P-174

Yeast two-hybrid assay를 사용한 Hipm 단백질과 돌연변이 HrpN 단백질간의 interaction 분석

창원대학교 미생물학과, 배영민; Cornell 대학교 식물병리학과, Marshall L. Hayes and Steve V. Beer

Analysis of the interaction of the mutant HrpN proteins with Hipm protein by yeast two-hybrid assay

Department of Microbiology, Changwon University, Young-Min Bae; Deaprtment of Plant Pathology and Plant-Microbe Biology, Cornell University, Marshall L. Hayes and Steve V. Beer

Objectives : To test the interaction between the apple Hipm protein and the mutant HrpN proteins of the Erwinia amylovora

Materials and Methods

① **Materials** : Hipm protein of Malus x domestica, mutant HrpN proteins of Erwinia amylovora (N1, $S_{138}Q_{139}N_{140}D_{141}D_{142}S_{143}$ → NAAIRS; N5, $L_{162}K_{163}M_{164}F_{165}S_{166}E_{167}$ → NAAIRS; N6, $I_{168}M_{169}Q_{170}S_{171}L_{172}F_{173}$ → NAAIRS)

② **Methods** : Yeast two-hybrid assays were performed as follows. S. cerevisiae cells carrying appropriate plasmids were plated on SD agar with or without leucine, incubated at 30℃ and growth was checked after 6 days. S. cerevisiae cells carrying additional copies of p8op-lacZ were plated on SD agar containing leucine and X-gal, incubated at 30℃ and checked for development of typical green color after 6 days.

Conclusion

It was found previously by the pear fruit assay that the mutant HrpN proteins, N5 and N6, lost pathogenecity though N1 mutant protein remained pathogenic. The activities of HrpN-Hipm and HrpN-HrpN interactions of those N1, N5 and N6 proteins were tested by the yeast two-hybrid assay. The results indicate that all those three proteins retain the ability to interact with Hipm protein and themselves. These results also suggest that losing the HrpN-Hipm or HrpN-HrpN interactions are not the prerequisites of losing pathogenecity

---

Corresponding author : 배영민 E-mail : yominbae@changwon.ac.kr Tel : 055-213-3483

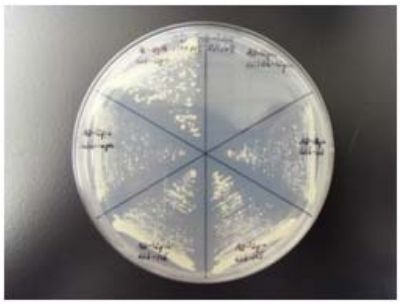

Figure 1. Growth of the S. cerevisiae EGY48 carrying pB42AD-hipm and various hrpN genes in the pGilda plasmid on the SD agar without supplemented leucine. Yeast cells were streaked on an SD agar without leucine and photographed 6 days after plating. Plated strains clockwise from the top are yeast cells carrying pB42AD-hipm and pGilda-hipm, pB42AD-hipm and pGilda-N1, pB42AD-hipm and pGilda-N5, pB42AD-hipm and pGilda-N6, pB42AD-hipm and pGilda-hrpN, and pB42AD-hrpN and pGilda-hipm.

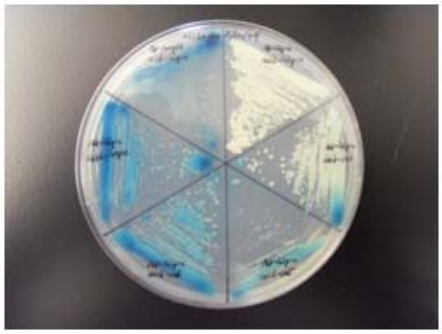

Figure 2. Growth of the S. cerevisiae EGY48 carrying p8op-lacZ, pB42AD-hipm and various hrpN genes in the pGilda plasmid on the SD agar with supplemented leucine. Yeast cells were streaked on an SD agar containing leucine and X-gal. Photograph was taken 6 days after plating. Plated strains clockwise from the top are yeast cells carrying pB42AD-hipm and pGilda-hipm, pB42AD-hipm and pGilda-N1, pB42AD-hipm and pGilda-N5, pB42AD-hipm and pGilda-N6, pB42AD-hipm and pGilda-hrpN, and pB42AD-hrpN and pGilda-hipm. Additionally, all these strains carry p8op-lacZ plasmid.