ABSTRACT

The pleiotropic effect of WD-40 domain containing proteins on cellular differentiation and production of secondary metabolites in *Streptomyces coelicolor*

WD-40 domains, also known as beta-transducin repeats, are highly conserved repeating amino acid units, which are found in a wide variety of eukaryotic proteins that have a range of different functions. In the late 1990s, the first WD-40 containing proteins were identified in prokaryotes, however the knowledge about their function is scarce.

Streptomyces coelicolor is a gram-positive bacterium with complicated morphological and physiological differentiation in the course of its life cycle. The genome of *Streptomyces coelicolor* encodes 6 potential genes encoding proteins with WD-repeat motifs. To determine the function of two of these WD-40 genes (*wdpB* and *wdpC*), the deletion replacement mutants in both genes were prepared. Both mutants exhibited medium-dependent phenotypes, which are markedly evident on modified R3 plates. Phenotypic studies revealed that deletion of *wdpB* gene resulted in substantial reduction of aerial hyphae formation and reduced production of undecylprodigiosin. In addition, the hyphae of Δ*wdpB* mutant were unusually branched and showed the signs of precocious lysis. Delayed spore-containing hyphae were irregularly septated. ΔwdpC deleted mutant demonstrated precocious lysis of hyphae and delayed sporulation with straight hyphae without typical curling of the aerial hyphae in early stages of sporulation. Its disruption resulted in the reduction of an antibiotic undecylprodigiosin and delayed actinorhodin production. Whole-genome transcription analysis revealed that deletion of *wdpB* affected the expression of genes responsible for aerial hyphae differentiation (*ram* cluster, chaplins, rodlins, *nepA* gene), sporulation (*whiH*, *whiI* and *rsfA*) and biosynthetic gene clusters for secondary metabolites (calcium-dependent antibiotic , coelichelin, carotenoids, geosmin and methylisoborneol). Transcriptional analysis suggested that WdpB is involved in repression its own expression and neighbouring SCO5954 gene either directly or indirectly. The deletion of *wdpC* resulted in downregulation of sporulation gene *whiE-ORFIII* and several biosynthetic gene clusters coding for secondary metabolites (actinorhodin, calcium-dependent antibiotic and *cpk* gene cluster). Similary to WdpB, WdpC is also involved, ether directly or indirectly, in repression its own expression and neighbouring SCO2245 gene and several other genes (SCO2217, SCO4214 and operon SCO4173-5). Overexpression of *wdpB* gene in wild type strain did not affect the phenotype, whereas overexpression of *wdpC* resulted in the increase of actinorhodin production. In addition, mutant strain with higher gene dosage of *wdpC* showed opossite trend of relative gene expression of selected genes than that of ΔwdpC-disrupted mutant. Both tested genes seemed to be constitutively expressed. Whereas expression of WdpC was temporally controlled, reaching a maximum level concurrently with the formation of spores, the presence of WdpB protein was not established.

The results obtained suggest that both genes studied have pleiotropic effect on the production of secondary metabolites and play an important role in celullar differentiation.