

Figure 1A. Restriction endonuclease analysis of the pET-28a/P38 prokaryotic expression plasmid. Lane M: KB Ladder. Lane 1: pET-28a/P38 plasmid digested with NdeI and XhoI. Lane 2: Undigested pET-28a/P38 plasmid.

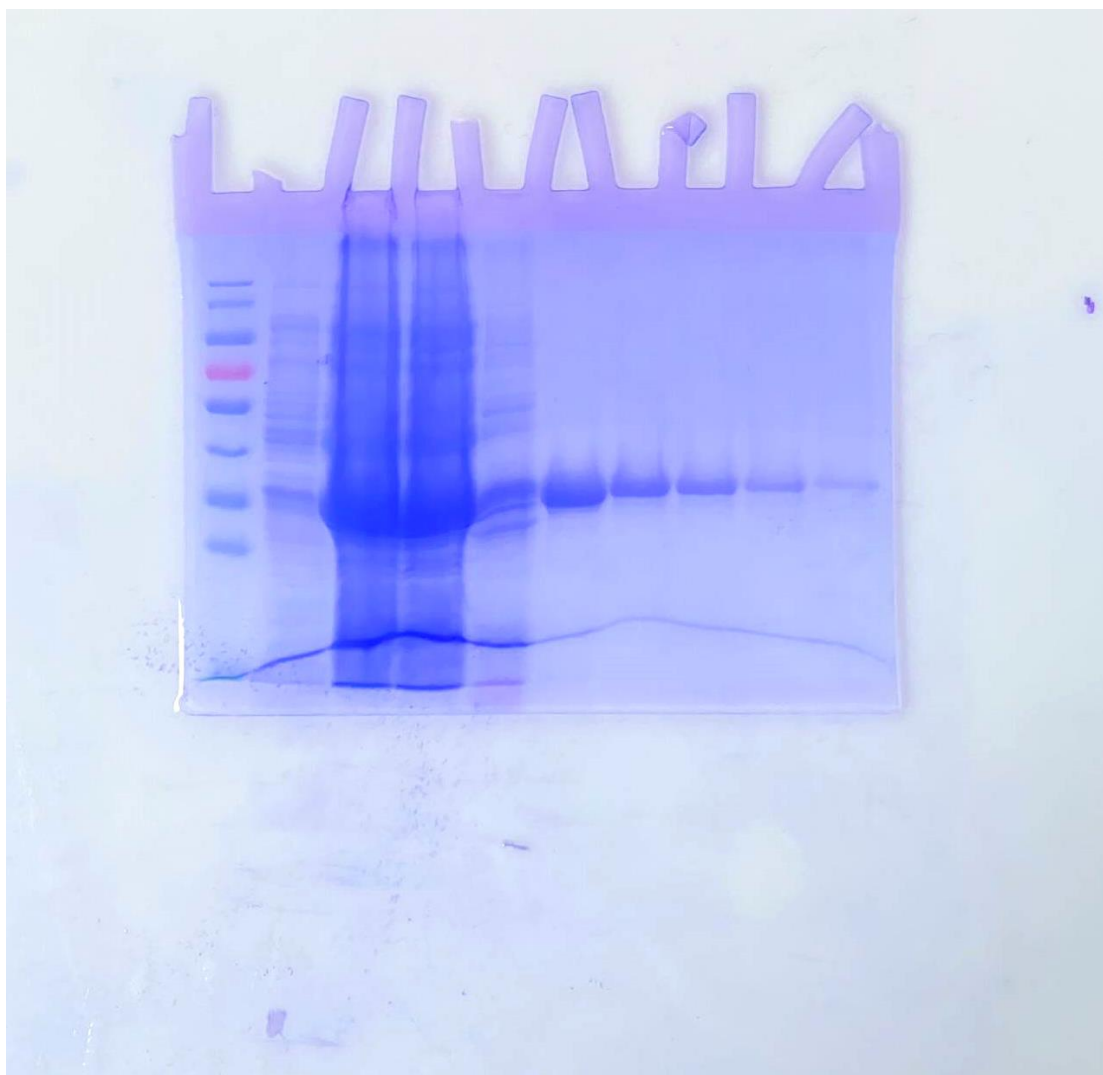


Figure 1B. SDS-PAGE analysis of recombinant P38 protein expression and purification. Lane 1: Total protein from *E. coli* BL21(DE3) cells cultured at 37°C without IPTG induction. Lanes 2 and 3: Supernatant protein from lysed *E. coli* BL21(DE3) cells harboring the pET-28a/P38 plasmid and induced with 0.6 mM IPTG at 37°C. Lane 4: Precipitated protein from lysed *E. coli* BL21(DE3) cells harboring the pET-28a/P38 plasmid and induced with 0.6 mM IPTG at 37°C. Lanes 5-9: Purified recombinant P38 protein eluted with 60, 80-, 90-, 100-, and 150-mM imidazole solution, respectively.

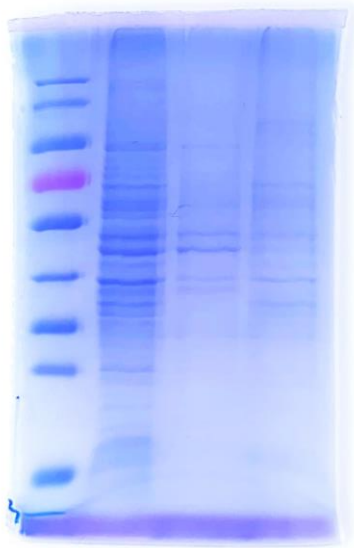


Figure 2A. This panel shows the SDS-PAGE result of protein profiles of the different *M. penetrans* fractions. Lane M: Protein marker. Lane 1: Total *M. penetrans* cell lysate. This lane shows the complex mixture of all proteins present in the cells. Lane 2: Cytoplasmic protein fraction. Lane 3: Membrane protein fraction.



Figure 2B. This panel uses the anti-rP38 antibody to detect P38 protein specifically within the fractions shown in Panel A. The presence of a band at ~ 38 kDa in lane 1 (Total protein) confirms the presence of P38 in the whole-cell lysate. Lane 2 (Cytoplasmic protein) shows a faint band, suggesting a small amount of P38 might be present in the cytoplasm, or possibly due to cross-contamination or non-specific binding of the antibody. Lane 3 (Membrane protein) shows a strong band, confirming the predominant localization of P38 in the membrane fraction.