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HISTIDINE DECARBOXYLASE ACTIVITY IN LACTIC ACID BACTERIA FROM WINE

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Summary : *Histidine decarboxylase activity was investigated in 21 strains of lactic acid bacteria isolated from Argentinian wines. This activity is not widely distributed between them, and occurs significatively only in some strains of Lactobacillus hilgardii.*

L.hilgardii 5w was selected for the study on the basis of its constitutive expression. Glucose is necessary for histidine transport. Maximum activity is observed at 37°C and pH 4.0. Enzyme activity is inhibited by SO_2 and ethanol at concentrations usually found in wine. L-malic acid and citric acid act as stimulators of the activity.

INTRODUCTION

Histamine is a biogenic amine found in many animal and plant tissues as well as in various food and beverages. It is generally accepted that the presence of biogenic amines in food may be related to the metabolic activity of decarboxylase-positive bacteria on its precursor aminoacid (VOIGT and EITENMILLER, 1977).

Food containing biologically active amines produce undesirable physiological reactions. Histamine has been implicated as the causative agent in a number of food poisoning episodes, particularly in fish poisoning (LIBER and TAYLOR, 1978 ; EDWARDS et *al.,* 1987) and physiological distress caused by drinking contaminated red wine (OUGH et *al.,* 1987). However, even if the histamine contents of most of these foods do not readily explain its apparent toxic effect, these foods may also contain other substances that potentiate the action of biogenic amine (PARROT and NICOT, 1966). Alcohol can potentiate the effects of histamine as it facilitates diffusion of amines through the gut wall and it may also interfere with the degradation of histamine. The presence of histamine in wine is interesting from a technological point of view. Certain authors have related high levels of biogenic amines to a low quality index of the product or to a defective elaboration (CERUTTI and REMONDI, 1972). Due to the fact that wine is a widely consumed product in many countries, the wines histamine contents may be regulated.

The aim of this work is to determine the histidine decarboxylase enzyme in lactic acid bacteria isolated from Argentinian wines and to study one of these bacteria and the effect of different factors on it activity.

MATERIALS AND METHODS

I — MICROORGANISMS

The lactic acid bacteria selected for this work (table I) were isolated from Argentinian wines (MANCA de NADRA and STRASSER de SAAD, 1987, 1989 ; STRASSER de SAAD and MANCA de NADRA, 1987).

TABLE I

Histidine decarboxylase activity in lactic acid bacteria from wine

Strain		Histidine decarboxylase activity (nmol CO_2.min^{-1}.mg dry $weight^{-1}$)			
		Basal medium		Basal medium + His[a]	
		-	+Glc[b]	-	+Glc[b]
Pediococcus pentosaceus	9p	0.08	0.08	0	0
	10p	0.34	0	0.05	0.05
	E1p	0.36	0	0	0
	X2p	0.6	0	0	0
	12p	0	0	0	0
	13p	0.41	0.24	0.15	0.12
	Xp	0	0	0	0
	E3	0.32	0	0	0
	E2p	0.41	0	0	0.32
	E5p	0	0	0	0
Leuconostoc œnos	X2L	0.32	0	0	1.0
	ST	0.08	1.2	2.0	3.7
	L2	0	0.82	0.21	3.2
	m	0.48	0	0	0.43
Lactobacillus hilgardii	X1B	0.98	7.1	0.07	12.3
	5w	1.8	20.7	0	19.7
	5s	0.97	5.8	0	32.6
	7k	0	15.7	0	20.7
	6C	0	13.3	0	27.7
	6D	0	28.6	1.4	39.3
	N5L	0	17.7	2.8	25.3

[a] : 5 g/l histidine added to the culture medium ; [b] : 16 mM glucose added to the reaction mixture.

II — CULTURE MEDIUM AND GROWTH CONDITIONS

The basal medium used was MRS broth (DE MAN et *al.,* 1960) which contained (g/l): Proteose peptone, 10 ; beef extract, 10 ; yeast extract, 5 ; dextrose, 20 ; sorbitan monooleate complex, 1 ; ammonium citrate, 1 ; magnesium sulphate, 0.1 ; manganese sulphate, 0.05 ; Na_2HPO_4, 2 and tomato juice, 150 (ml/l) in distilled water.

The histidine concentration was 5 g/l, when added. The pH was adjusted to 4.8 with 10 M KOH before sterilization. The medium was sterilized for 15 min at 121°C.

Filter - sterilized, absolute ethanol was added to the cooled sterilized medium to yield the desired concentration. The inoculum was standardized to an optical density of 1.0 at 560 nm and added at 10 p. cent (v/v). Cultures were incubated at 30°C for 18 h.

III — HISTIDINE DECARBOXYLASE ACTIVITY

The enzymatic activity was determined by the respirometric method of GALE (1940). Cells from 100 ml of culture were collected by centrifugation at 8000 g per 15 min. The pellet was washed and resuspendend in 15 ml of 0.2 M acetate buffer pH 4.0. 5.0 ml of aliquots were taken for dry - weight determination.

The reaction mixture contained 0.2 ml of 0.2 M histidine, 1 ml of cell suspensions (side arm) and 0.2 M of acetate buffer pH 4.0 to give a final volume of 2.5 ml. Specific activity was expressed as nanomoles of CO_2 released per minute and per milligram of cell dry - weight. In all cases, a blank with glucose and without histidine was used to eliminate the CO_2 resulting from glucose fermentation by *L. hilgardii* 5w. When added, glucose concentration was 16 mM. The concentrations of the additives : ethanol, SO_2, L-malic acid and citric acid are indicated for each experiment.

RESULTS AND DISCUSSION

The lactic acid bacteria that develop during vinification in Cafayate (Argentina) wines belong to the genera *Lactobacillus, Pediococcus* and *Leuconostoc.* (MANCA de NADRA and STRASSER de SAAD, 1987 ; STRASSER de SAAD and MANCA de NADRA, 1987).

The histidine decarboxylase activity was investigated in 21 strains of lactic acid bacteria from wines composed of 10 strains of *Pediococcus pentosaceus*, 4 strains of *Leuconostoc oenos* and 7 strains of *Lactobacillus hilgardii* (table I).

Significative levels of enzymatic activity were not detected in *Pediococus pentosaceus* or *Leuconostoc oenos* strains, while this activity was observed in *Lactobacillus hilgardii* strains when 16 mM glucose was added to the reaction mixture. The presence of histidine decarboxylase activity in *Lactobacilli* has been previously reported (BAUMGART et *al.,* 1979 ; RODWELL, 1953 ; LAGEBORG and CLAPPER, 1952).

In 1985, SUMMER et *al.* reported that *Lactobacillus buchneri* from Swiss cheese, due to histamine production, was involved in a food poisoning outbreak. The presence of histidine in the basal medium increased the enzymatic activity of the strains except for *L. hilgardii* 5w. This is in accordance with the fact that catabolic bacterial decarboxylases are induced by high substrate concentrations (GALE, 1946 ; MORRIS and FILLINGAME, 1974).

For the characterization of enzyme activity, 5w strain was selected on the basis of this constitutive expression. The enzymatic activity showed a linear relationship when studied as a function of the histidine concentration. From Lineweaver Burk plot a Km value of 60 mM was calculated. The behavior is in agreement with that observed in *Lactobacillus* 30a (CHANG and SNELL, 1968) but differs from that reported for *L. buchneri* St2A by JOOSTEN and VAN BOEKEL (1988). In *L. hilgardii* 5w, the presence of glucose in the reaction mixture could facilitate the substrate transport.

The optimum concentration of glucose in the reaction mixture for histidine decarboxylase activity in cells grown with or without histidine is shown in table II. In both cases the activity was at a maximum with 16 mM glucose but it was lower in the cells grown in the presence of 5 g/l histidine. The reason for this effect is not clear. To determine the possible role of glucose in histidine transport, the enzymatic activity was studied in the presence of metabolic inhibitors (table III). Iodoacetate, an inhibitor of glyceraldehyde phosphate dehydrogenase, produced an inhibition of 84 p. cent. This effect could be due to the absence of histidine transport due to the lack of ATP energy. DCCD, the proton-translocating ATPase inhibitor, at a concentration of 0.5 and 1.0 mM, reduced enzymatic activity by 57 p. cent and 87 p. cent respectively. The ATPase system would be necessary for histidine active transport.

TABLE II

Influence of glucose concentration on the histidine decarboxylase activity from *L. hilgardii* 5w

Glucose concentration[a] (mM)	Specific activity of histidine decarboxylase (nmol.min^{-1}.mg dry weight^{-1})	
	Basal medium[b]	Basal medium + 5g/l histidine
0	0	0
1.6	1.2	1.7
4.0	5.0	5.0
16.0	18.3	16.4
32.0	7.5	6.0

a : glucose added to the reaction mixture ; **b** : cells grown in MRS + 15 p. cent tomato juice.

TABLE III

Histidine decarboxylase inhibition by metabolic inhibitor

Metabolic inhibitor	(mM)	% Inhibition
N, N'-dicyclohexylcarbodiimide	0.5	57
	1.0	87
Iodoacetate	1.0	84
Valinomicine	0.022	65
Carbonylcyanide-m-chlorophenylhidrazine	1.0	95

Addition of ionophores produced an inhibition of 65 p. cent (valinomicine) and 95 p. cent (CCCP) of enzymatic activity indicating that a proton motive force mediates the histidine transport. This tends to indicate that the transport system for L-histidine in *L. hilgardii* 5w is an ATP-dependent system in which ATPase and a proton motive force are involved. Glucose is necessary to supply energy to the system. These results are supported by the fact that for other lactic acid bacteria it has been shown that the accumulation of amino acids is an energy dependent process (THOMAS et *al.,* 1969 ; POOLMAN et *al.,* 1987).

The effect of temperature on histidine decarboxylase activity is shown in figure 1. Maximum activity was observed at 37°C. The optimum pH value for the enzymatic activity is 4.0. JOOSTEN and VAN BOEKEL (1988) reported maximun activity at pH 4.4 and 37°C for *L. buchneri* St2A.

The influence of many compounds naturally present in the wine (organic acids, ethanol) or added to it (SO_2) on histidine decarboxylase activity was investigated. The addition of 2.5 p. cent ethanol to the reaction mixture produced an inhibition of 100 p. cent and the addition of 20 mg/l SO_2 inhibited the enzyme activity by 95 p. cent (figure 2). Figure 3 shows the effect of citric acid and malic acid. At concentrations normally found in wine, a stimulation of histidine decarboxylase activity was observed with a maximum at 4.0 g/l of L- malic acid or 200 mg/l of citric acid.

The histidine decarboxylase activity was determined during the growth of *L. hilgardii* 5w in a basal medium (figure 4). Maximum activity was observed at the middle of logarithmic phase of growth and the level remained constant until the end of this period thereafter decreasing. When histidine was added to the culture medium the enzyme activity was lower which is confirmed by the data of table I.

We can conclude that histidine decarboxylase is not widely distributed between lactic acid bacteria isolated from Argentinian wines and is only present in some strains of *Lactobacillus hilgardii.* Among these strains the enzyme levels increased in the presence of histidine except for *L. hilgardii* 5w.

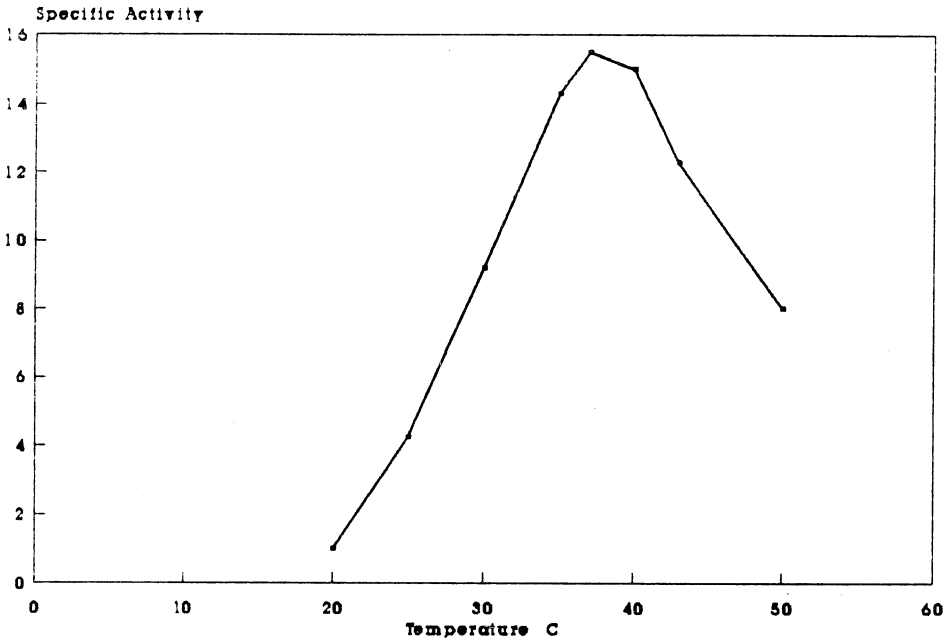

Fig. 1 — Influence of temperature on histidine decarboxylase activity of washed cell suspensions of *L. hilgardii* 5w

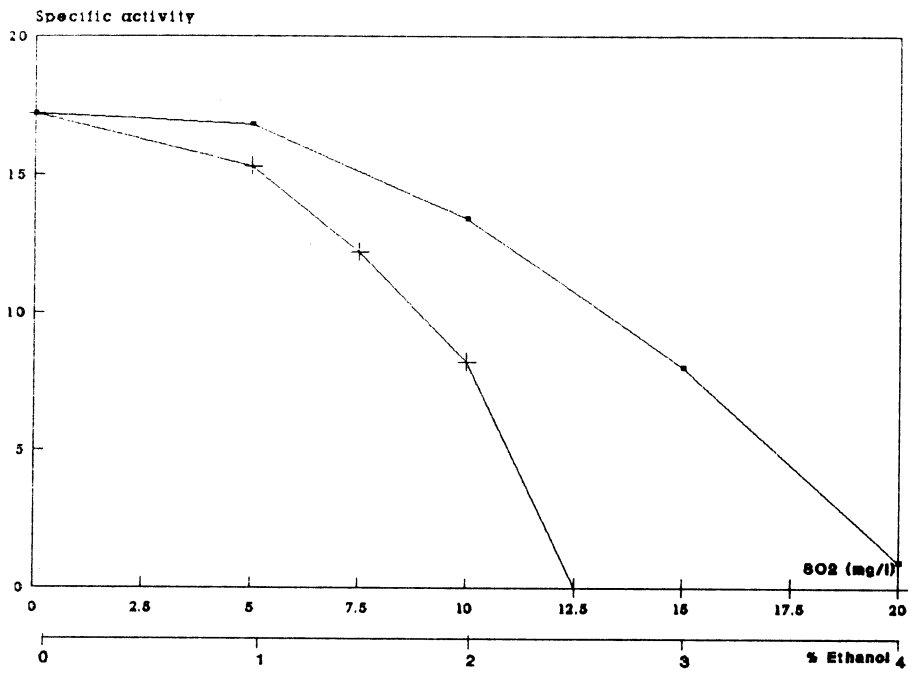

Fig. 2 — Influence of SO_2 (closed square) and ethanol (plus sign) on histidine decarboxylase activity of washed cell suspensions of *L. hilgardii* 5w

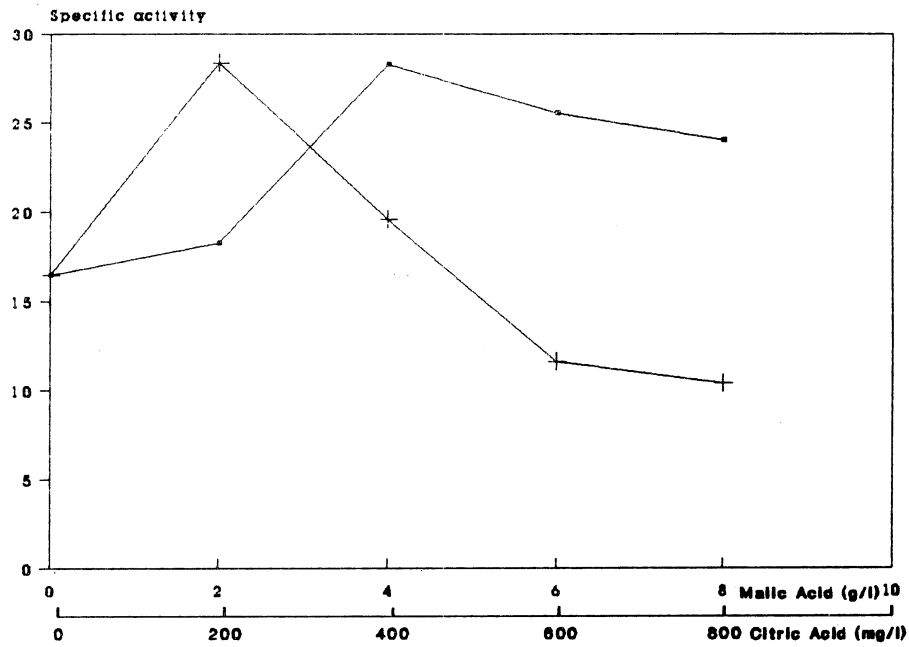

Fig. 3 — Influence of L-malic acid (closed square) and citric acid (plus sign) on histidine decarboxylase activity of washed cell suspensions of *L. hilgardii* 5w

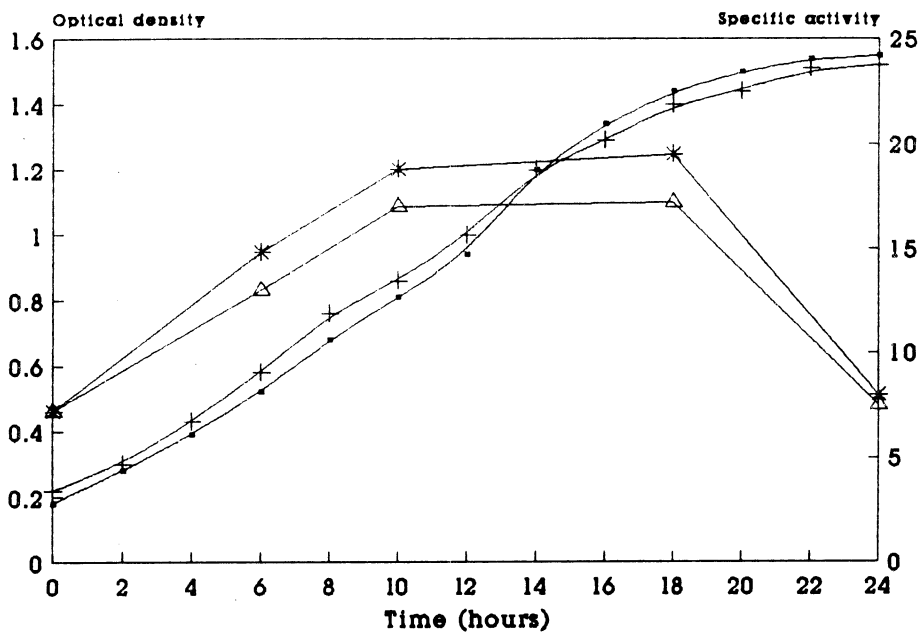

Fig. 4 — Histidine decarboxylase activity as a function of *L. hilgardii* 5w growth

In Basal medium: growth,(closed square); enzyme activity,(asterisk) ;
In Basal medium + 5 g/l histidine: growth, (plus sign); enzyme activity, (triangle)

The enzyme activity of the 5w strain requires glucose to supply the energy for the transport system of histidine. Its enzyme activity is inhibited by SO_2 and ethanol at concentrations naturally found in wine. Added to the reaction mixture, citric acid and L-malic acid produce stimulation of the enzyme activity. In order to consider the histamine formation from histidine by *L. hilgardii* 5w in wine, these effects have to be taken in account. Histidine decarboxylase accordingly should contribute to a small degree to the histamine content in wine.

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