

Characterization of a L-serine dehydratase activity from *Streptococcus faecalis*

Marta E. FARIAS *, Ana M. STRASSER de SAAD **,
Aída A. PESCE de RUIZ HOLGADO **, and G. OLIVER **

CERELA, Centro de Referencia para Lactobacilos, Chacabuco 145, 4000 Tucumán, Argentina and
Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina

Summary

Streptococcus faecalis sp. produces pyruvate and ammonia from L-serine via a specific L-serine dehydratase. The apparent Michaelis constant for L-serine is 50 mM and the V_{max} is 142 nmol of pyruvate formed per minute and per mg of protein. Maximum enzymatic activity is observed at 40 °C and pH 8 in 100 mM phosphate buffer. L-serine is the sole substrate. D-serine, L-threonine and glycine are competitive inhibitors, L-cysteine acts as a non-competitive inhibitor.

The enzyme seems to be a very labile protein ; it is inactivated by dilution, dialysis and temperature. Pyridoxal phosphate is not required for maximum activity.

L-serine dehydratase is strongly inhibited by Cu^{2+} , Zn^{2+} , Hg^{2+} , p-chloromercuribenzoate, Tris, borate and acetate. The enzymatic activity is stimulated by Fe^{2+} ions.

Key words : *Streptococcus faecalis* - L-serine dehydratase - Serine catabolism.

Résumé

Caractérisation d'une activité L-sérine déshydratase de Streptococcus faecalis

Streptococcus faecalis sp. produit du pyruvate et de l'ammoniac par une L-sérine déshydratase spécifique, à partir de L-sérine.

La constante apparente de Michaelis pour la L-sérine est de 50 mM et la vitesse maximum de 142 nmoles de pyruvate formé par minute et par mg de protéine. L'activité enzymatique est maximum à 40 °C et à pH 8 dans du tampon phosphate 100 mM.

La L-sérine est le seul substrat de la réaction. La D-sérine, la L-thréonine et la glycine sont des inhibiteurs compétitifs. La L-cystéine est un inhibiteur non-compétitif.

* Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

** Career Investigator of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

L'enzyme semble être une protéine très labile ; elle est inactivée par dilution, dialyse et par la température.

Le pyridoxal phosphate n'est pas exigé pour obtenir une activité maximum.

La L-sérine déshydratase est fortement inhibée par les ions Cu^{2+} , Zn^{2+} et Hg^{2+} et par le P-chloromercuribenzoate. L'activité est aussi inhibée par les tampons Tris, borate et acétate.

L'ion Fe^{2+} a un effet stimulant sur l'activité enzymatique.

Mots-clés : *Streptococcus faecalis* - L-sérine déshydratase - Catabolisme de la sérine.

Introduction

Among the microorganisms which play a role in cheese ripening, the enterococci and specially *Streptococcus faecalis* are involved. Their importance has been shown in manufacture of soft Italian, Cheddar and some Swiss cheeses. This species is found in natural cheese or it is introduced as starter cultures (KOSIKOWSKI, 1978). Although other aspects of their metabolism and its effect on the appearance of flavor constituents is poorly understood. Ammonia is, according to DUMONT and ADDA (1978) the only volatile amine to play a genuine role in cheese flavoring, and this compound is produced by *S. faecalis* sp. from L-serine.

The enzymatic, non oxidative deamination of L-serine to pyruvate and ammonia by microorganisms has been known since 1938 (GALE and STEPHENSON, 1938). L-serine dehydratase and L-threonine dehydratase had long been considered to be the same enzyme. However, since the work of BENZIMAN *et al.* (1960) and CARTER and SAGERS (1972), who proved the existence of a L-serine dehydratase specific for L-serine in *Clostridium acidiurici*, similar activities have been reported in extracts of other microorganisms, such as *Arthrobacter globiformis* (BRIDGELAND and JONES, 1965 ; GANNON *et al.*, 1977), *Escherichia coli* K₁₂ (ISENBERG and NEWMAN, 1974), *Bacillus cereus*, *Salmonella typhimurium* (RASKO *et al.*, 1969), *Pseudomonas cepacia* (WONG and LESSIE, 1979), *Bacillus alvei* (GRIFFITHS and DE MOSS, 1970), *Corynebacterium* sp. (MORIKAWA *et al.*, 1974), *Klebsiella aerogenes* (VING and MAGASANIK, 1981), and *Brevibacterium linens* (HAMOUY, 1983). The existence of the enzyme in the Genus *Lactobacillus*, has been demonstrated in a prior work (FARIAS *et al.*, 1985) in *Lactobacillus murinus* ATCC 35020. In *Streptococcus*, on the other hand, the existence of this enzyme has not yet been demonstrated. The present paper described the general properties of the L-serine dehydratase from *Streptococcus faecalis* sp.

I. Materials and methods

A. Organism and culture medium

The strain utilized was *Streptococcus faecalis* sp. isolated from digestive human tract. CRL 663.

The culture medium (LAPTg₁₀) was described by RAIBAUD *et al.* (1956) (glucose, 10 g ; yeast extract, 10 g ; peptone, 15 g ; tryptone, 10 g ; Tween 80, 1 ml ; distilled water, 900 ml). The pH was adjusted to 6.5 with 0.2 N NaOH. The medium was sterilized by autoclaving for 20 min at 120 °C.

B. Preparation of cell-free extract

The cells of *S. faecalis*, grown in 2.000 ml of LAPTg medium at 37 °C were harvested, after 8 h, by centrifugation at 7.000 g for 15 min at 4 °C. The pellet was washed twice in 0.1 % β -mercaptoethanol, then, resuspended in 100 mM potassium phosphate buffer to 40 % (w/v) and disrupted in a French-press. The cell-free extract was obtained by centrifugation at 30.000 g for 30 min, at 4 °C.

C. Enzyme Assay

Reaction mixtures (final volume of 2 ml) containing 100 mM L-serine ; 100 mM potassium phosphate buffer pH 8 and 0.1 ml cell-free extract, were incubated at 40 °C. The reaction was stopped by rapidly pipetting 100 μ l aliquots into 500 μ l of 10 % (w/v) Trichloroacetic acid (TCA). L-serine dehydratase activity was determined by assaying the pyruvate produced by the method of FRIEDEMANN and HAUGEN (1943).

The mixture was held in an ice bath for 30 min and was then centrifugated at 5,000 g for 10 min at 4 °C. 500 μ l of 0.1 % (w/v) 2-4-dinitrophenylhydrazine (DNPH) in 2 N HCl were added to 500 μ l of supernatant. After 5 min at ambient temperature, 2.5 ml of 2 N NaOH were added and the absorbance at 540 nm was measured.

Specific activity of L-serine dehydratase is expressed as nmoles of pyruvate formed per minute per milligram of protein. The protein concentration was determined by the method of LOWRY *et al.* (1951) with bovine serum albumin as a standard.

II. Results

A. Kinetics parameters and specificity of L-serine dehydratase

L-serine dehydratase activity was detected in cell-free extracts of *Streptococcus faecalis* grown in LAPTg medium.

The quantity of pyruvate formed was proportional to protein concentration used. In the presence of 100 mM L-serine and 1 mg of protein, pyruvate formation was linear for 20 min.

The rate of enzyme activity was studied as a function of L-serine concentration at 40 °C and pH 8. From the Lineweaver-Burk curve (fig. 1), the following values were calculated : apparent Km for L-serine 50 mM and Vm for L-serine deamination 142 nmol \cdot min⁻¹ per mg of protein.

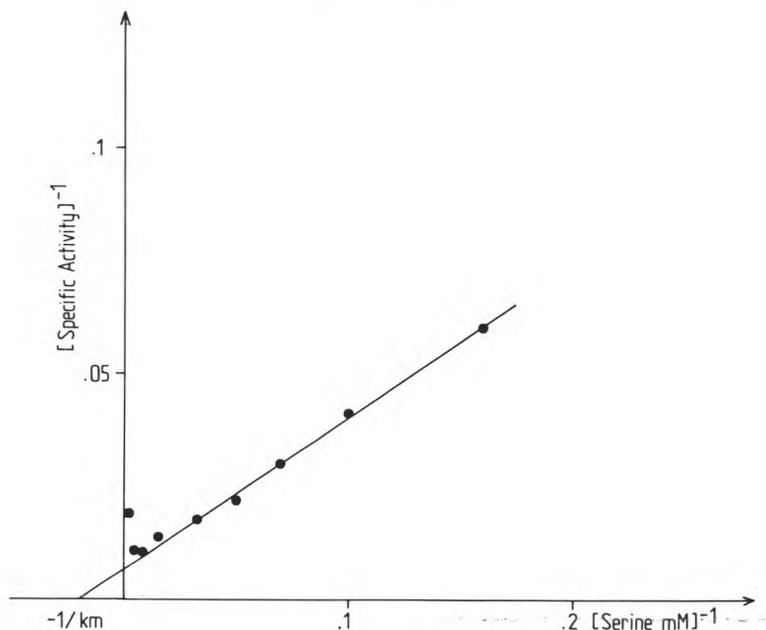


FIG. 1

Graphic determination (Lineweaver-Burk) of kinetic constants of L-serine dehydratase activity.

Détermination graphique (Lineweaver-Burk) des constantes cinétiques de la L-sérine déshydratase.

Pyruvate was not produced when D-serine or L-threonine were added to the reaction mixtures in the absence of L-serine.

The enzymatic activity was not modified by the presence of exogenous pyridoxal-5'-phosphate (PLP).

B. Effect of pH and temperature

Enzymatic activity was optimal at pH 8 in 100 mM phosphate buffer. For the test of activity at acid or alkaline pH values, it was not possible to use acetate, Tris or Borate buffers, since they inhibited the enzymatic activity. From Dixon plot (fig. 2) the following pK values were calculated, for the free enzyme : pK_a 7.05 and pK_b 8.5, and the pK of the ES complex 6.65.

Pyruvate production was maximal at 40 °C (fig. 3). The apparent activation energy of the reaction, ΔG^* , was calculated from Arrhenius plot and found to be about 13.200 cal mol⁻¹ (inset fig. 3).

Thermostability was investigated by preincubation of cell-free extract for 10, 20 and 30 min at different temperatures before the rate of enzymatic activity was assayed.

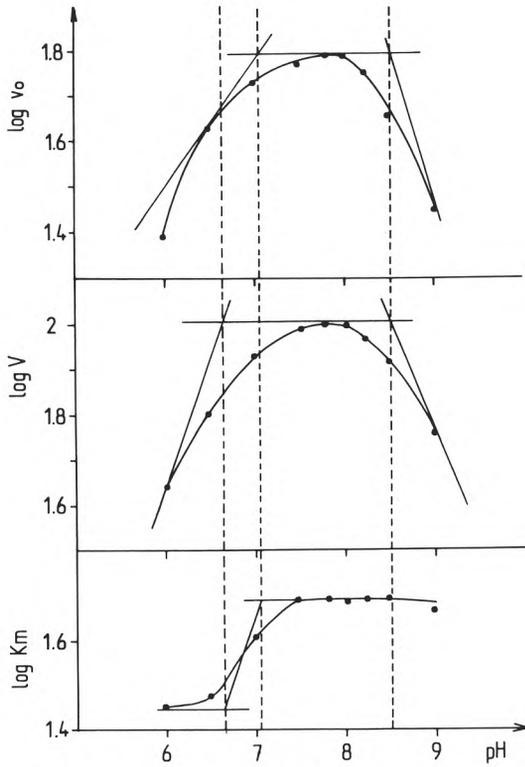


FIG. 2

*Effect of pH on K_m , V and v_o , for L-serine dehydratase activity.
Effet du pH sur K_m , V et v_o , pour l'activité L-sérine déshydratase.*

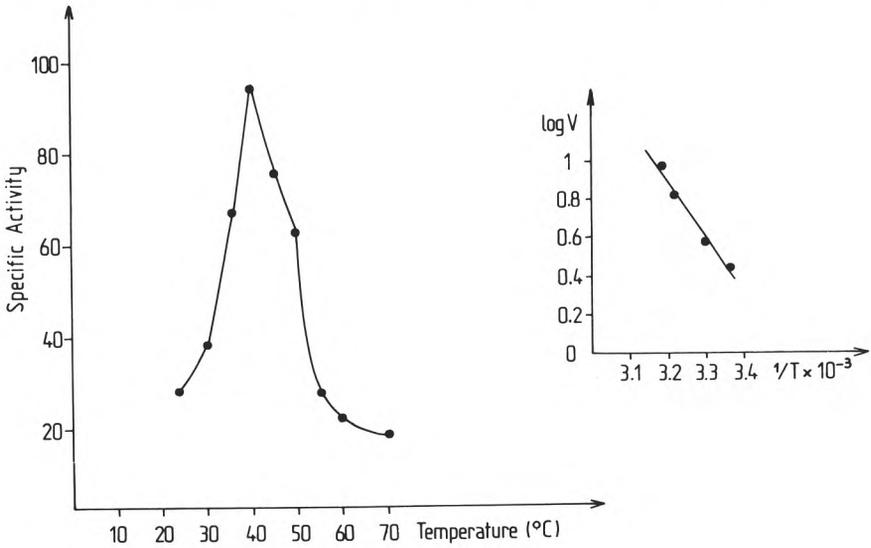


FIG. 3

*Effect of temperature on L-serine dehydratase activity. Inset figure : Arrhenius plot.
Effet de la température sur l'activité L-sérine déshydratase.*

The results in figure 4 shown a notable loss of activity from 30 °C (30 % in 10 min). This effect was not observed when the enzymic preparation was preincubated in the presence of 100 mM L-serine. At 60 °C the activity was only 10 % of the original, while at 70 °C, the enzyme was completely inactive.

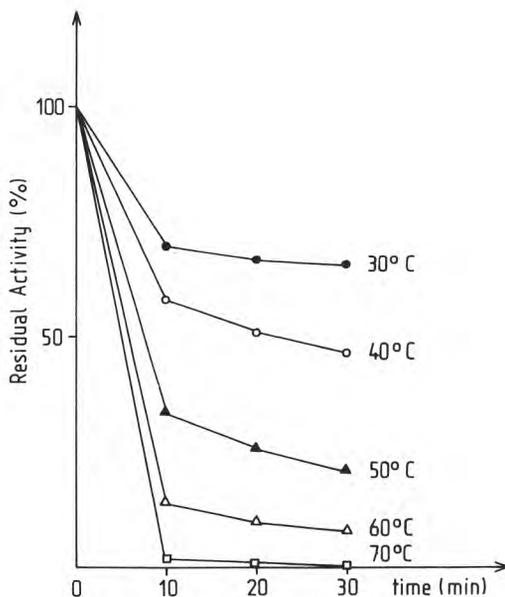


FIG. 4

*Thermostability of L-serine dehydratase activity.
Stabilité thermique de l'activité L-sérine déshydratase.*

C. Stability

When the cell-free extract was dialysed against 100 mM phosphate buffer, pH 8, for 6 h at 4 °C, a rapid loss of activity was observed (95 %). Different substances were added to the buffer to prevent the inhibition caused by dialysis. No protection was observed by 10 mM L-cysteine, β -mercaptoethanol or dithiothreitol. Thermal stability was studied by following the L-serine dehydratase activity during storage at different temperatures (fig. 5).

The half-life of the enzyme at 4 °C was approximately 24 h. At 0 and - 20 °C, after 3 days, the activity was only 10 and 35 %, respectively. No protection was observed by 10 mM dithiothreitol or glutathion (reduced form). At - 70 °C, the enzyme retained 100 % of the original activity after 90 days storage.

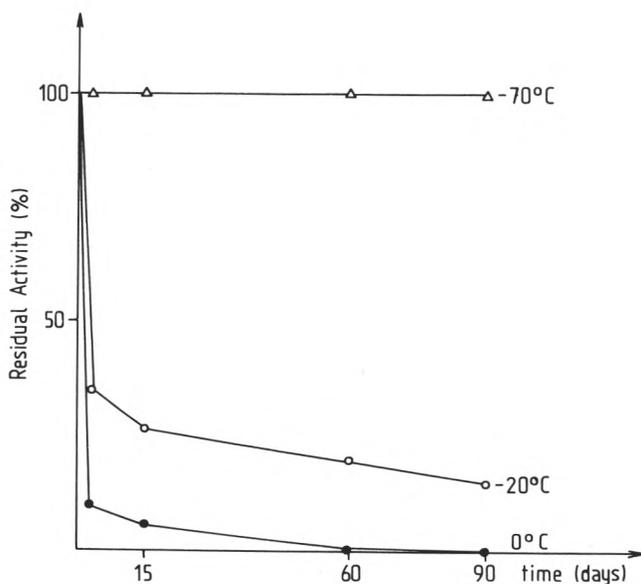


FIG. 5

Stability of L-serine dehydratase activity during storage.
Stabilité de l'activité L-sérine déshydratase pendant la conservation.

TABLE 1

Effect of cations on L-serine dehydratase activity
Effet des cations sur l'activité L-sérine déshydratase

Cation (1 mM)	Specific activity ^(a)	
	Before dialysis	After dialysis
—	112	6
Fe ²⁺	136	6
Fe ³⁺	113	5
Mg ²⁺	109	6
Mn ²⁺	92	13
Zn ²⁺	74	0
Cu ²⁺	68	0
Hg ²⁺	10	0
Fe ²⁺ + DTT ^(b)	110	20

(a) nmoles of pyruvate/min/mg of protein.

(b) dithiothreitol.

D. Effect of cations and substrate analogues

The results in Table 1 shown the effect of cations on enzymatic activity. In non dialysed cell-free extract, these results do not take into account the concentration of inorganic ions in the preparation which were not determined. Among the cations tested, only Fe^{2+} stimulate the enzymatic activity. Cu^{2+} , Zn^{2+} and Hg^{2+} acted as inhibitors of enzymatic activity. Mg^{2+} , Mn^{2+} and Fe^{3+} had no effect. Similar behavior was observed by these ions at a concentration 10 mM.

After dialysis, activation was observed by Mn^{2+} (50 %) and by Fe^{2+} plus dithiothreitol (DTT) (70 %), but the original activity was not recupered.

D-serine, L-threonine and glycine, were competitive inhibitors of L-serine dehydratase activity, while L-cysteine (fig. 6) acted as a non-competitive inhibitor (table 2).

P-chloromercuribenzoate (PCMB) was a potent inhibitor of the enzymatic activity (table 3). In the presence of 2 mM inhibitor, the activity decreased 75 %.

TABLE 2
Effect of substrate analogues on L-serine dehydratase activity
Effet des analogues du substrat sur l'activité L-sérine déshydratase

Inhibition	Inhibition-type	Ki (mM)
D-serine	competitive	30
L-threonine	competitive	2
glycine	competitive	20
L-cysteine	non-competitive	8

TABLE 3
Effect of PCMB on L-serine dehydratase activity
Effet du PCMB sur l'activité L-sérine déshydratase

PCMB (mM) ^(a)	Specific activity ^(b)
0	82.5
0.5	80.0
1	49.5
2	24.7
4	12.7

(a) p-chloromercuribenzoate.

(b) nmoles of pyruvate/min/mg of protein.

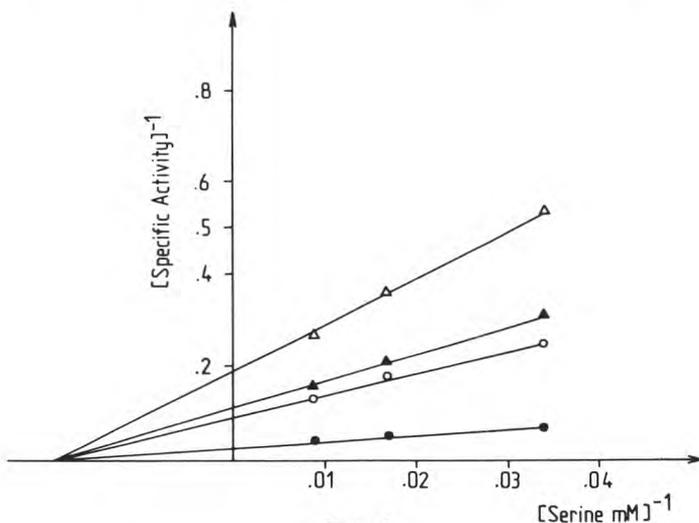


FIG. 6

Inhibition of L-serine dehydratase activity by L-cysteine.
 L-cysteine concentration : ●—●, 0 ; ○—○, 12.5 mM ;
 ▲—▲, 25 mM ; △—△, 50 mM.

Inhibition de l'activité L-sérine déshydratase par la L-cystéine.
 Concentration de la L-cystéine : ●—●, 0 ; ○—○, 12,5 mM ;
 ▲—▲, 25 mM ; △—△, 50 mM.

III. Discussion

L-serine dehydratase is present in cell-free extract of *S. faecalis*, and it is specific for L-serine.

This is a Michaelis type enzyme as serine dehydratases from *Clostridium acidurici* (BENZIMAN *et al.*, 1960), *Brevibacterium linens* (HAMOUY and DESMAZEAUD, 1985), and *Lactobacillus murinus* (FARIAS *et al.*, 1985).

The apparent K_m (50 mM) is not exceptional ; the same value was reported for the enzyme from *B. linens* (HAMOUY and DESMAZEAUD, 1985) ; the enzyme from other species such as *Pseudomonas cepacia* (WONG and LESSIE, 1979), *Escherichia coli* K₁₂ (NEWMAN and KAPOOR, 1980) and *Lactobacillus murinus* (FARIAS *et al.*, 1985) has a K_m value of same order of magnitude, 2×10^{-2} M ; 42×10^{-2} M, and 83.3×10^{-2} M, respectively. Similar values were reported for L-serine dehydratase from eukaryotes (SUDA and NAKAGAWA, 1971 ; SELIM and GREENBERG, 1959).

The optimum pH for L-serine dehydratase is generally slightly alkaline. The value obtained for *S. faecalis* enzyme was in agreement with those reported for L-serine dehydratase from *Corynebacterium* (MORIKAWA *et al.*, 1974) ; *Cl. acidurici* (SAGERS and CARTER, 1971) ; *Klebsiella aerogenes* (VINING and MAGASANIK, 1981), and *P. cepacia* (WONG and LESSIE, 1979).

The pH value was lower than that obtained for *B. linens* (HAMOUY and DESMAZEAUD, 1985) and higher than that reported for *L. murinus* (FARIAS *et al.*, 1985).

The two last enzymes, are thermolabile proteins; same behavior was observed in *S. faecalis* enzyme. However, L-serine dehydratase from *S. faecalis* was very stable during storage at -70°C .

Usually, serine dehydratase enzymes are very sensible to procedures such as dialysis or dilution. The enzyme from *S. faecalis* was not an exception.

The non-competitive inhibition by L-cysteine is interesting since this aminoacid is generally cited as a competitive inhibitor of the enzyme (ALFOLDI *et al.*, 1968; SUDA and NAKAGAWA, 1971; MORIKAWA *et al.*, 1974; GANNON *et al.*, 1977).

This behavior was only observed in *B. linens* (HAMOUY and DESMAZEAUD, 1985). L-cysteine would act as a chelator. Inhibition produced by L-cysteine, HgCl_2 and PCMB would by tend to show that a metal ion, probably Fe^{2+} participates in the enzymatic catalysis; this has been shown in other cases (BENZIMAN *et al.*, 1960; CARTER and SAGERS, 1972; NEWMAN and KAPOOR, 1980; HAMOUY and DESMAZEAUD, 1985). In addition Fe^{2+} is absolutely necessary for L-serine dehydratase activity in *Cl. acidiurici* (CARTER and SAGERS, 1972). Fe^{2+} stimulated L-serine dehydratase activity from *S. faecalis*, after dialysis, slight reactivation was obtained by Fe^{2+} in the presence of DTT.

D-serine, glycine and L-threonine acted as a competitive inhibitors. This behavior is in accordance with the chemical structures of these aminoacids.

PLP has no effect on the enzymatic activity. This was observed too in *Cl. acidiurici* (SAGERS and CARTER, 1971) and in *Arthrobacter globiformis* (GANNON *et al.*, 1977). This cofactor cannot reactivate the enzyme after dialysis as was observed too in *B. linens* (HAMOUY and DESMAZEAUD, 1985). The most interesting finding reported here is the first description of L-serine dehydratase activity exhibited by bacteria of the genus *Streptococcus*.

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References

- ALFOLDI L., RASKO I., KEREKES E., 1968. L-serine deaminase of *Escherichia coli*. *J. Bacteriol.*, **96**, 1512-1518.
- BENZIMAN M., SAGERS R.D., GUNSALUS I.C., 1960. L-serine specific dehydratase from *Clostridium acidurici*. *J. Bacteriol.*, **79**, 474-478.
- BRIDGELAND E.S., JONES K.M., 1965. L-serine dehydratase of *Arthrobacter globiformis*. *Biochem. J.*, **94**, 1-24.
- CARTER J.E., SAGERS R.D., 1972. Ferrous ion-dependent L-serine dehydratase from *Clostridium acidurici*. *J. Bacteriol.*, **109**, 757-763.
- DUMONT J.P., ADDA J., 1978. Flavour formation in dairy products. In : *Progress in Flavour research*. Ed. Lang D.G. and Nurstein H.E., London Applied Sciences Publishers, 245-262.
- FARIAS M.E., STRASSER DE SAAD A.M., PESCE DE RUIZ HOLGADO A., OLIVER G., 1985. Evidence for the presence of L-serine dehydratase in *Lactobacillus murinus*. *J. Gen. Appl. Microbiol.*, **31**, 563-567.
- FRIEDEMANN T.E., HAUGEN G.E., 1943. The determination of Keto-acids in blood and urine. *J. Biol. Chem.*, **147**, 415-422.
- GALE E.F., STEPHENSON M., 1938. Factors influencing the activity of DL-serine deaminase in *Bacterium coli*. *J. Biol. Chem.*, **32**, 392-404.
- GANNON F., BRIDGELAND E.S., JONES K.M., 1977. L-serine dehydratase from *Arthrobacter globiformis*. *Biochem. J.*, **161**, 345-355.
- GRIFFITHS S.K., DE MOSS R.D., 1970. Physiological comparison of L-serine dehydratase and tryptophanase from *Bacillus alvei*. *J. Bacteriol.*, **101**, 813-820.
- HAMOUY D., 1983. *Etude du catabolisme de la sérine chez Brevibacterium linens*. Thèse, Université Paris XI.
- HAMOUY D., DESMAZEAUD M.J., 1985. Characterization of an L-serine dehydratase activity in permeabilized cells of *Brevibacterium linens* ATCC 9175. *Lait*, **65**, 103-121.
- ISENBERG S., NEWMAN E.B., 1974. Studies on L-serine deaminase of *Escherichia coli* K₁₂. *J. Bacteriol.*, **118**, 53-58.
- KOSIKOWSKI F., 1978. In *cheese and Fermented Milk Foods*. 2nd edition. New York, Kosikowski F.V. and Associates, 16-48, 179-212, 228-260, 413-446.
- LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265-275.
- MORIKAWA Y., NAKAMURA N., KIMURA K., 1974. Purification and some properties of L-serine dehydratase of *Corynebacterium* sp. *Agric. Biol. Chem.*, **38**, 531-537.
- NEWMAN E.B., KAPOOR V., 1980. *In vitro* studies on L-serine deaminase activity of *Escherichia coli* K₁₂. *Can. J. Biochem.*, **58**, 1292-1297.
- RAIBAUD P., DICKINSON A.B., SACQUET E., CHARLIER H., MOCQUOT G., 1966. La microflore du tube digestif du rat. I. Techniques d'étude et milieux de culture proposés. *Ann. Microbiol.*, **110**, 568-590.
- RASKO I., KEREKES E., ALFOLDI L., 1969. Properties of L-serine deaminase from *Salmonella typhimurium* and *Bacillus cereus*. *Acta Microbiol. Hung.*, **16**, 237-244.
- SAGERS R.D., CARTER J.E., 1971. L-serine dehydratase (*Clostridium acidurici*). In : *Methods in Enzymology*, XVII (B). Ed. Tabor H. and Tabor C.W., New York, Academic Press, 351-356.
- SELIM A.S.M., GREENBERG D.M., 1959. An enzyme that synthesizes cystathionine and deaminates L-serine. *J. Biol. Chem.*, **234**, 1474-1480.
- SUDA M., NAKAGAWA H., 1971. L-serine dehydratase (rat liver). In : *Methods in Enzymology*, XVII (B). Ed. Tabor H. and Tabor C.W., New York Academic Press, 346-351.
- VINING L.C., MAGASANIK B., 1981. Serine utilization by *Klebsiella aerogenes*. *J. Bacteriol.*, **146**, 647-655.
- WONG H.C., LESSIE T.G., 1979. Hydroxyaminoacid metabolism in *Pseudomonas cepacia* : role of L-serine deaminase in dissimilation of serine, glycine and threonine. *J. Bacteriol.*, **140**, 240-245.