Functional Analysis of Conserved Hypothetical Proteins from the Antarctic Bacterium, *Pedobacter cryoconitis* Strain BG5 Reveals Protein Cold Adaptation and Thermal Tolerance Strategies

ABSTRACT

Pedobacter cryoconitis BG5 is an obligate psychrophilic bacterium that was first isolated on King George Island, Antarctica. Over the last 50 years, the West Antarctic, including King George Island, has been one of the most rapidly warming places on Earth, hence making it an excellent area to measure the resilience of living species in warmed areas exposed to the constantly changing environment due to climate change. This bacterium encodes a genome of approximately 5694 proteincoding genes. However, 35% of the gene models for this species are found to be hypothetical proteins (HP). In this study, three conserved HP genes of P. cryoconitis, designated pcbg5hp1, pcbg5hp2 and pcbg5hp12, were cloned and the proteins were expressed, purified and their functions and structures were evaluated. Real-time quantitative PCR analysis revealed that these genes were expressed constitutively, suggesting a potentially important role where the expression of these genes under an almost constant demand might have some regulatory functions in thermal stress tolerance. Functional analysis showed that these proteins maintained their activities at low and moderate temperatures. Meanwhile, a low citrate synthase aggregation at 43 ◦C in the presence of PCBG5HP1 suggested the characteristics of chaperone activity. Furthermore, our comparative structural analysis demonstrated that the HPs exhibited cold-adapted traits, most notably increased flexibility in their 3D structures compared to their counterparts. Concurrently, the presence of a disulphide bridge and aromatic clusters was attributed to PCBG5HP1's unusual protein stability and chaperone activity. Thus, this suggested that the HPs examined in this study acquired strategies to maintain a balance between molecular stability and structural flexibility. Conclusively, this study has established the structure– function relationships of the HPs produced by P. cryoconitis and provided crucial experimental evidence indicating their importance in thermal stress response.