Genetics and Molecular Research

Immunological characteristics of outer membrane protein omp31 of goat *Brucella* and its monoclonal antibody

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Genet. Mol. Res. 14 (4): 11965-11974 (2015)
Received January 14, 2015
Accepted June 9, 2015
Published October 5, 2015
DOI http://dx.doi.org/10.4238/2015.October.5.10

ABSTRACT. We examined the immunological characteristics of outer membrane protein omp31 of goat *Brucella* and its monoclonal antibody. Genomic DNA from the M5 strain of goat *Brucella* was amplified by polymerase chain reaction and cloned into the prokaryotic expression vector pGEX-4T-1. The expression and immunological characteristics of the fusion protein GST-omp31 were subjected to preliminary western blot detection with goat *Brucella* rabbit immune serum. The *Brucella* immunized BALB/c mouse serum was detected using purified protein. The high-potency mouse splenocytes and myeloma Sp2/0 cells were fused. Positive clones were screened by enzyme-linked immunosorbent assay to establish a hybridoma cell line. Mice were inoculated intraperitoneally with hybridoma cells to prepare ascites. The mAb was purified using the n-caprylic acid-ammonium sulfate method. The characteristics of mAb were examined using western blotting and enzyme-linked immunosorbent assay. A 680-base pair band was observed after polymerase chain reaction. Enzyme digestion identification and sequencing showed that the pGEX-4T-1-omp31

prokaryotic expression vector was successfully established; a target band of approximately 57 kDa with an apparent molecular weight consistent with the size of the target fusion protein. At 25°C, the expression of soluble expression increased significantly; the fusion protein GST-omp31 was detected by western blotting. Anti-omp31 protein mAb was obtained from 2 strains of *Brucella*. The antibody showed strong specificity and sensitivity and did not cross-react with *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium tuberculosis*, or *Bacillus pyocyaneus*. The pGEX-4T-1-omp31 prokaryotic expression vector was successfully established and showed good immunogenicity. The antibody also showed strong specificity and good sensitivity.

Key words: *Brucella*; Expression; omp31; Outer membrane protein; Purification

