Cloning, expression, and purification of PcBG5HP1, a conserved hypothetical protein related to thermal stress response in antarctic bacterium, Pedobacter cryoconitis BG5

ABSTRACT

The psychrotolerant bacterium, Pedobacter cryoconitis BG5, has been discovered to encode numerous protein-coding genes that are crucial for thermal adaptation. However, more than 35% of these protein-coding genes for this species are classified as hypothetical proteins (HP). These HPs are proteins whose existence has been predicted, but empirical evidence of their expression in vivo remains lacking. Thus, this research aims to generate a high-quality protein specimen suitable for protein assays and structural biology analyses for future studies. To achieve this, an in vitro analysis was conducted, in which the proteins were cloned, expressed in Escherichia coli, and purified through a two-step purification process. Cultures of P. cryocinitis BG5 cells were retrieved from the glycerol stock and successfully cultivated in LB broth medium at a temperature of 20°C following a three-day incubation period. The targeted gene was successfully amplified, subjected to functional annotation and physicochemical analysis. The targeted genes revealed a 37 kDa conserved HP containing an alcohol and glucose dehydrogenase domain designated as Pcbg5HP1 and annotated with oxidoreductase activity, indicating the bacteria's higher capacity for adaptation to low temperature environments. The recombinant Pcbg5HP1 proteins were successfully overexpressed in their soluble form at 37°C. Subsequently, a soluble protein was obtained through a two-stage purification process involving a column with a his-tag and gel filtration. The identification and validation of the peptide sequences of the purified recombinant protein were successfully achieved through the utilisation of MALDI-TOF-MS analysis. Conclusively, this study has established an efficient workflow to produce high-quality samples of conserved HP from P. cryoconitis BG5, which are well-suited for subsequent protein assays and structural biology analyses.