## 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 Stransferase (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 [v-32P]ATP vielded 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  $[\gamma^{-32}P]$ ATP. Disruption of chromosomal *afsK-g* had no effect on A-factor or streptomycin production, irrespective of the culture conditions. The afsK*q* disruptants did not form aerial mycelium or spores on media containing glucose at concentrations higher than 1%, but did form spores on mannitol- and glycerolcontaining media; this suggests that *afsK-q* is essential for morphogenesis in the presence of glucose. Introduction of *afsK-g* restored aerial mycelium formation in the disruptants. The phenotype of *afsR-q* disruptants was similar to that of *afsK*q disruptants; introduction of *afsR-q* 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.