer *Lact. plantarum* OG 025-5, were used to study aspartokinase activity in cell extracts under different growth conditions. Both strains showed virtually the same aspartokinase activities when growing in DSM and in DSM plus L-threonine (Table 2). However, when growing in DSM containing L-lysine plus L-threonine, the specific activity of aspartokinase in cell extracts was almost double in the mutant with respect to its original wild-type strain (0·034 and 0·018 μmol min^{-1} mg^{-1}, respectively) (Table 2).

DISCUSSION

This investigation has validated the use of AEC resistance as a method for obtaining spontaneous mutants of *Lact. plantarum* that overproduce lysine. More importantly, it has also

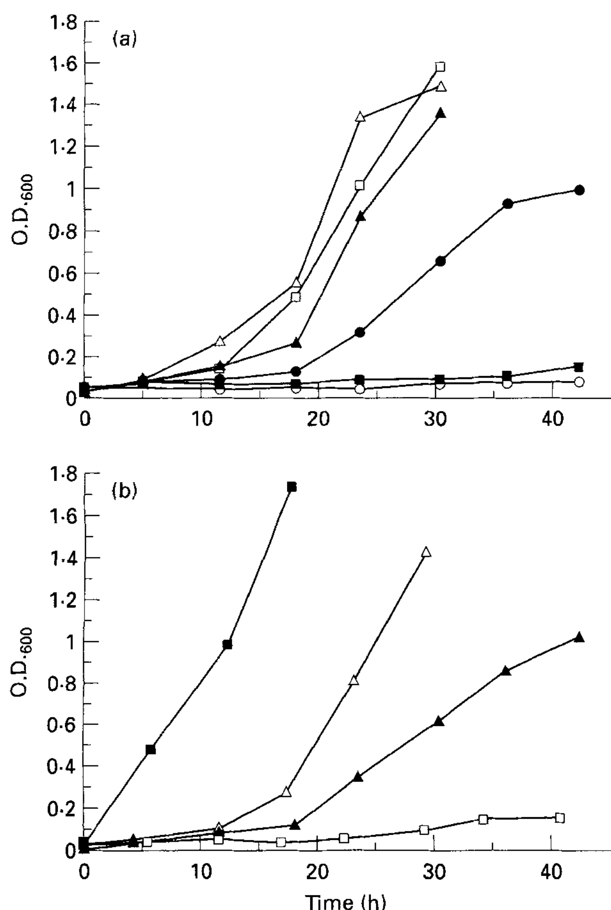

Fig. 3 Effect of aspartate-family amino acids on the growth of *Lactobacillus plantarum* OG 025, a wild-type strain isolated from an ogi fermentation. (a) Optical density at 600 nm (O.D.$_{600}$) of cultures growing in a defined synthetic medium (DSM) (●) and in DSM plus either L-lysine (0·5 mg ml^{-1}) (■), L-lysine (1 mg ml^{-1}) (○), L-threonine (1 mg ml^{-1}) (▲), L-methionine (1 mg ml^{-1}) (△) or L-homoserine (1 mg ml^{-1}) (□). (b) O.D.$_{600}$ of cultures growing in DSM containing 1 mg ml^{-1} each of either L-lysine plus L-threonine (▲), L-lysine plus L-methionine (□), L-lysine plus L-homoserine (△) or a combination of L-lysine, L-threonine and L-methionine (■)

revealed differences in the regulation of lysine biosynthesis between *Lact. plantarum* and other bacteria. Thus, aspartokinase activity in cell extracts of wild-type as well as lysine-overproducing mutants of all the *Lact. plantarum* strains tested was not sensitive to inhibition by either lysine, threonine, methionine or a combination of lysine and threonine as has been reported for most aspartokinases from *Escherichia coli* (Cohen 1983), *Bacillus subtilis* (Rosner and Paulus 1971), *Brevibacterium flavum* (Sano and Shiio 1970), *Coryne. glutamicum* (Cremer *et al.* 1988, 1991) and other bacteria (Umbarger 1978). Such resistance to feedback inhibition has also been reported for aspartokinase II of *E. coli* K12 (Cohen 1983). Rather than being inhibitory, methionine enhanced aspartokinase activity in *Lact. plantarum in vitro* (Fig. 2), the first time an observation of this type has been made, to our knowledge. In *B. flavum* and *Coryne. glutamicum*, release of feedback inhibition by aspartokinase leads to overproduction of lysine by the respective mutants (Sano and Shiio 1970; Cremer *et al.* 1991). This does not seem to be the case with lysine-overproducing *Lact. plantarum* strains.

The growth inhibition caused by addition of high amounts of lysine to DSM indicated that this amino acid plays a key role in the regulation of some essential biosynthetic pathway in *Lact. plantarum*, most probably the aspartate family amino acids biosynthetic pathway. This inhibition could be alleviated by addition of either homoserine or threonine, indicating that biosynthesis of all these amino acids is indeed connected by a common pathway, as in other micro-organisms (Umbarger 1978). Homoserine is the usual branch point compound for the methionine and threonine biosynthetic pathways (Umbarger 1978). Surprisingly, however, threonine could also alleviate the growth inhibition caused by lysine without any methionine in the culture medium. This could indicate a role of threonine in the regulation of the pathway.

Significantly, when lysine was present in high amounts in the growth medium, and its growth inhibitory effects were alleviated by threonine (without which no growth was observed), aspartokinase activity in cell extracts was always lower than in those from the same growth medium without lysine (Table 2). However, only 34% (0·018 μmol min^{-1} mg^{-1}) of the activity remained in cell extracts from the wild-type strain grown in the presence of lysine, in contrast to 68% (0·034 μmol min^{-1} mg^{-1}) in the lysine-overproducing mutant derived from the same strain. This difference in aspartokinase specific activities between wild-type and mutant strains may indicate that the mutants, in general, are more resistant to the regulatory effects of lysine, and that this is the key to lysine overproduction observed in these mutants. As regulation by inhibition of the aspartokinase activity has apparently been discarded, it is tempting to speculate that regulation of this biosynthetic pathway in *Lact. plantarum* is at the level of gene expression. This is currently being investigated in this laboratory. We are also seeking to determine whether more than one aspartokinase is present in *Lact. plantarum.*

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Table 2 Specific activity of aspartokinase in cell extracts of *Lactobacillus plantarum*

Strain	Growth medium*	Aspartokinase specific activity (μmol min^{-1} mg^{-1})
OG 025 wt	DSM	0·053 ± 0·004†
OG 025 wt	DSM + Thr	0·047 ± 0·003
OG 025 wt	DSM + Lys + Thr	0·018 ± 0·005
OG 025-5	DSM	0·050 ± 0·003
OG 025-5	DSM + Thr	0·048 ± 0·003
OG 025-5	DSM + Lys + Thr	0·034 ± 0·003

wt, Wild-type strain; DSM, defined synthetic medium.
*L-Amino acids were added at a concentration of 1 mg ml^{-1} each to the DSM and cells were harvested after overnight incubation at 30°C.
†Values are means ± standard deviation from at least three independent experiments.

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REFERENCES

Akinrele, I. A. (1970) Fermentation studies on maize during the preparation of a traditional African starch-cake food. *Journal of Science and Food Agriculture* **21**, 619–625.

Anon. (1985) *Difco Manual for Microbiology Culture Media: Dehydrated Culture Media and Reagents for Microbiology*, 10th edn. pp. 1055–1066. MI: Difco Laboratories Inc.

Black, S. and Wright, N.G. (1955) β-Aspartokinase and β-aspartyl phosphate. *Journal of Biological Chemistry* **213**, 27–38.

Buckenhüskes, H.J. (1993) Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. *FEMS Microbiology Reviews* **12**, 253–272.

Cohen, G.N. (1983) The common pathway to lysine, methionine and threonine. In *Amino acids: Biosynthesis and Genetic Regulation* ed. Hermann, K.M. and Somerville, R.L. pp. 147–171. Reading, MA: Addison-Wesley Publishing Co.

Cremer, J., Treptow, C., Eggeling, L. and Sahn, H. (1988) Regulation of enzymes of lysine biosynthesis in *Corynebacterium glutamicum*. *Journal of General Microbiology* **134**, 3221–3229.

Cremer, J., Eggeling, L. and Sahn, H. (1991) Control of the lysine biosynthesis sequence in *Corybacterium glutamicum* as analyzed by overexpression of the individual corresponding genes. *Applied and Environmental Microbiology* **57**, 1746–1752.

De Man, J.C., Rogosa, M. and Sharpe, M.E. (1960) A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology* **32**, 130–135.

Flodin, N.W. (1993) Lysine supplementation of cereal foods: a retrospective. *Journal of the American College of Nutrition* **12**, 619–625.

Kalinowski, J., Cremer, J., Bachman, B., Eggeling, L., Sham, H. and Puhler, A. (1991) Genetic and biochemical analysis of the aspartokinase from *Corynebacterium glutamicum*. *Molecular Microbiology* **5**, 1197–1204.

Kandler, O. and Weiss, N. (1986) Genus *Lactobacillus* Beijerink 1901, 212. In *Bergey's Manual of Systematic Bacteriology*, Vol. 2, ed. Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. pp. 1209–1234. Baltimore: Williams and Wilkins.

Ledesma, O.V., de Ruiz Holgado, A.P. and Oliver, G. (1977) A synthetic medium for comparative nutritional studies of lactobacilli. *Journal of Applied Bacteriology* **42**, 123–133.

Lu, Y., Chen, Y. and Paulus, H. (1991) Identification of *aecA* mutations in *Bacillus subtilis* as nucleotide substitutions in the untranslated leader region of the aspartokinase II operon. *Journal of General Microbiology* **137**, 1135–1143.

McFeeters, R.F. and Kuang-Hua Chen (1986) Utilization of electron acceptors for anaerobic mannitol metabolism by *Lactobacillus plantarum*. Compounds which serve as electron acceptors. *Food Microbiology* **3**, 73–81.

Morishita, T., Deguchi, Y., Yajima, M., Sakurai, T. and Yura, T. (1981) Multiple nutritional requirements of lactobacilli: genetic lesions affecting amino acid biosynthesis pathways. *Journal of Bacteriology* **148**, 64–71.

Odunfa, S.A. and Adeyele, S. (1984) Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. *Journal of Cereal Science* **3**, 173–180.

Oyewole, O.B. and Odunfa, S.A. (1990) Characterization and distribution of lactic acid bacteria in cassava fermentation during fufu production. *Journal of Applied Bacteriology* **68**, 145–152.

Patte, J.-C. (1983) Diaminopimelate and lysine. In *Amino Acids: Biosynthesis and Genetic Regulation* ed. Hermann, K.M. and Somerville, R.L. pp. 147–171. Reading, MA: Addison-Wesley Publishing Co.

Rosner, A. and Paulus, H. (1971) Regulation of aspartokinase in *Bacillus subtilis*: the separation and properties of two isofunctional enzymes. *Journal of Biological Chemistry* **246**, 2965–2971.

Sano, K. and Shiio, I. (1970) Microbial production of L-lysine III. Production by mutants resistant to *S*-(2-amino ethyl)-L-cystein. *Journal of General and Applied Microbiology* **16**, 373–391.

Shiio, I. and Miyajima, R. (1969) Concerted inhibition and its reversal by end products of aspartate kinase in *Brevibacterium flavum*. *Journal of Biological Chemistry* **65**, 849–859.

Thierbach, G., Kalinowski, J., Bachmann, B. and Puhler, A. (1990) Cloning of a DNA fragment from *Corynebacterium glutamicum* conferring aminoethyl cysteine resistance and feedback resistance to aspartokinase. *Applied Microbiology and Biotechnology* **32**, 443–448.

Umbarger, H.E. (1978) Amino acid biosynthesis and its regulation. *Annual Reviews of Biochemistry* **47**, 533–606.