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## MOLECULAR CHARACTERIZATION OF Bradyrhizobium diazoefficiens TRANSCRIPTIONAL REGULATOR (PhaR) PROTEIN

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Bradyrhizobium diazoefficiens is a soil bacterium that can live within soybean root nodules and under free-living conditions. It accumulates polyhydroxybutyrate (PHB) in both states, with the PhaR protein being a key regulator for PHB metabolism. Previous transcriptomic and proteomic studies of a phaR mutant compared to the wild type, both grown under microaerobic conditions with mannitol, showed that PhaR has a pleiotropic function and regulates not only PHB metabolism, but also central carbon and nitrogen allocation pathways, as well as universal stress and motility proteins. Interestingly, PhaR also modulates the microaerobic-responsive regulatory network by activating the expression of fixK 2 and repressing nifA, both encoding two transcription factors relevant for microaerobic lifestyle. In this study, we applied a multidisciplinary approach to dissect the molecular mechanism of the PhaR regulator, including an in silico DNA motif prediction, analysis of its oligomeric state, and PhaR-DNA interaction assays. We then identified two conserved PhaR binding motifs (PhaR box): a 12bp regular pattern containing a conserved GCx(3)GC sequence present at single or multiple locations within the promoter region of target genes, and a novel, alternative, and longer 22-bp pattern also enriched in G and C. Purified recombinant PhaR protein effectively interacted with either of PhaR box type, thus leading to the identification of novel 7 PhaR direct targets in addition to phaP1, the model target for PhaR, encoding one of the phasins of B. diazoefficiens. Interestingly, the functional mutagenesis of the phaP1 promoter which harbors two regular patterns, revealed that both are important for PhaR interaction as tetramer and that the double GC sequence in tandem plays a key role in this interaction. These findings suggest that regulation mediated by PhaR appears to be complex and that other players may modulate the function of this regulator.

Palabras clave: Polyhydroxybutyrate-PhaR protein-DNA-protein interactions