## 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 $\left[\gamma-{ }^{32} P\right] A T P$ 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 $\left[\gamma-{ }^{32} \mathrm{P}\right] A T P$. Disruption of chromosomal $\operatorname{afs} K-g$ had no effect on A-factor or streptomycin production, irrespective of the culture conditions. The afs $K$ $g$ disruptants did not form aerial mycelium or spores on media containing glucose at concentrations higher than $1 \%$, but did form spores on mannitol- and glycerolcontaining media; this suggests that $a f s K-g$ is essential for morphogenesis in the presence of glucose. Introduction of $a f s K-g$ restored aerial mycelium formation in the disruptants. The phenotype of $a f s R$ - $g$ disruptants was similar to that of afs $K$ $g$ disruptants; introduction of $a f s R-g$ 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. coelicolorA3(2) it is conditionally involved in secondary metabolism.


