An AfsK/AfsR system involved in the response of aerial mycelium formation to glucose in Streptomyces griseus

Abstract

In *Streptomyces coelicolor* A3(2), a protein serine/threonine kinase (AfsK) and its target protein (AfsR) control secondary metabolism. AfsK and AfsR homologues (AfsK-g and AfsR-g) from *Streptomyces griseus* showed high end-to-end similarity in amino acid sequence with the respective *S. coelicolor* A3(2) proteins, as determined by cloning and nucleotide sequencing. AfsK-g and a fusion protein between AfsK-g and thioredoxin (TRX–AfsK-g) produced in high yield as inclusion bodies in *Escherichia coli* were solubilized with urea, purified by column chromatography and then refolded to an active form by dialysis to gradually remove the urea. AfsR-g was also fused to glutathione *S*-transferase (GST–AfsR-g); the fusion product in the soluble fraction in *E. coli* was purified. Incubation of AfsK-g or TRX–AfsK-g in the presence of [γ-^{32}P]ATP yielded autophosphorylated products containing phosphoserine and phosphothreonine residues. In addition, TRX–AfsK-g phosphorylated serine and threonine residues of GST–AfsR-g in the presence of [γ-^{32}P]ATP. Disruption of chromosomal *afsK-g* had no effect on A-factor or streptomycin production, irrespective of the culture conditions. The *afsK-g* disruptants did not form aerial mycelium or spores on media containing glucose at concentrations higher than 1%, but did form spores on mannitol- and glycerol-containing media; this suggests that *afsK-g* is essential for morphogenesis in the presence of glucose. Introduction of *afsK-g* restored aerial mycelium formation in the disruptants. The phenotype of *afsR-g* disruptants was similar to that of *afsK-g* disruptants; introduction of *afsR-g* restored the defect in aerial mycelium formation on glucose-containing medium. Thus the AfsK/AfsR system in *S. griseus* is conditionally needed for morphological differentiation, whereas in *S. coelicolor* A3(2) it is conditionally involved in secondary metabolism.