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Analysis of protein expression and a new prokaryotic expression system for goat (*Capra hircus*) spermadhesin Bdh-2 cDNA

J.B. Cajazeiras[1], L.M. Melo[1], E.S. Albuquerque[1], G. Rádis-Baptista[2], B.S. Cavada[3] and V.J.F. Freitas[1]

[1]Laboratório de Fisiologia e Controle da Reprodução, Universidade Estadual do Ceará, Fortaleza, CE, Brasil
[2]Instituto de Ciências do Mar, Labomar, Universidade Federal do Ceará, Fortaleza, CE, Brasil
[3]Laboratório de Moléculas Biologicamente Ativas, Biomol-Lab, Universidade Federal do Ceará, Fortaleza, CE, Brasil

Corresponding author: V.J.F. Freitas
E-mail: vjff@pq.cnpq.br

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ABSTRACT. Low purification efficiency and incomplete characterization of male goat (buck) spermadhesins (Bdhs) prompted us to develop an effective system to produce recombinant Bdhs (rBdhs). Bdh-2 cDNA was inserted into a prokaryotic expression plasmid, pTrcHis TOPO. The pTrcHis-Bdh-2 system was constructed to produce a His_6 fusion protein in *Escherichia coli* Top10 cells. Recombinant clones were selected by growth in ampicillin-enriched medium, PCR amplification and nucleotide sequencing. The inserted cDNA was completely identified and recombinant protein synthesis was monitored by SDS-PAGE, followed by immunoblotting with monoclonal anti-His antibody. Expression of insoluble rBdh-2 was achieved at 0.1 to 2.0 mM IPTG, after 2 to 6 h of induction. Significantly increased production of rBdh-2 ($P < 0.01$) occurred with 1.5 mM IPTG after 2 h of induction, and with 0.3 mM IPTG after 4 h in culture. Among the induction times investigated, a period of 6 h gave the lowest levels of rBdh-2 production; with a 6-h

incubation, there were no significant differences in rBdh-2 production for the various concentrations of IPTG tested ($P > 0.05$). The apparent molecular weight of rBdh-2 was 15.85 ± 0.09 kDa, calculated by image analysis of membranes. This is similar to the theoretical molecular weight of 15.5 kDa predicted from the nucleotide sequence. Prior to this study, expression of recombinant goat spermadhesin had never been reported. Thus, an effective prokaryotic rBdh-2 expression system was developed in order to provide an adequate tool for studying biofunctions of goat spermadhesins.

Key words: *Capra hircus*; Spermadhesin; Bodhesin; Recombinant protein; cDNA; Prokaryotic expression

