Ⅲ-14

식물 병원성 그람음성 세균의 병원성을 일으키는 제3유형 분비체계 저해제의 탐색

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Search of inhibitors for type III secretion system responsible for the virulence of phytopathogenic Gram-negative bacteria

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실험목적 (Objectives)

The type III secretion system (TTSS) constitutes a common virulence system present in many pathogenic Gram-negative bacteria that cause diseases in human, animal, and plant hosts by injecting effector proteins into the cytosol of host cells. TTSSs are understood to play essential roles in the virulence of phytopathogenic Gram-negative pathogens, including Burkholderia, Erwinia, Pseudomonas, Ralstonia, and Xanthomonas. Furthermore, many researches have revealed that several components of the TTSS are conserved between different species. In addition, it is reported that TTSS inhibitors do not suppress the bacterial growth but selectively attenuate the virulence incurred by virulence factors. Therefore, TTSS is a good model system for development of novel antibacterial agents that attack phytopathogenic bacteria.

To search and isolate TTSS inhibitors of Xanthomonas axonopodis from natural resources and chemical libraries, we have established an in vitro assay system consisting of HpaG as a virulence factor and Gus as a reporter, and selected three hit compounds from microbial secondary metabolites and chemical libraries. Among them, the TTSS inhibitory activity and bacterial growth inhibition patterns of a compound were examined.

재료 및 방법 (Materials and Methods)

TTSS assay consisting of HpaG and GUS bases on the fact that when Xanthomonas cultures are grown under TTSS-inducing conditions (a medium shift from LB to XVM2), they secrete a distinct set of proteins via TTSS into the culture supernatant. The absorbance of supernatant containing test compound after GUS

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staining was detected at 590 nm. Nalidixic acid was used as positive control.

The minimum inhibitory concentrations (MICs) of compound against various phytopathogenic bacteria were determined by a two-fold serial broth microdilution method using trypticase soy broth. The antibacterial activities against phytopathogenic bacteria were determined by using various bacteria such as Xanthomonas axonopodis, Pseudomonas syringae, Burkholderia glumae, Ralstonia solanacearum, and Xanthomonas oryzae. The plates were incubated 28-37 °C for 24 hr. The MIC was defined as the lowest concentration of compound which completely inhibited the growth of the organism when compare to a control plate containing no compound.

실험결과 (Results)

In order to search and isolate TTSS inhibitors of Xanthomonas axonopodis from natural resources and chemical libraries, an in vitro assay system consisting of HpaG and GUS was established. Through this assay system, three candidates from 3,000 microbial metabolites and 1,000 chemical libraries were selected and one candidate showed a potent inhibitory activity (IC50=0.3 μg/ml) against the TTSS of Xanthomonas axonopodis. The compound did not show the antifungal activities against various phytopathogenic bacteria such as Xanthomonas spp., Pseudomonas, Burkholderia, and Ralstonia with the MIC values of over 512 μg/ml. The inhibition of TTSS protein secretion was not result of inhibition of bacterial growth, as viable counts of bacteria were the same in the presence or absence of the compound. Isolation and purification of other TTSS inhibitors and in vivo bioassay for these compounds are under investigation.