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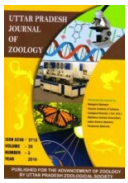

IMPACT OF LACTOPEROXIDASE SYSTEM ON *Listeria monocytogenes*

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

ARTICLE INFORMATION

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Short Research Article

ABSTRACT

Listeria monocytogenes is highly present in raw milk and dairy waste .It's also present in cheese received from retail shops. Objective of this experiment was to study the inhibition of *Listeria monocytogenes* by lactoperoxidase system in milk. Lactoperoxidase, potassium thiocyanate, glucose and glucose oxidase were added as per required concentration to generate the L-P system. *Listeria monocytogenes* strain 4e was taken under study and *Escherichia coli* strain NCTC 9703 was taken as positive control in the experiment. Colony count of *Listeria monocytogenes* fell from an average of 2×10^4 c.f.u /ml to an average of 2.5×10^3 c.f.u/ml within 5 days of storage. As per the study, non-lethal effect of L-P system was observed on *L. monocytogenes.*

Keywords: Listeria monocytogenes; food borne pathogen; pathogenic bacteria; lactoperoxidase system.

1. INTRODUCTION

Listeria monocytogenes is a food borne pathogen. Seeliger [1] observed *L. monocytogenes* as a causative agent of 'granulomatosis infanseptica' which was subsequently confirmed by Potel [2]. Since then, *L.monocytogenes* emerged as an important cause of prenatal infection and death of human infants. Listeriosis is regarded also as zoonoses [3]. Transmission of this organism occurs from animals to man during handling of newborn calves, infected dogs and drinking of infected milk. An attempt was made in this study to check the possibility of inhibiting *Listeria monocytogenes* by lactoperoxidase system. The lactoperoxidase-thiocyanate-hydrogen peroxide system is a natural antimicrobial system present in milk [4]. Lactoperoxidase catalyzes the oxidation of thiocyanate by hydrogen peroxide yielding short-lived oxidation products [5], hypothiocyanite ions [6] and higher oxyacids which are responsible for the antibacterial effect of the system.

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2. REVIEW OF LITERATURE

2.1 Report on Inhibition of *Listeria monocytogenes*

Carminati et al. [7] examined the inhibition of *L. monocytogenes* by bacteriocin-like inhibitor *streptococcus lactis*. Harris et al. [8] also evaluated 14 bacteriocin producing strains from genera *Lactobacillus, Leuconostoc, Pediococcus* and *Lactococcus* for their stability to inhibit the growth of 8 strains of *L. monocytogenes.* Ahmad and Marth [9] examined acid injury of *L. monocytogenes* in 0.3% and 0.5% acetic acid,citric acid and lactic acid solutions at 13 and 15 deg C. The Lactoperoxidase system (LP-s) is a natural antimicrobial system present in milk and other biological fluids. Its' function has been extensively reviewed [10,11]. The lacto peroxidase system activated by glucose oxidase proved to be bacteriostatic to *L. monocytogenes* inoculated in UHT milk supplemented with glucose [12]. Denis et al. [13] also studied antibacterial activity of lacto-peroxidase system on *L. monocytogenes* in trypticase soy broth, UHT milk and French soft cheese. W. Bibi and M. R. Bechmann [14] also studied the antibacterial effect of the lacto-peroxidase thiocyanate-hydrogenperoxide on the growth of *Listeria spp.* in skim milk. J. P. Perraudin [15] observed that Lactoperoxidase concentration was linked to lactation period. Fontec et al. [16] found the Thiocyanate concentrations (between 1 and 15 µg/ml) within the range in the milk samples as described in the literature. J. P. Zapico et al. [17] also studied the inhibition effect of L-P system on *L. monocytogenes* strain Ohio at 30 deg C and found the difference in population increase between activated and control milk of 3 log after 24 hour.

3. MATERIALS AND METHODS

3.1 Materials

The strain of *L. Monocytogenes* (serotype 4e)was collected from Indian Veterinary Research Institute, Izatnagar, U.P. (India). *Escherichia coli* (strain NCTC 9703) culture was collected from Bose Institute, Calcutta (India).

3.2 Methods

Sample preparation: Sterile litmus milk (8.9 ml) was taken in 30 ml sterile screw capped polystyrene universal containers. A decimal dilution was prepared in milk from overnight incubated culture (at 37 deg C) of *Listeria minocytogenes* strain 4e grown in nutrient broth and overnight incubated culture (at 37 deg C) of *Escherichia coli* strain NCTC 9703 grown in nutrient broth. Subsequently, 0.1 ml was taken from both the solutions.

Lactoperoxidase system: Lactoperoxidase, potassium thiocyanate, glucose and glucose oxidase were added to test samples according to the concentrations given: Lactoperoxidase 27 mg/litre of a 40-50 units /mg (lyophilized) preparation, potassium thiocyanate 84 mg/litre, glucose 10 g/litre and glucose oxidase 1.5 mg/litre of 1 x 10^5units /g (lyophilized) preparation. Sterile distilled water was added to make a final volume of 10 ml.All samples were prepared in duplicates and stored at 22 deg C up to the 5^{th} day.

Sampling: Duplicate samples were analysed after the samples were mixed well on a vortex mixer for 1 minute and a decimal dilution series was prepared in quarter strength Ringers solution containing 0.1% proteose peptone. Colonies of *Listeria monocytogenes* was enumerated by spreading of 0.1 ml of an appropriate solution and undiluted milk in duplicate over the dried surface of selective media (trypticase soy agar containing potassium thiocyanate and nalidixic acid).Incubation was done at 37 deg C for 2 days .Colonies of *Escherichia coli* were enumerated in similar way by spreading onto EMB agar. Incubation was done at 37 deg C for 2 days. The counts of both bacteria were taken daily upto the 5^{th} day. pH of samples was determined using a standard pH meter.

4. RESULTS AND DISCUSSION

In untreated control sample, *L. monocytogenes* grew over the initial 48 hrs from 10^4 c.f.u./ml to reach a maximum population of 10^8 c.f.u /ml which remained relatively constant until 5^{th} day.

Escherichia coli grew over the entire period of storage for an initial level of 10^4 c.f.u/ml to 10^8 c.f.u/ml after 5 days. The average colony counts for 5 days of storage is given in Table 2.

L. monocytogenes reduced very slowly from 10^4 c.f.u/ml to 10^3 c.f.u/ml over the 5 days period in the samples which were treated with L-P system. The average colony counts of *L.monocytogenes* on different days of storage are given in Table 3.

Whereas *E. coli* was reduced by a population size of 10^2 c.f.u/ml immediately after the treatment with lactoperoxidase. The growth inhibition of *Escherichia coli* with L-P system as noted in the present study corroborates earlier reports [18]. The killing of *E. coli* was an effective positive control for L-P system function. The average colony counts of *E. coli* on different days of storage are given in Table 4.

Table 1. Results of untreated (Control) sample for *Listeria monocytogenes*

L. monocytogenes	Storage time (days)					
	0	**1**	**2**	**3**	**4**	**5**
Colony count (c.f.u/ml)	$2x10^4$	$1.5x10^6$	$2x10^8$	$2.5x10^8$	$3x10^8$	$6x10^8$
Log_{10} c.f.u/ml	4.30	6.17	8.30	8.39	8.47	8.77

Table 2. Results of untreated (Control) sample for *Escherichia coli*

E. coli	Storage time (days)					
	0	**1**	**2**	**3**	**4**	**5**
Colony count (c.f.u/ml)	$3x10^4$	$3.5x10^5$	$3.5x10^6$	$4x10^7$	$5x10^8$	$6.5x10^8$
Log_{10} c.f.u/ml	4.47	5.54	6.54	7.60	8.69	8.81

Table 3. Effect of lactoperoxidase system (Treated sample) on the growth of *Listeria monocytogenes*

L. monocytogenes	Storage time (days)					
	0	**1**	**2**	**3**	**4**	**5**
Colony count (c.f.u/ml)	$2x10^4$	$5x10^3$	$4x10^3$	$3.5x10^3$	$3x10^3$	$2.5x10^3$
Log_{10} c.f.u/ml	4.30	3.69	3.60	3.54	3.47	3.39

Table 4. Effect of Lactoperoxidase system (Treated sample) on the growth of *Escherichia coli*

E. coli	Storage time (days)					
	0	**1**	**2**	**3**	**4**	**5**
Colony count (c.f.u/ml)	$3x10^4$	$3x10^2$	$2.5x10^2$	$2x10^2$	$1.25x10^2$	$1x10^2$
Log_{10} c.f.u/ml	4.47	2.47	2.39	2.30	2.09	2.0

The non-lethal effect on *Listeria Monocytogenes* by means of L-P system was almost clear. It may be suggested that *L. monocyogenes* produced substances that effectively neutralized the antimicrobial reaction products. In a study, Rodriguez, Tomillo, Nunez and Medina, [19] found that activation of the L-P system (at 8°C) was responsible for a reduction of 0.37 log units of *L. monocytogenes* compared to the control. In similar study, Seifu et al. [20]reported a decrease of 0.12 log units *L. monocytogenes* in L-P activated indigenous milk compared to the control which had an increase of 0.92 log units after 6h at ambient temperatures. Lactoperoxidase catalyzes the oxidation of thiocyanate by hydrogen peroxide yielding hypothiocyanite ions [21]. There are various reports concerning mechanisms of resistance to hypocyanate ($OSCN^-$). It states that an enzyme which catalyses the oxidation of $NADH_2$ by $OSCN^-$ [22,23] and an increase in cell sulphydryl groups that rapidly reduce $OSCN^-$ to SCN^- [24]. Other possible resistance mechanisms include novel $OSCN^-$ resistant respiration system (Mickelson, 1966) and a phosphoenolpyruvate dependent phosphor transferase system sugar transport mechanism which is resistant to $OSCN^-$ [25].

5. CONCLUSION

Regarding inhibition of *L.monocyogenes* by L-P system, it was observed that colony count of the organism had fallen from an average of 2 x 10^4 c.f.u /ml to an average of 2.5 x 10^3 c.f.u/ml within 5 days of storage. However, a few associated factors such as food components, water activity, type and load of associated microbial flora were not considered in this research. The efficiency of LP system mainly depends on the temperature which directly determines the antibacterial activity. Further, the results described in this study suggest that L-P system may be effective in milk and milk products to assist in inhibition of *L. monocytogenes*. Further research is needed to assess the possible interactions between the substrates, the enzymatic activity and efficiency of the LP system to see the potential effect of L-P system towards inhibition of *L.monocytogenes* like pathogenic bacteria.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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