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# A Comparison of Two Methods for the Extraction of Lactoferrin-binding Proteins from *Streptococcus uberis*

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**Abstract :** Lactoferrin-binding proteins (LBP) has not been well characterized in *Streptococcus uberis* isolated from milk of bovine mastitis and to date this protein is considered to be an important virulence factor in Streptococcal mastitis. To determine the more efficient extraction method of LBP from four *S. uberis* strains, we used two different extraction methods (mutanolysin and sodium dodecyl sulfate) in this study. Bacterial proteins extracted were electrophoresed by 10% polyacrylamide gels in the presence of sodium deodecyl sulfate and gels were transferred onto nitrocellulose membrane. Rabbit anti-bovine lactoferrin antibody and HRP-conjugated donkey anti-rabbit IgG antibody were used to detect LBP. This Western blotting analysis demonstrates that extraction method with SDS extracted 110 kDa and 112 kDa LBPs more efficiently compared to the mutanolysin extraction method.

***Key words* :** sodium dodecyl sulfate, mutanolysin, lactoferrin-binding protein.

## Introduction

Lactoferrin-binding proteins (LBP) are cell-surface associated bacterial proteins. Fang and and Oliver (2) identified LBP in *Streptococcus uberis*, however, to date this protein has not been well characterized. A recent study (1) suggested that there were different immunological reactions on immunoblots with antisera against acid extracted and mutanolysin extracted M-protein of *Streptococcus equi*, and the amino acid composition varied depending on extraction methods used. Mutanolysin and sodium dodecyl sulfate (SDS) have been used extensively to extract surface proteins of Gram positive bacteria (7).

These methods appeared to have little, if any, influence on conformational epitopes of the surface protein (1). However, prior to purification and subsequent characterization of LBP, we needed to determine the optimal method for extraction of LBP from *S. uberis*. Thus, the present study was conducted to compare potential differences in the efficiency of extraction of *S. uberis* LBP with mutanolysin or SDS by SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting.

## Materials and Methods

Four strains of *S. uberis* were used in this study. Three strains (UT888, UT366 and UT102) are originally isolated from dairy cows with mastitis in our laboratory in the University of Tennessee. *S. uberis* UT888 has been used extensively for experimental challenge studies to induce clinical mastitis and *S. uberis* UT366 is more virulent and causes clinical mastitis with systemic signs (8). *S. uberis* UT102 was isolated from a cow with subclinical mastitis and *S. uberis* ATCC 13387 was obtained from the American Type Culture Collection.

Bacterial surface proteins from cell pellets were extracted from 0.2% SDS in 0.1 M phosphate buffered saline (PBS, pH 7.4; NaCl 8 g, KCl 0.2 g, $Na_2HPO_4$ 1.44 g, $KH_2PO_4$ 0.24 g in D.W. 1*l*) following the method described by Fang and Oliver (2). Briefly, each strain of *S. uberis* was grown in Todd-Hewitt broth (THB, Difco) at 37°C for overnight. After centrifugation, bacteria were resuspended in PBS (pH 7.2). Bacterial pellets were washed three times with sterile PBS, and surface proteins were extracted using 0.2% SDS (Bio-Rad; 30 mg wet weight of bacteria per 100 μ*l* of 0.2% SDS) for 1 h at 37°C In the mutanolysin extraction method, a modified procedure described by Galan and Timoney (3) was used. Briefly, bacterial cells were suspended (1 g/2μ*l*) in 50 mM phosphate buffer, pH 7.2, containing 0.5 M sucrose and 10 mg/μ*l* lysozyme (Sigma). The resulting suspension was divided into 2 ml aliquots and 250 units of mutanolysin (N-acetylmuramidase, Sigma) were added per aliquot. The suspension was then allowed to incubate for 1 h on a shaking incubator at 37°C Bacteria were pelleted by aliquoting the suspension into microcentrifuge tubes and centrifuging for 5 min. Supernatant of each were removed carefully and stored at –20°C

Bacterial proteins (10 μg per lane) were electrophoresed on 10% polyacrylamide gels in the presence of SDS as described by Laemmli (6). Gels were either stained with Coomassie brilliant

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blue or transferred onto nitrocellulose membrane using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad) (5). Unbound sites on blots were blocked with 3% casitone in PBST. Blots were probed with Lf (5 mg/m*l*; Sigma) in PBST containing 0.1% casitone (Difco) for 6 h at 4°C, followed by four washes with PBST. Procedures for further probing of blots with rabbit anti-bovine Lf antibody and HRP-conjugated donkey anti-rabbit IgG antibody were as described previously (2). Blots without probing with Lf and rabbit anti-bovine Lf antibody were included as negative controls.

## Results and Discussion

In this experiment, bacterial surface proteins were extracted with 0.2% SDS or mutanolysin. Bacterial surface proteins were electrophoresed by SDS-PAGE and stained with Coomassie brilliant blue. When surface proteins were extracted with 0.2% SDS-detergent and evaluated by SDS-PAGE, 110 kDa and 112 kDa protein bands were extracted more efficiently compared to the mutanolysin extraction method (Fig 1). In Western blot analysis, LBP bands in SDS extracts, particularly 110 and 112 kDa, were much stronger than those of mutanolysin extracts (Fig 2). Several different methods had been used to extract streptococcal surface proteins (1,4,7). Hammerschmidt *et al.* (4) extracted bacterial surface proteins with 3-[(3-cholamidopropyl)-dimethylammonio-1-propanesulfonate] and identified the pneumococcal surface protein A of *Streptococcus pneumoniae* as a lactoferrin-binding protein.

Boschwitz *et al.* (1) extracted M-protein from *Streptococcus equi* using different extraction methods such as alkaline, acid, mutanolysin, and SDS. Some extraction methods altered or destroyed conformational epitopes, however, mutanolysin and SDS appeared to have little, if any, influence on conformational epitopes of bacterial surface proteins (1).

In conclusion, results of this study suggest that SDS extracts 110 kDa and 112 kDa protein of interest more efficiently and this extraction method appears to be a suitable method to extract LBP for purification and subsequent characterization.

**Fig 1.** Proteins separated by SDS-PAGE after extraction of bacterial surface proteins with 0.2% SDS or mutanolysin. S: 0.2% SDS extraction, M: mutanolysin extraction, Lane 1: Molecular marker, Lane 2: UT387, Lane 3: UT102, lane 4: UT102, Lan 5: UT366, Lane 6: UT366, Lane 7: UT888, Lane 8: UT888, Lane 9: Molecular weight marker, Lane 10: UT387.

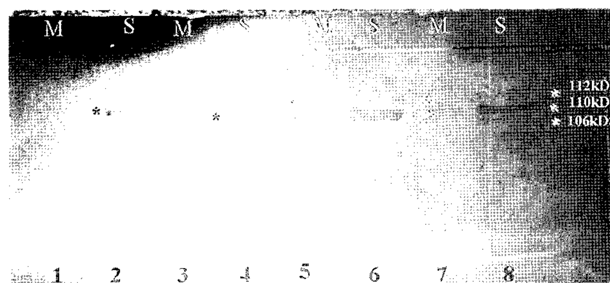


**Fig 2.** Western blot of proteins separated by SDS-PAGE after extraction of bacterial surface proteins with 0.2% SDS or mutanolysin. M: Mutanolysin extraction, S: SDS extraction, Lane 1: UT 387(M), Lane 2: UT387(S), Lane 3: UT102(M), Lane 4: UT102(S), Lane 5: UT366(M), Lane 6: UT366(S), Lane 7: UT888(M), Lane 8: UT888(S).

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# Streptococcus uberis의 락토페린 결합단백질 추출을 위한 두 가지 방법의 비교

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**요 약** : 락토페린 결합단백질(Lactoferrin-binding proteins, LBP)은 젖소유방염 원인균인 *Streptococcus uberis*의 막단백질로서 그 특성에 관해서는 잘 규명되어 있지 않지만, 특히 최근에는 스트렙토코커스성 유방염의 독성인자로서 중요시되고 있다. 본 연구에서는 *S. uberis* 네 가지 균주를 대상으로 LBP를 보다 효율적으로 추출하기 위하여 mutanolysin 및 sodium dodecyl sulfate(SDS)를 이용한 두 가지 다른 추출 방법을 사용하였다. 추출된 세균단백질을 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)로 전기영동을 하였고, 겔을 니트로셀룰로스 막으로 이동시켰다. Rabbit anti-bovine lactoferrin 항체와 HRP-conjugated donkey anti-rabbit IgG 항체를 사용하여 LBP를 검출하였다. 이러한 웨스턴 블롯팅 분석을 통해 SDS 추출법이 mutanolysin 추출법에 비해 보다 효율적으로 110 kDa 및 112 kDa의 LBP를 추출할 수 있음을 증명하였다.

**주요어** : sodium dodecyl sulfate, mutanolysin, 락토페린 결합단백질.