TISSUE EXPRESSION AND CONSTRUCTION OF PROKARYOTIC EXPRESSION SYSTEM OF GALLINACIN-2

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Introduction

The alarming increase of drug-resistant bacteria and banning of antimicrobial growth promoters world wide make the search for novel means of fighting bacterial infections and promoting animal growth imperative (Van den Bogaard and Stobberingh, 2000). The beta-defensin may be the ideal substitutes for bactericidal agents and antimicrobial growth promoters. Gallinacins (Gal) in poultry are functional equivalents of mammalian beta-defensins, which constitute an integral component of the innate immune system. The reported biological activities of beta-defensin included anti-microbial, antiviral, anti-tumor，immunomodulation effects, and growth promotation (Ganz, 2003; Peng et al., 2007). Gallinacin-2 (Gal-2), expressed in the respiratory tract and bone marrow of chicken, is abundant in cells that are involved in the innate immune response against microbial infections (Sugiarto and Yu, 2004). To our knowledge, it has been mentioned rarely that the tissue expression of Gal-2 of single comb white leghorn (SCWL) and the prokaryotic expression of Gallinacin-2, especially in pMAL-c2X system. The objective of this study was research the tissue expression of Gal-2 of SCWL and construction of prokaryotic expression system of Gallinacin-2 using pMAL-c2X.

Materials and Methods

Tissue expression of Gal-2. Total RNA were prepared from chicken marrow, kidney, liver, brain, heart, bursa of Fabricius, spleen and lung of SCWL by using the Trizol kits (Promega, USA), respectively. The upstream primer (5'-GCGAATTCCATGA GGATTCTTTACC-3') with a *EcoR* I restriction site at the *gal-2* start codon and downstream primer (5'-GCTCTAGATCATGCATTC CAAGGC-3') with a *Xba* I restriction site at the stop codon(underlined bases), were designed to amplificate Gal-2 gene accoding the DNA sequence (Genbank Accession No. P46158). Reverse transcription (RT) was performed at 42°C for 1 h, followed by heat inactivation for 5 min at 95°C. The cDNAs were amplified in sequential cycles. The annealing conditions consisted of an initial 5-min denaturation step at 94°C; 33 cycles of 40 sec at 94°C, 45 min at 58°C, 50 sec of 72°C; and a final extension step of 72°C for 7 min. The RT products were resolved by electrophoresis with 2.0% agarose gels，and verified by DNA sequencing.

Construction of prokaryotic overexpression. To construct a maltose-binding protein (MBP)-Gal-2 overexpression plasmid, the *gal-2* gene was amplified using PCR. The PCR fragment was subsequently cloned into the *EcoR* I- *Xba* I restriction sites of pMAL-c2X (New England Biolabs) to generate the recombinant plasmid pMAL-gal-2. The correct sequence of the insert was confirmed by PCR. Optimal production of MBP- pMAL-gal-2 was obtained when mid-exponential-phase cells (OD_{600}=0.6) were induced with 0.3 mM IPTG for 2 h at 37 °C. Harvested cells were disrupted by sonication and the soluble fraction loaded onto an amylose resin (New England Biolabs) equilibrated with 50 mMTris-HCl (pH 7.5). MBP-Gal-2 was eluted with 10% maltose in 50 mM Tris-HCl (pH 7.5). The purity of fractions was assessed by SDS-PAGE.

Results and Discussion

Our results showed that *gal-2* of SCWL could express conctrctively in marrow and lung (Fig1), which was consistent with the results obtained by Zhao et al.(2001). The prokaryotic expression system of Gallinacin-2 using pMAL-c2X was constructed successfully, and the MBP-Gal-2 was overexpressed induced with IPTG (Fig 2). As shown in Fig 2, the fusion protein with MBP (MBP-Gal-2) corresponding to a molecular mass of 50 kDa, which was not dectected in the uninduced *E. coli*, appeared in induced *E. coli*. SDS-PAGE analysis of the crude extracts indicated that the fusion protein accounted for 30.6 % of the total cell protein. Respecting to the molecular mass of MBP(42.5 kDa), the molecular mass of Gal-2 with the expected 7.5 kDa was calculated, which was consistent with the molecular mass calculated accroding to the amino acids sequence of Gal-2.

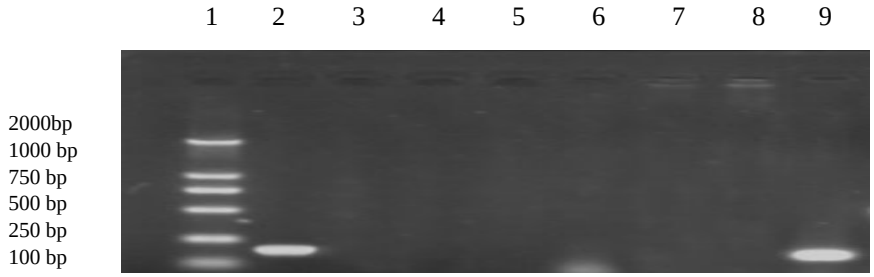

Fig.1.Tissue expression of Gal-2.1.mark DL2000; 2.marrow; 3.kidney; 4.liver; 5.brain; 6.heart; 7. bursa of Fabricius; 8.spleen; 9.lung

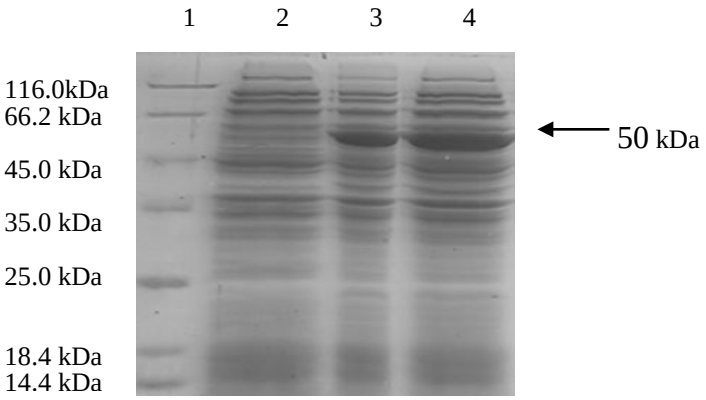

Fig. 2. protein electrophoresis.1. protein Mark; 2.uninduced cell;3.induced cell; 4.crude extract

Conclusions

Gal-2 of single comb white leghorn could express constructively in marrow and lung. The prokaryotic expression system of Gallinacin-2 using pMAL-c2X was constructed successfully, which were to get ready to the reseach of Gal-2 on substitutes for bactericidal agents and antimicrobial growth promoters.

References

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