TRANSMISSION ELECTRON MICROSCOPY OF LYTIC ACTION OF EQUINE MILK LYSOZYME ON *MICROCOCCUS LYSODEIKTIKUS* CELLS

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ABSTRACT

Lyophilized *Micrococcus Lysodeiktikus* cells suspended in phosphate buffer demonstrated typical anatomy of bacterial cells comprising thick cell wall, plasma membrane and cytoplasmic organelle mesosome when viewed under transmission electron microscopy. Treatment of *M. lysodeiktikus* cells with equine milk lysozyme caused progressive disintegration of peptidoglycan, a predominant constituent o cell wall, in presence of electrolytes at 25°C. The lytic action took 7 minutes to complete.

INTRODUCTION

Lysozyme (Muraminidase, N-acetylmuramyl-hydrolase, E.C. 3.2.1.17) is an extraordinary antibacterial immunoprotein (Ekstrand, 1989), which comes in different secretions of body i.e., milk, saliva, tears, etc. However, it was first found in hen's egg white. Lysozyme activity depends not only on the concentration of enzyme but also on its origin. Lysozyme isolated from bovine and equine milk has a far greater lytic activity on the most sensitive bacteria *Micrococcus lysodeiktikus* than that isolated from egg white (Vakil *et al.*, 1969).

The lytic activity of equine milk lysozyme measured by a turbidimetric method follows a simple kinetic reaction which shows a linear curve in the initial seven minutes of reaction and thereafter, maintains a plateau (Sarwar, 1995). It was considered worthwhile to study the lytic action of equine milk lysozyme on *M. lysodeiktikus* with the help of transmission electron microscopy.

MATERIALS AND METHODS

Equine milk sample was centrifuged at 15000 U/minute for 15 minutes in Biofuge A. One ml of milk serum was collected with the help of a micropipette. Attempt was made to avoid fat layer and sediments deposited at bottom. 30 µl of milk serum was mixed with 1770 µl of 0.9% NaCl solution to get 1:60 dilution. Lyophilized *M. lysodeiktikus* cells ATCC 4698 (Lot. 109F68O881) were dissolved in filtered phosphate buffer (1:5). The dilutions were used as prescribed by Sarwar (1995) for determination of lysozyme activity in mare's milk with modified turbidimetric method. Both of the diluted samples of milk serum and cell suspension were incubated at 25°C for 10 minutes to maintain a uniform temperature.

After incubation, 25 µl of equine milk serum was added in 1.5 ml of *M. lysodeiktikus* cell suspension allowing the reaction to proceed for specific time intervals i.e., 2 and 7 minutes and fixed with Kornowsky fixative mixture. The control samples were fixed, however, without adding equine milk serum. After fixation, the solution was centrifuged at 10,000 U/minute for 15 minutes to form a pellet of cells. The collected pellet was washed with 0.1M sodium cacodylate buffer and incubated overnight at 4°C with same buffer, post-fixation was carried out in osmium tetroxide solution for 2 hours. After post-fixation, the samples were dehydrated in alcohol with last step in propylenoxide, infiltered in ERL-resin/ propylenoxide-series (Spurr, 1969), and transferred to plane embedding shells. Ultrathin Sections were cut with the help of an ultracut-E-ultramicron, and examined with Zeiss EM109 transmission electron microscope.

RESULTS AND DISCUSSION

Morphology

M. lysodeiktikus cells appeared normal when viewed in ultrathin sections prepared without treatment of equine milk serum. The cell wall, cytoplasmic membrane, and the membranous organelle present in cytoplasm (Mesosome) were clearly visible (Fig. 1a). The cell wall of *M. lysodeiktikus* appears to be a thick amorphous structure typical of gram positive

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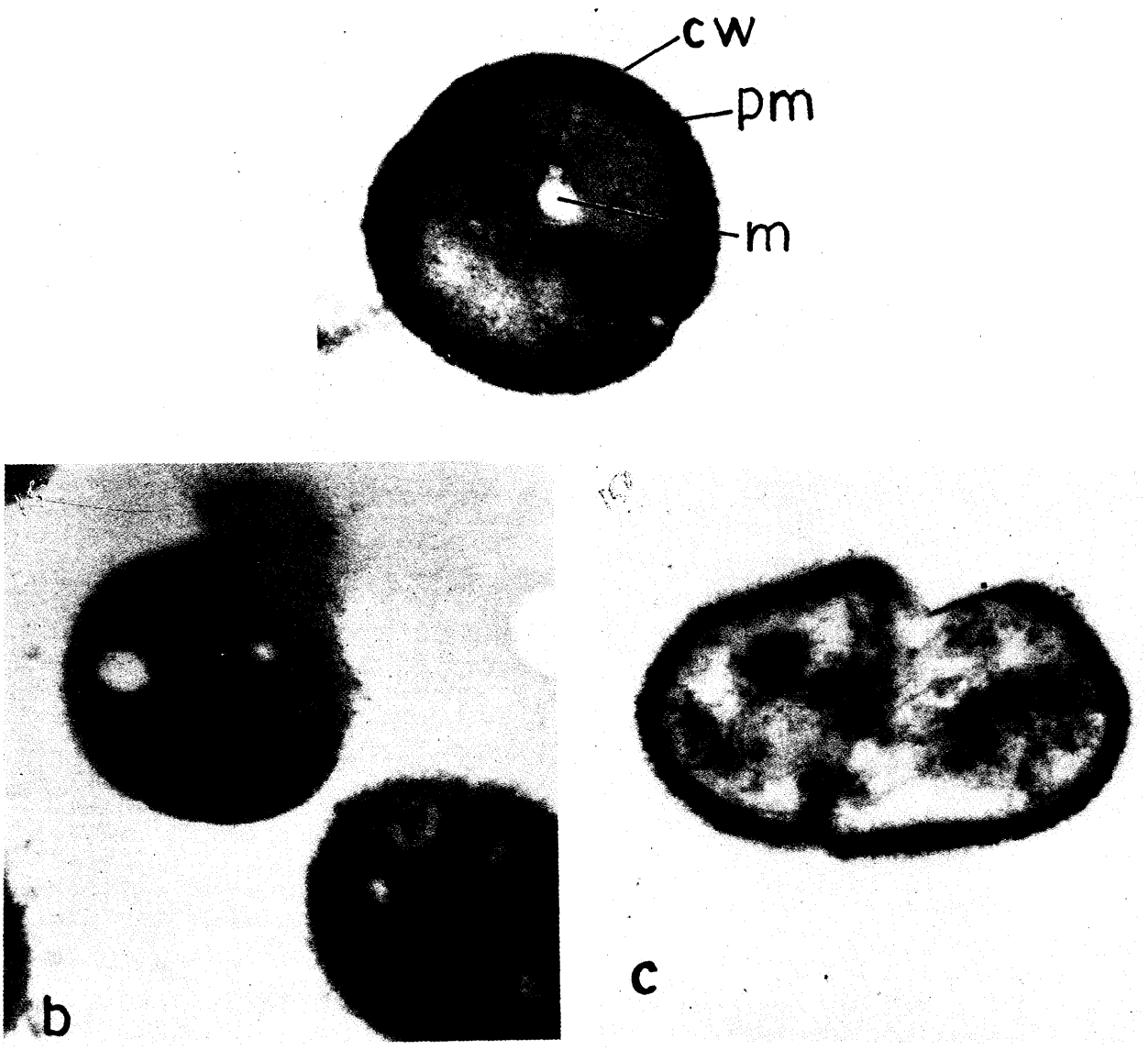

Fig. 1. Electron micrographs of *Micrococcus lysodeiktikus* (a) showing unlysed cell demonstrating intact thick cell wall plasma membrane, and mesosome(m). x 50,000; (b) lysed cells after 2 minutes of reaction with equine milk serum, x 50,000, cytoplasmic contents are bubbling out; (c) lysed cells after 7 minutes of reaction with equine milk serum., x 50,000, cytoplasmic contents are washed off.

bacteria. This terminology has been adopted here from Salton and Chapman (1962).

Lytic effect of equine milk lysozyme on *M. lysodeiktikus*.

Fig. 1(b) illustrates ultrathin sections of *M. lysodeiktikus* treated with equine milk serum for two minutes. Both the cell wall and plasma membrane were missing in damaged part under the action of lysozyme. Cytoplasmic contents had started to bubble out of the cell boundary. Samples allowed to react for seven minutes with equine milk serum are presented in Fig.1 (c) which demonstrated that the cell wall and plasma membrane were missing in damaged part of the cell.

The cytoplasmic contents had completely washed off following the disruption of cell wall and plasma membrane. The bio-anatomical studies of this organism indicated that cell wall was predominantly comprised of peptidoglycan polymer murein (Jolles, 1965) which is sufficiently accessible to the lysozyme to account for the considerable and rapid hydrolysis of the ß-1,4 glycosidic bonds.

Fig. 2: (a) and (b) illustrates the overviews of intact cells before treatment and lysed cells after treatment with equine milk lysozyme.

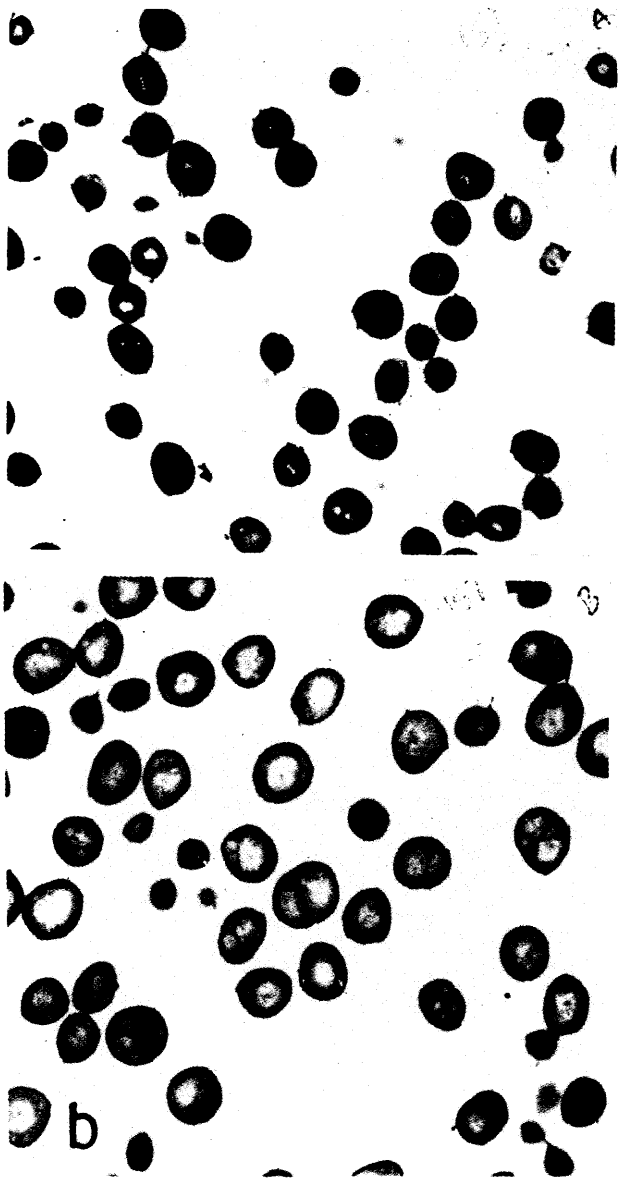

Fig. 2: Electron micrographs of *Micrococcus lysodeikticus* cells (a) before treatment with equine milk serum. x 12,000, nearly all cells are unlysed; (b) after 7 minutes of reaction with equine milk serum. x 7,000, nearly all cells are lysed.

CONCLUSIONS

The equine milk serum treatment provides a gentle method for complete and rapid progressive disintegration of cell wall which ultimately causes lysis of the cell. Nearly all the cells lysed under the action of equine milk lysozyme at 25°C in the presence of electrolytes took a maximum of seven minutes to complete. EM studies showed that lysis of the cell wall of *M. lysodeiktikus* under the action of equine milk serum can be morphologically exploited.

ACKNOWLEDGMENTS

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REFERENCES

Ekstrand, B., 1989. Antimicrobial factors in milk. Food Biotechnology, 3:105-126.

Jolles, P., 1965. Chemie und biochemie der Lysozyme. Chimia, 19:176.

Sarwar, A., 1995. Untersuchungen zur Lysozymaktivität in der Milch von der Stuten im Verlauf der frÜhen Laktationsperiode unter BerÜcksichtigung des Fruchtbarkeits- und Hormonstatus sowie anderer Einflußfaktoren. Hannover, Tierärztliche Hochschule, Diss.

Salton, M.R.J. and Chapman, J.A., 1962. Isolation for membrane-mesosome structure for *Micrococcus lysodeiktikus*. J. Ultrastructure Res., 6:489-498

Spurr, A.R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Res., 26:31-43.

Vakil, J.R., Chandan, R.C., Parry, R.M. and Shahani K.M., 1969. Susceptibility of several microorganisms to milk lysozyme. J. Dairy Sci, 52:1192-1197.