*Asian Journal of Chemistry* Vol. 15, No. 2 (2003), 1149–1151

*NOTE*

# Antibacterial Activity of Lactoperoxidase from Sheep Colostrum

P.V JOSEPH†, JYOTHIS MATHEW††, CLARAMMA JACOB‡ and M. HARIDAS*
*Department of Life Sciences, Kannur University*
*Thalassery Campus, Palayad-670 661, India*

Various immune factors of colostrum have been reported to be associated with antibacterial activity. Therefore investigations were carried out with a view to examine the antibacterial action of the enzyme lactoperoxidase from the colostrum of sheep. The lactoperoxidase was purified from colostrum of sheep using ion exchange chromatography on C.M Sephadex C-50 and gel filtration chromatography on G-100 (Sigma). Purified lactoperoxidase was found to have antibacterial action against a number of disease causing bacteria.

**Key words: Sheep lactoperoxidase, Antibacterial activity, Disc diffusion.**

The presence of lactoperoxidase (LP) in secretion of mammary, salivary, lacrimal and harderian glands may constitute a naturally occurring defence mechanism in the body to inhibit bacterial proliferation[1-3]. The involvement of LP in the inhibition of bacterial growth was first suggested by Hanssen[4] and was demonstrated by Wright and Tramer[5]. LP has been recognised as an effective antimicrobial agent for many years and has been used extensively as a bactericidal agent in reducing microflora in milk[6, 7]. The enzyme LP together with hydrogen peroxide and thiocyanate ions comprise an antibacterial system in milk[8-12]. In biological fluids, LP catalyzes the peroxidation of endogenous thiocyanate ions ($SCN^-$) into the antibacterial hypothiocyanite ions ($OSCN^-$)[13-15] and serves as a component of the natural immunological defence system of mammals. In the present study, LP was isolated from sheep colostrum and its antibacterial properties were investigated for exploring the possibilities of developing it as an agent to help fight against diseases.

**Purification of sheep lactoperoxidase (sLP):** Sheep colostrum was collected from Sheep Breeding Research Station, Sandhinalla, Tamil Nadu. Defatting of colostrum was done by centrifuging at 10000 rpm for about 20 min in a refrigerated centrifuge. Defatted colostrum was diluted in 1 : 1 ratio with 0.05 M *tris*-HCl buffer, pH 8. Diluted colostrum was bound to swelled CM Sephadex C-50 (Sigma) and ion-exchange chromatography was done by NaCl gradient elution. The presence of sLP containing fractions was detected by 2,2′-azino bis

†Department of Zoology, K.E College, MG University, Mannanam-686 561, Kottayam , India.
††School of Bio Sciences, MG University, Kottayam-686 560, India.
‡Department of Zoology, Alphonsa College, MG University, Pala, Kottayam-686 574, , India.

(3-ethyl benzthiazoline-6-sulphonic acid) (ABTS) assay method[16]. The LP got eluted at 0.1 M NaCl concentration. Fractions containing sLP were concentrated by poly ethylene glycol (PEG)-20000 and gel filtration was done on Sephadex G-100 (Sigma). The fractions were collected and detected for the presence of sLP using ABTS assay method. Fractions with sLP were pooled, lyophilized and checked for purity by sodium dodecyl sulphate-poly acrylamide gel eletrophoresis (SDS-PAGE).[17]

**Disc diffusion studies:** The antibacterial property of sLP was studied by disc diffusion method. The bacterial strains were procured from Sree Chithira Thirunal Institute of Medical Sciences and Technology, Thiruvananthapuram. The organisms tested were *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi A, Shigella dysenteriae, Citrobacter freundii, Shigella sonnei, Staphylococcus albus* and *Serratia marcescens.*

For preparing protein solution for soaking the discs, 0.1 mL of 10 mM hydrogen peroxide, 0.1 mL of 10 mM potassium thiocyanate and 0.2 mL of protein were mixed and allowed to react for 1 min. After filter sterilization, 20 L of this mixture containing 6.3 g protein was applied on to blank sterile disc with 6 mm diameter (Hi-Media Laboratories Ltd., Bombay). Discs were used after drying them in an incubator at 37°C.

The nutrient broth cultures of the organisms, grown at 37°C for approximately 3–4 h were used as inocula.

Mueller Hinton agar plates were prepared[18] and lawned with the inocula using sterile cotton swabs dipped in nutrient broth culture. The discs were then applied to the lawned plates. Sterile discs impregnated with filter sterilized heat denatured protein (75°C for 3 min) were also used as control. After overnight incubation at 37°C, examination of plate for inhibitory zone around each disc was done. The diameter of the inhibitory zone around each disc was measured. Bacterial strains which showed a zone of inhibition > 12 mm were considered sensitive; the strains which showed an inhibitory zone between 10–12 mm were considered weakly sensitive and those which showed an inhibitory zone < 10 mm were considered resistant.

The results of disc diffusion studies against the bacterial strains are shown in Table-1. All the tested organisms showed a zone of inhibition 16 mm and were considered sensitive to LP. The antibacterial activity of LP from camel milk has already been reported[19]. It has been reported that the purified goat LP was found to have antibacterial action against many of the disease causing bacteria[20]. From the studies it is learnt that a potent bacterial inhibitory LP is present in sheep colostrum. Since many of the tested bacteria are pathogens, showing multiple drug resistance, the possibility of utilizing sLP as an antimicrobial agent against these pathogens can be a subject of further study. One major advantage of the application of antibacterial protein is that in principle they are biodegradable and biocompatible. In addition, since patients normally use these proteins, no severe side effects are anticipated even when they are administered in high dose.

In the present study only one strain, as the representative of each species, was included. Since bacteria show strain to strain variation in sensitivity to antimicrobial agents, a detailed study with a number of strains has to be conducted

to arrive at a final conclusion on the suitability of sLP as an antibacterial agent of significant status.

TABLE-1
RESULT OF DISC DIFFUSION STUDIES AGAINST BACTERIAL STRAINS

| Bacteria | Zone of Inhibition (mm) |
|---|---|
| *Escherichia coli* | 18 |
| *Kebsiella pneumoniae* | 19 |
| *Staphylococcus albus* | 17 |
| *Citrobacter freundii* | 20 |
| *Salmonella typhi* | 17 |
| *Salmonella paratyphi A* | 18 |
| *Shigella sonnei* | 18 |
| *Shigella dysenteriae* | 17 |
| *Serratia marcescens* | 16 |

## REFERENCES


1. S.J. Klebanoff, W.H. Clem and R.G. Luebke, *Biochem. Biophys. Acta,* **117**, 63 (1966).
2. M. Morrison and P.Z. Allen, *Science*, New York, **152**, 1626 (1966).
3. B. Reiter and J.D. Oram, *Nature,* **216**, 328 (1967).
4. F.S. Hanssen, *Br. J. Exp. Path.*, **5,** 271 (1924).
5. R.C. Wright and J. Tramer, *J. Dairy Res.*, **25**, 104 (1958).
6. T.M. Aune and E.L Thomas, *Eur. J. Biochem.*, **80**, 209 (1977).
7. K.M. Pruitt and J. Tenovuo, The Lactoperoxidase System, Chemistry and Biological Significance, Marcel-Dekker, New York, p. 143 (1985).
8. A. Portmann and J.E. Auclair, *Dairy Science,* **21**, 2638 (1959).
9. G.R. Jago and M. Morrison, *Proc. Soc. Ex. Bio. Med.*, **111**, 585 (1962).
10. R.R. Slowey, S. Eidelman and S.J. Klebanoff, *J. Bacteriol.*, **96**, 575 (1968).
11. W.F. Steele and M. Morrison, *J. Bacteriol.*, **97**, 635 (1969).
12. D. McC. Hogg and G.R. Jago, *J. Biochem.*, **117**, 779 (1970).
13. C.B. Hamon and S.J. Klebanoff, *J. Exp. Med.*, **137,** 438 (1973).
14. H. Hoogendoorn, J.P. Plessens, W. Scholtes and L.A. Stoddard, *Caries Res.*, **11,** 77 (1977).
15. D. Charles and R. Bernard, *J. Biol. Chem.*, **258,** 14166 (1983).
16. J.S. Shindler and W.G. Bardsley, *Biochem. Biophys. Res. Communs.*, **67,** 1307 (1975).
17. U.K. Laemmli, *Nature*, **277**, 680 (1977).
18. Monica Cheesbrough, Medical Laboratory Manuel for Tropical Countries, Vol. 2: Microbiology, Butterworth Co., U.K. (1985).
19. E.I. Agamy, R. Roger, Amin Ismail, C.P. Champage and Robbert Assaf, *Eur. J. Biochem.*, **59**, 169 (1992).
20. M.J. Benoy, N.K. Manoj and M. Haridas, *Ind. J. Exp. Biol.*, **36,** 808 (1998).
21. M. J. Benoy, E. Antony, B. Sreekumar and M. Haridas, *Life Sciences,* **66,** 2433 (2000).


(*Received*: 22 December 2002; *Accepted*: 22 January 2003) AJC-2997