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Study on Aerial Hyphae Formation Mechanism of Siderophore in the Cell of *Streptomyces coelicolor*

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Research aims

Since iron acquisition is a vital process for bacteria to survive, more than 100 enzymes functioning in primary and secondary metabolism possess iron-containing cofactors such as iron-sulfur clusters or heme groups. In aqueous solution, iron exists as ferrous ion Fe (II), or ferric ion Fe (III). The ferric ion is constantly insufficient in natural environments because the solubility of ferric ion is as low as 10^{-18} μM in water of biological pH, while optimum growth of one bacterial cell needs 1 μM of iron[1]. In order to utilize such a low concentration of ferric ion in natural habitats, bacteria excrete low molecular compounds called siderophores, which have the ability to chelate ferric ion. After chelating ferric ion, the ferric siderophore is taken up into cells via receptors located across the cell membrane[2].

Iron uptake systems of pathogenic bacteria using siderophores have been well studied for the sake of establishing efficient clinical treatments because iron acquisition is critical process for pathogen to survive in host cells. In fact, the secretion of some siderophores was reported to enhance the virulence of the pathogenic bacteria[3]. Due to these biological significances, many siderophores including pyochelin[4], yersiniabactin[5], petrobactin[6], and mycobactin[7] were isolated from pathogenic bacteria. Regarding unique siderophore of *Streptomyces*, Umezawa *et al.*[8] reported about isolation of foroxymithine from *S. nitrosporeus* in 1985 and recently a related peptide siderophore coelichelin was found in *S. coelicolor* by combination of genome-mining technique and chemical analysis[9,10].

Streptomyces scabies is a plant pathogenic bacterium which causes serious damage to potato plants. Therefore many studies about this bacterium, including the whole genome sequencing project, have been performed for decades to understand the infection mechanism[11, 12]. The phytotoxin named thaxtomin A was isolated from *S. scabies*. This toxin was shown to be a direct virulence factor to plants[13] and was recently reported to cause apoptosis in plant cells[14]. The necrogenetic factor, nec1, was also distributed in *S. scabies* and related strains[15–17].

It was reported that the presence of deferoxiamine enhanced the production of antibiotics in *Streptomyces griseus*. In order to clarify the role of siderophore in the bacterial cell, we attempted to isolate siderophore-binding proteins from *S. coelicolor* using deferoxiamine, a commercially available siderophore, as a probe.

Methods

The affinity column was prepared by coupling deferoxiamine (Sigma-Aldrich) to the Affi-Gel affinity supports (Bio-Rad) in MOPS buffer at pH 7.5. The bacterium *S. coelicolor* was cultured in ISP2 liquid media at 27°C for 5 days. The cells were harvested by centrifugation, and washed with distilled water. Cellular proteins were extracted from the washed cells by subjecting the cells to ultrasonication in 10% SDS-containing buffer. The proteins in the cell extract were precipitated with trichloroacetic acid. The precipitated proteins were dissolved in Tris buffer. The protein solution was loaded onto the affinity column, followed by elution of putative deferoxiamine-binding proteins with distilled water and 0.1 N NaOH solution. Each fraction was analysed by SDS-PAGE.

Results

The result of SDS-PAGE was shown in Fig. 1. As shown by the arrow, a thick protein band (approximately 50 kDa) was observed in the fraction eluted with 0.1 N NaOH. In order to identify the protein, the mass-fingerprinting method was applied after SDS-PAGE. The Fig. 2 illustrates the MOF-MS spectrum of fragments generated by trypsin-digestion of the protein. As a result of MASCOT web-

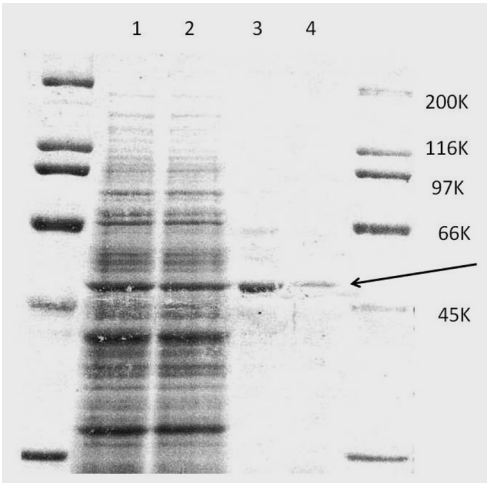

Fig. 1. SDS-PAGE analysis of protein fractions.
Lane 1, cellular protein fraction; lane 2, flowthrough fraction; lane 3, protein fraction eluted with distilled water; lane 4, protein fraction eluted with 0.1 N NaOH.

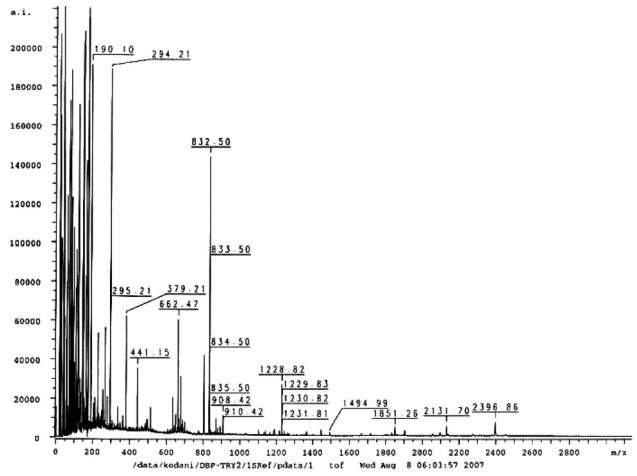

Fig. 2. TOF-MS spectrum of peptides recovered after tryptic digestion of the 50 kDa protein.

search, the protein was identified as the dihydrolipoamide dehydrogenase of *S. coelicolor*.

It has been reported that the *S. coelicolor* dihydrolipoamide dehydrogenase had the ability to catalyze oxidation of dihydrolipoamide in an NAD^+ dependent manner. In this study, we attempted to isolate deferoxiamine-binding proteins in *S. coelicolor* by using the deferoxiamine-immobilized affinity column. As a result, we obtained the dihydrolipoamide dehydrogenase of *S. coelicolor* as a putative binding protein for deferoxiamine. However, this was most likely due to the structural similarity between deferoxiamine and dihydrolipoamide.

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