

Sequences and cloning and expression of the BMSA1 and BmSA5-1-1 proteins

1. BMSA1

By connecting BMSA1 gene to vector PGEX-4T-3, it was recombined, expressed and purified in *E. coli* BL21 (DE3). The molecular size of recombinant BMSA1 fused with GST tag was about 59 kDa. Hamsters were immunized with recombinant BMSA1 and the positive serum obtained could recognize the natural protein of *B. microti* with a molecular size of about 33 kDa.

Babesia microti secreted antigen 1, BmSA1

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Majority -----TAFAAFLAFONISPVLSAGGSGGNGGGGGHQEQNNANDSSNPTGAGGQPNNESKGGAVKLDLDLDMKETKQVCTTNTKLVGKAKSKLNKL
secreted --SFKPTII..... 98
surface ..... 74
BmGP12 MVSFKPTII..... 100
BmN1-9 ..... 91
32 ..... 68
BmN1 .....MFLFKSI.IPSFI.....FLLMSV.AF.GDTD.G.SGTAG.....LVRLVKDL.ISFFSNLIYLEY.K 58
Majority EG-ESHKEYVAEKTKEIDEKNGGFENLVKIEKGGKIK-VPADTG----AEVDAVDDGVAGALSLLSSDISAIKTLTDDVSEKVENLKDDEASATEHTD
secreted ..... 192
surface .....R..... 188
BmGP12 ..... 194
BmN1-9 ..... 185
32 KDK.AE.D.MD.K.LN.K.VEDAIA.LVHEE.FEGK.EQPNIQE.K.PVT.EEY.NEMAEA.TA.TEL.A.E.YR.HSS.SG.NTF.D.188
BmN1 HD--GY.D.IE.N.K.....L.NEY.Y-I.E.....S.FFINKKEIIEVFEKVPNAL.KYIS.V.E.YN.AYDT.EG.A.IEF.YEC 137
Majority IKEKATLLQESCNG-IGTILDKLA--EYLN-NDTTQNIKKEFDERKGGHLSLTKIKVENKDEYVTHFRDMATEAQNAVEVKGAIIDAVVARRGAENLDVD
secreted ..... 288
surface ..... 284
BmGP12 ..... 290
BmN1-9 ..... 287
32 R.L.EM.VK.L.A-FDS.IIPITID.LIKD.EDVKQK.EA..Q.KVEA.P..K...NS.LK.IA..NG.AE...IL.TL.SS..EKEVE.N 267
BmN1 K.R..GIFFQILFAPC.SLVNY.IS-.FGG-DN.YED..EV.HNYSRAIA..I.IF.R.G..N...E...D.VKG.IE..N.I..VL.SNIRDSGYVT. 235
Majority DILFNSLSTLLDITIIETSR-AYLPQVAFALLSSVAMFLF-----
secreted ..... 326
surface ..... 328
BmGP12 ..... 328
BmN1-9 G..DPE---KSL.KQT-----K.R.KDQGE.C 233
32 EAF.....I.DV.A.K.NI.KIVLT..TTIGLAA.V 306
BmN1 NII.I.SFKSV.SI.NKIAQINVSN.IIPG.KALILSV.LILITIA 280
    
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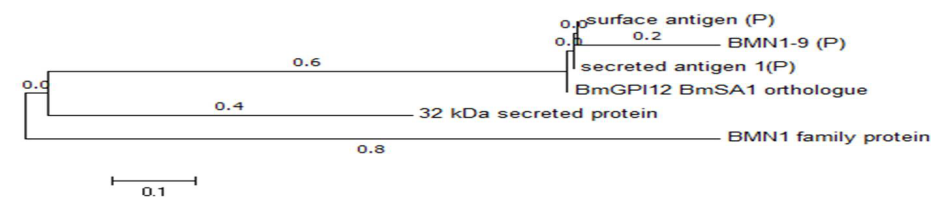


Fig. 1.1 Comparison of the homologous sequence of BMSA1 and the N-J tree



FIG.2 The PCR results of BmSA1

M: DNA Marker; 1: BmSA1(906bp)

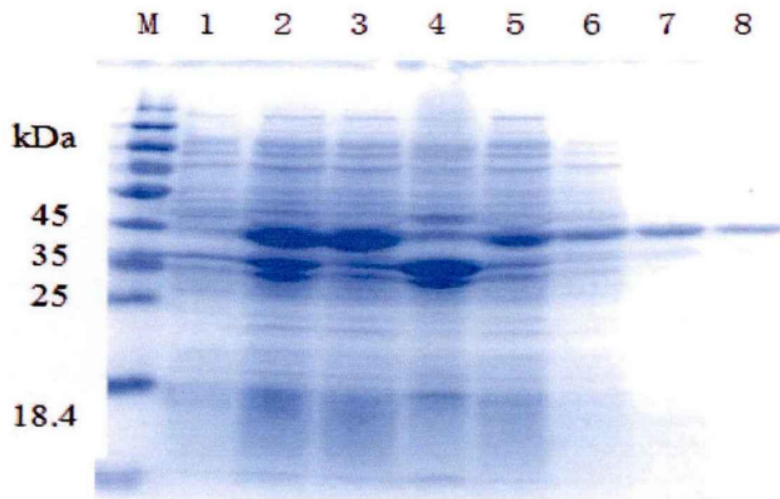


FIG.3 SDS-PAGE analysis of BmSA1 induced expression

M: Protein marker, 1:Pre-induced protein, 2:induced protein, 3: supernatants of induced protein, 4: precipitation of induced protein., 5-8: purified protein in different concentration

2. BmSA5-1-1

Babesia microti BmSA5-1-1 gene was inserted into pET28a vector through *Bam*HI and *Xho*I. The size of the inserted gene was 378 bp, and the pET28a expression vector was constructed. The constructed expression vector was transformed into BL21 (DE3) competent cells, and the induced band was consistent with the expected molecular weight of 15 kDa.

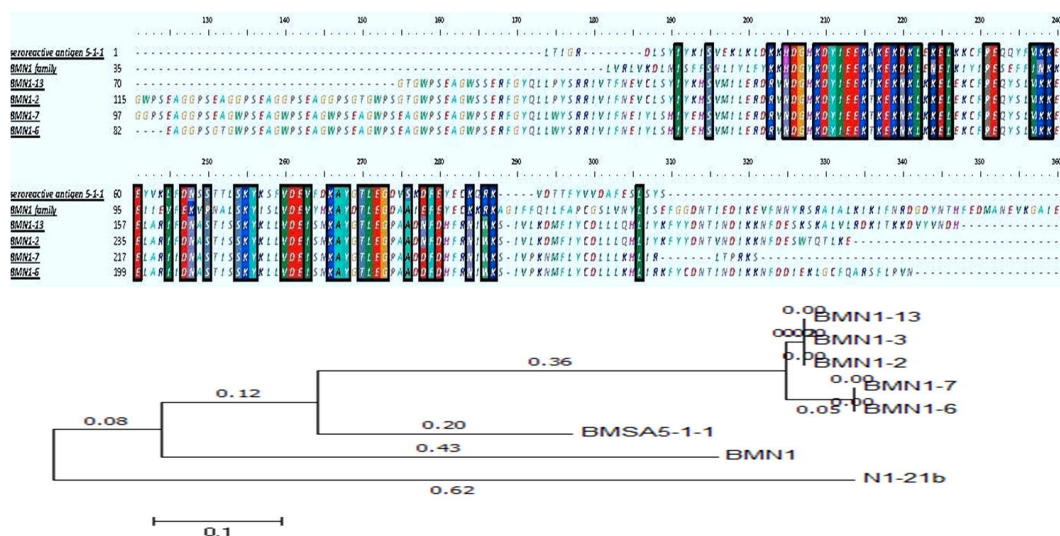


Fig.2.1 Comparison of the homologous sequence of BMSA5-1-1 and the N-J tree

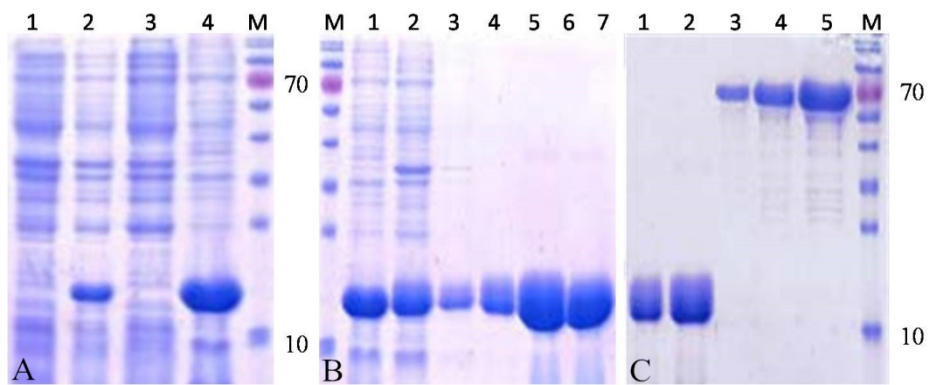


Figure 2 .2 Cloning and expression of the BmSA5-1-1 protein

- A- M: Protein marker, A1: Pre-induced protein, A2: Induced protein, A3: Supernatant of induced protein, A 4: Precipitation of induced protein.
- B- M: Protein marker, B1: Pre-purified protein, B2: Flow in liquid of protein, B 3-7: Purified protein in different concentration.
- C- M: Protein marker, C1: 1ul Recombinant BmSA5-1-1 protein, C2: 2ul Recombinant BmSA5-1-1 protein, C3: 1ug BSA standard protein, C4: 2ug BSA standard protein, C5: 4ug BSA standard protein.