Expression, purification and band-shift assays of the two regulatory proteins ModE1 and ModE2 from *Herbaspirillum seropedicae*

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Molybdenum is an essential trace element required for the activity of several enzymes. It is present in the iron-molybdenum cofactor of nitrogenase and the molybdopterin cofactor of other molybdoenzymes. Molybdenum is usually transported by a high-affinity ABC-type uptake system encoded by the *modABC* genes. The endophytic β-proteobacterium *Herbaspirillum seropedicae* has two *modABC* gene clusters and two probable regulator proteins (ModE1 and ModE2). Analysis of the amino acid sequence of the ModE1 protein identified two domains: a DNA- binding domain containing a helix-turn-helix motif (HTH) at the N-terminal and the molybdate -binding domain at the C-terminal. On the other hand, the amino acid sequence of ModE2 showed that this protein contains only the helix-turn-helix motif. A ModE1-His-tag fusion protein was over-expressed as inclusion bodies, solubilized with urea and purified by metal affinity chromatography after an on-column refolding step. ModE2 was also over-expressed as a soluble His-tag fusion protein and purified on a HiTrap chelating Ni^{2+} column. *In vitro* assays showed that both ModE1-His and ModE2 –His bound to the *modA2B2C2* promoter region. Our findings suggest that these proteins are involved in the regulation of *modA2B2C2* transcription in response to the molybdenum concentration.

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