



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Detection of histamine production by *Lactobacillus*

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ABSTRACT

Histamine causes many risks to human. Therefore, histamine detection in milk is important for infants. Beside milk components can be the relation to allergy, lactic acid bacteria existing in milk may be a cause. In the study, *Lactobacillus* produced histamine too low to detect by changing the color of from yellow to purple. However, thin layer chromatography (TLC), high performance liquid chromatography (HPLC) were set up to detect histamine at the detection level of histamine around 10 µg/ml in milk. The study clarified the histamine detection of lactic acid bacteria and warned milk products supplied with lactic acid bacteria should be used as soon as preparation.

Keywords: *Lactobacillus rhamnosus*, histamine, detection, thin layer chromatography, high performance liquid chromatography.

INTRODUCTION

Immune responses commonly relate to histamine, being a biogenic amine. Histamine also plays a role as a neurotransmitter controlling many actions in intestinal tract [1]. Histamine is synthesized from histidine via L-histidine decarboxylase as catalyst and vitamin B₆ as the substrate [2-5], then a carboxyl group will be removed and carbon dioxide will be released. Consequently, food that is rich of L-histidine and vitamin B₆ may produce histamine. Many histamine producing bacteria were studied [6] that depends on living conditions such as: animal, fish [7]. Up to now, *Bacillus subtilis* that is commonly used in food products or probiotic also produces histamine [8]. The histamine production by bacteria was detected by the changing the color to purple [3]. However, lactic acid bacteria (LAB) produce many kind acids leading to very low pH that makes histamine detection on agar plate not easy. Therefore, the study tried to use *Lactobacillus rhamnosus* as a model to find out the way to detect histamine production [8]. From that, this study pointed out the way to detect histamine produced in *Lactobacillus rhamnosus*.

EXPERIMENTAL SECTION

Detection of histamine production by indicator

Lactobacillus rhamnosus ATCC 11533 was used in the study. The *Lactobacilli* MRS medium and milk were added with 1.0% histidine, 0.006% bromocresol, 0.003% vitamin B₆ [9]. The pH was adjusted to 6.5 ± 0.2 in the practical culture media which then were sterilized at 121°C for 15 min. The bacterial inoculum of 10⁷ CFU was inoculated into broth at room temperature. Consequently, 10ml of culture were taken for analysis at sampling time of 10, 12, 13, 14 days.

Histamine extraction

Each 10ml culture was centrifuged to collect supernatant. Supernatant was filtered and shook up 5 mL chloroform (2:1) heated at 60°C. Repeated process was done in 3 times. Chloroform layers were gathered and continuously shook up with 5 ml ethanol (1:1). Ethanol layers were collected and evaporated completely.

Thin layer chromatography (TLC)

TLC method was used to detect histamine. Mobile phases were screened for detection. chloroform, methanol and ammonium were considered for experiment. The ratios of solvents were described in table 1. Silica plate gel 60 F₂₅₄ 25 Aluminium sheets 20x20 cm, Merck was used [10].

Table 1: The ratio of the solvents for histamine detection

	Chloroform	Methanol	Ammonium
Ratio	0	19	1
	0	18	2
	0	17	3
	0	16	4
	0	15	5
	12	7	1
	12	8	2
	12	9	1
	12	9	2
	12	9.2	2.2

The spots on the chromatogram were detected by spraying with ninhydrine. The dark purple spot and retention factor was considered as histamine.

High performance liquid chromatography

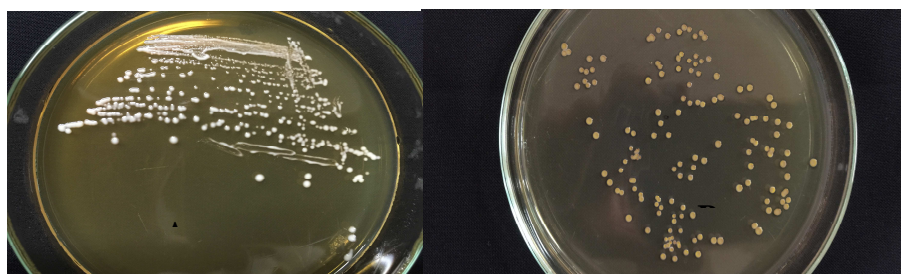
Histamine was analyzed using the HPLC method. The detection and quantitation by high performance liquid chromatography, using mobile phase water: acetonitrile (92:8) with flow rate 0.8ml per minute [11]. The samples were determined with ultraviolet detector at 210 nm and adopted a C18 column (Gemini 110A, 250×4.6, 5µm) for component separation; the analysis time for each sample was twenty minutes. The results were recorded by Shimadzu LC solution software (Tokyo, Japan).

RESULTS AND DISCUSSION**Morphological studies**

L. rhamnosus colonies had yellow color, not purple color as reported in many previous studies. Probably, *L. rhamnosus* produce organic acids in the media, leading low pH, therefore, although histamine increased, it couldn't neutralize pH to convert yellow to purple (Figure 1). Therefore, bromocresol modified in agar for histamine detection couldn't be used in histamine detection in *L. rhamnosus* or *Lactobacilli* genera. Actually, the size of colonies hardly changed which were varied from 2.0 to 3.5 mm. By using the pH indicator papers, pH of medium were measured approximately about 4 (Table 2). In milk agars, *Lactobacillus rhamnosus* showed deep- yellow colonies as well. The size of these colonies varied from 3.0 to 4.5 mm. The pH of medium changed from 6.5 to 5 (Figure 1, Table 2). To study on histamine production in *Lactobacilli*, many tests should be done, like thin layer chromatography, high performance liquid chromatography analysis.

Table 2: The color change and pH of experimental agars

Changes	Media	
	MRS	Milk
Color	yellow	yellow
pH	~4	~5

**Figure 1: Morphology of *L. rhamnosus* colonies on agar. (A): without supplement; (B) with supplements**

Thin-layer chromatography analysis

The best determination was recorded when the extraction was obtained from milk cultures. The optimal mobile phase was CHCl₃:CH₄O:NH₃ (2:9:2). From figure 2, by TLC analysis, extract from milk showed spots similarly to standard histamine (R_f=0.5).



Figure 2: TLC of samples prepared from modified milk. (1) standard histamine; (2) extract to detect histamine; (3) negative control

As showing in figure 2, *L. rhamnosus* could not produce histamine in modified MRS while histamine was produced in milk. Probably, milk is rich of nutrients and some other substrates that make *L. rhamnosus* produce histamine. With this study, it is careful to used long stored milk supplied with *L. rhamnosus*.

Histamine quantification

The extraction was introduced to high performance liquid chromatography (HPLC) for histamine detection and quantification. The data obtained from HPLC results was analyzed by comparison with the histamine standard. According to HPLC chromatogram, the retention time for histamine detection in the study was 8.724 min. The histamine concentration can be detected at the lowest 10 µg/ml (Table 3).

Table 3: The histamine concentrations obtained by the retention times

Incubation	Histamine (µg/ml)
Day 10	10.671±0.256
Day 11	12.637±0.517
Day 12	30.206±0.417
Day 13	30.685±0.690

Histamine production increased in the following days, suggesting that milk was a good nutrient source for LAB growth and histamine production.

CONCLUSION

The study gave a method to extract and detect histamine with low amount produced by LAB in milk, warned that milk products supplied with lactic acid bacteria should be used as soon as preparation.

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