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Studies on Proteases and the Protease Inhibitor Regulating Differentiation of *Streptomyces coelicolor*

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Streptomyces coelicolor is soil bacteria producing diverse secondary metabolites with morphological differentiation. Because of soil environment and complex life cycle, protease and protease inhibitor are considered as important factors in cellular events. The aim of the this study was to identify protease and protease inhibitor involved in differentiation of *Streptomyces coelicolor*. In order to identify the proteins that might be involved in morphological and physiological differentiation, the proteins prepared from supernatant of different growth phases in wild type (M600) strain and *bldA* mutant, were analyzed with proteomics. As a result, it was found that extracellular proteomes were apparently linked with *bldA* mutation. Among extracellular proteomes, 11 proteins found to be significantly down regulated in the *bldA* mutant, might be involved in differentiation. A protein encoded by SCO0762 was completely absent in *bldA* mutant. This protein was identified to be a *Streptomyces* trypsin inhibitor (STI) that belongs to the *Streptomyces* subtilisin inhibitor family (SSI), and it has been recognized to be implicated in differentiation of *Stretomyces* spp. It was revealed that *Streptomyces* trypsin inhbitor (STI) production is regulated by the *bldA*-AdpA dependant mode, and STI plays an inhibitory role in differentiation during the growth phase.

Protease related with STI, was searched for elucidating function of STI. Firstly, extracellular serine protease (ScoP1) was identified. From disruption and kinetics studies, it was considered that the ScoP1 might be responsible for the inactivation of STI. Furthermore, another serine protease (ScoP2) interacting with STI, was identified by yeast two hybrid system. This protease contains domain involved in translational modification. The site of protein-protein interaction was analyzed, and this analysis showed that domain involved in processing is important for interaction with STI..

It is proposed that proteases and protease inhibitor cascade (ScoP1-STI-ScoP2) lead to morphological differentiation and antibiotics production of *S. coelicolor* in programmed manner. STI might be inactivated by ScoP1 for the initiation of morphological differentiation and production of actinorhodin after growth phase, and then ScoP2 could be released from STI inhibition for translational modification of essential proteins involved in differentiation of *S. coelicolor*.