제 3강연장

1. An insertion sequence(IS) element from *Ralstonia solanacearum*; Its molecular characterisation and genetic variation between isolates

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Bacterial wilt caused by *Ralstonia solanacearum* E. F. Smith [formerly, *Pseudomonas solanacearum* (Yabuuchi *et al.*, 1992 and 1995)] is one of the most devastating bacterial plant diseases. *R. solanacearum* is a very heterogeneous species which appears to be continually extending its host range (Hayward, 1994). This is associated with extensive heterogeneity for biochemical characteristics (Hayward, 1964) and genomic features (Cook and Sequeira, 1994; Opina *et al.*, 1997). The large genetic variation in the pathogen, the apparent paucity of host resistance and the lack of knowledge of the ecology and evolution of *R. solanacearum* have hampered attempts to develop control strategies (Cook and Sequeira, 1994). Therefore, a better understanding of genetic variation and the mechanisms responsible for generating diversity in *R. solanacearum* are required.

Insertion sequence (IS) elements are bacterial mobile DNA elements which vary in length from 800 bp to 2500 bp. They have been found in multiple copies, which vary in number from just a few to a few hundred per genome, in many gram-negative and gram-positive bacteria and in the Archaebacteria (Galas and Chandler, 1989). IS elements contain one or more ORF(s) that encode a transposase and they are bordered by terminal inverted repeats (IRs) of 10~40 bp which are both required for transposition (Galas and Chandler, 1989). IS elements are agents in a wide range of genomic mutations and rearrangements such as deletion, inversion and insertion which are the products of transposition events (Galas and Chandler, 1989). The IS-mediated genomic rearrangements can result in modification of transcription of flanking genes, caused by the activity of the IS promoter or if they make hybrid promoters during the insertion event (Galas and Chandler, 1989).
Here, we report the molecular characterisation of an IS element in *R. solanacearum* ACH0158 strain and we investigate its genomic variation within a wide range of strains of the pathogen.

A novel insertion sequence element, designated IS102J has been identified in *Ralstonia solanacearum* ACH0158. IS102J is 860 bp in length, flanked by 21 bp of perfectly matched inverted repeats and contains one long open reading frame which encodes a putative transposase. IS102J is a member of the IS5 family of insertion sequences. Southern

hybridisation with IS102J sequences revealed that it is confined to biovar 2 strains where it is present in five to seven copies. Southern analysis of 17 biovar 2 strains using IS102J sequences as probe showed considerable similarity between strains but also revealed significant genetic diversity. The strain-specificity and genomic heterogeneity observed suggests that this, and perhaps other, IS elements could play an important role in the generation of genetic variation in *R. solanacearum*.

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2. Molecular Cloning of Two Pathogenesis-Related Protein Genes from *Nicotiana glutinosa* L. and Their Expression During Plant Disease Resistance

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During the expression of resistance to plant pathogen infections, a wide variety of biochemical and physiological responses are induced. We were to understand a large number of changes in the processes involved in hypersensitive response (HR) and systemic acquired resistance (SAR). Therefore we cloned pathogenesis-related protein (PR-1 and β-1,3-glucanase) cDNAs from *Nicotiana glutinosa* cDNA library and used them for investigating the molecular changes during the expression of plant defense response against pathogens, and for screening of plant resistance activation by monitoring of their transcripts. Through screening of cDNA library constructed from *N. glutinosa* showing systemic resistance using PCR products as a probe, PR-1 cDNAs were obtained. Nucleotide sequence analysis revealed that PR1-10 clone contained a complete copy of PR-1 mRNA, and showed 95% homology with PR-1 gene from *N. tabacum* cv. Samsun NN. Deduced amino acid sequences showed 90% identity with that from *N. tabacum* cv. Samsun NN, and 61% with that of tomato and 50% with that of barley. Northern blot analysis showed that the PR-1 mRNA was induced by TMV-inoculation and SA-treatment in *N. tabacum* cv. Samsun NN. Chemicals, such as β-aminobutyric acid, $CuSO_4$ and Na_3PO_4, also induced PR-1 mRNA in tobacco plant, when they were treated. It was demonstrated that the expression of PR-1 mRNA by $CuSO_4$ is possibly SA-independent process. cDNA of β-1,3-glucanase, which hydrolyses β-1,3-glucan of fungal cell wall, was cloned from *N. glutinosa* cDNA library as using a PCR amplified DNA fragment as a probe. GN-3 clone was a portion of 3'-region of β-1,3-glucanase cDNA 800bp in size with 171 amino acids coding region, and untranslated regions. This clone had 90% nucleotide homology with β-1,3-glucanase gene from *N. tabacum* cv. BY4. Deduced amino acid sequence from GN-3 clone had 91% identity with β-1,3-glucanase of *N. tabacum* cv. BY4, 58% with that of *Lycopersicon esculentum* and 51% with that of *Glycine max*. Northern blot analysis revealed that expression of β-1,3-glucanase mRNAs were induced by TMV-infection and SA-treatment. In addition to that this gene was highly induced by β-aminobutyric acid, and $CuSO_4$, which were known as inducers of plant disease resistance. To investigate the induction of several defense related genes in plants during expression of plant disease resistance, we used temperature-sensitive nature of TMV-resistance in tobacco as a model system. Induction of PR genes (PR-1 and β-1,3-glucanase) by TMV-infection was suppressed at 32℃ and

induction by salicylic acid-treatment was also highly suppressed at high temperature (32℃). Suppression of PR gene expression by high temperature is considered to be regulated at both downstream and upstream of salicylic acid synthesis in the SAR expression pathways. Shifting temperature from 32℃ to 24℃ caused to recover expression of PR genes suppressed by high temperature (32℃) within 2 - 4 hr after shifting in both TMV-infected and salicylic acid-treated plants. But it was not until 12hr after temperature shift that salicylic acid was synthesized in TMV-infected tobacco leaves. It is suggested that there may be another signal transduction pathway different from salicylic acid-mediated pathway in induction of PR gene expressions during the activation of SAR in plants.

SAR in plants to pathogen infections accompanys with accumulation of PR proteins. In consideration of these properties, we were to screen induction of plant disease resistance by monitoring expression of PR genes in transcription levels. Slot blot analysis showed that PR gene transcripts were induced by treatments of ethyl acetate-extracts from endophytic bacterial cultures and northern blot analysis confirmed these results. By slot and northern blot analyses, six strains of bacteria showing induction of PR gene transcriptions were selected and cultured in a large scale. When they were treated to several plants, plants were protected from different pathogens by 50% (tomato late blight) compared to non-treated plants. *In vitro* test revealed that cultures of these strains showed no antimicrobial activity to tested microbes at all. It is suggested that protection effects against plant pathogens were resulted from activation of defense-related responses such as PR protein accumulation, not from direct antimicrobial activity against pathogens.

3. 몇가지 식물병원세균이 생산하는 Pectate Lyase의 특성과 *pel* 유전자 분리

한 광 섭
충청남도 농촌진흥원 식물환경과

식물에 무름병을 일으키는 *Erwinia carotovora* subsp. *carotovora*, *E. chrysanthemi*, *E. rhapontici*, *Pseudomonas marginalis*, 십자화과 채소에 검은빛 썩음병을 일으키는 *Xanthomonas campestris* pv. *campestris*가 생산하는 pectate lyase (Pel)의 특성을 비교하고, 인공배지에서 Pel을 생산하지 않는 *E. rhapontici*의 *pel* 유전자를 분리하여 이 유전자가 *E. rhapontici*의 병원성에 미치는 영향등에 관하여 조사하고자 본 연구를 수행하였다.

Polygalacturonate yeast-extract agar(PYA) plate 상에서 Pel 활성을 확인한 결과 18균주중 10균주는 Pel 활성이 아주 미약하거나 없었고, 4균주는 낮은 Pel 활성을 나타냈으며, *E. carotovora* subsp. *carotovora*, *E. chrysanthemi*, *P. marginalis*와 *X. campestris* pv. *campestris*는 높은 Pel 활성을 나타냈다. Minimal salts(MS) 배지에 16종의 탄소원을 각각 첨가하여 배양하였을 때에 그중 pectin과 polygalacturonate에서 가장 높은 Pel 활성을 나타냈고, D-raffinose등 13종의 탄소원을 각각 첨가하여 배양하였을 때에는 미약한 Pel 활성을 나타냈으며, arabinose를 첨가하여 배양하였을 때에는 4균주 모두 Pel 활성이 전혀 없었다. 온도에 따른 Pel 활성은 4균주 모두 20℃에서 배양하였을 때 Pel 활성이 가장 높았다.

E. carotovora subsp. *carotovora*, *E. chrysanthemi*와 *P. marginalis*는 20℃ 에서 감자 괴경조직의 붕괴속도가 가장 빨랐고, 조직붕괴량도 많았으며, *X. campestris* pv. *campestris*는 25℃에서 감자 괴경조직의 붕괴속도가 빠르고 조직 붕괴량도 많았다. *E. carotovora* subsp. *carotovora*의 접종에 의해 붕괴된 상추조직내에서의 Pel 활성은 접종 2일 후에 7.6 unit/㎖, *E. chrysanthemi*에 의해 붕괴된 배추조직내에서의 Pel 활성은 접종 3일 후에 4.3 unit/㎖, *P. marginalis*에 의해 붕괴된 상추조직에서의 Pel 활성은 접종후 4일 만에 7.5 unit/㎖, *X. campestris* pv. *campestris*에 의해 접종된 배추의 붕괴된 조직에서는 접종 3일 후에 3.6 unit/㎖로 높게 나타났다.

E. rhapontici 는 minimal salts glycerol(MSG) 배지에서는 Pel을 전혀 생산하지 못하였으나, 배추 세포벽을 첨가하여 배양하였을 때에는 Pel 활성이 1.1 unit/㎖로 비교적 높게 나타났고, *E. carotovora* subsp. *carotovora*, *E. chrysanthemi*, *P. marginalis*와 *X. campestris* pv. *campestris*는 모두 (MSG)배지에 배추 세포벽을 첨가하여 배양하였을 때 Pel 활성이 약 1.5~3배 정도 증가 하였으며, MSG 배지에 pectin과 PGA를 배추 세포벽과 함께 첨가하여 배양하였을 때 Pel 활성이 증가되는 것으로 나타났다. 또한 *E. carotovora* subsp. *carotovora*는 배추 세포벽을 Ethyl ether로 멸균한 후 MSG 배지에 첨가하여 배양하였을 때에는 2.1 unit/㎖로 비교적 높은 Pel 활성을 나타냈으나, 고온고압 멸균 후 배추 세포벽을 MSG 배지에 첨가하여 배양하였을 때는 Pel 활성이 1.5 unit/㎖로 MSG 배지에서 배양하였을 때와 유사한 Pel 활성을 나타냈다. 세포벽의 열처리온도가 높아짐에 따라서 감소하는 경향을 보였고, 80℃이상의 온도에서 30분간 열처리한 배추 세포벽을 MSG배

지에 첨가하여 배양하였을 때는 Pel 활성이 전혀 없었다.

E. carotovora subsp. *carotovora*, *E. chrysanthemi*, *P. marginalis*와 *X. campestris* pv. *campestris*는 모두 MSG 배지에서 배양하였을 때보다 $CaCl_2$ 와 $MgCl_2$ 를 첨가하여 배양하였을 때가 Pel 활성이 증가하는 것으로 나타났고, 0.5%에서 보다 2.0%에서 Pel 활성이 약간 높게 나타났다.

E. carotovora subsp. *carotovora*로 부터 *pel* 1 유전자가 cloning된 pPEL 1은 *E. carotovora* subsp. *carotovora* 의 *pel* 1 유전자 (Chatterjee, 1995)와 95% 이상의 homology를 나타냈고, 3종류의 제한효소를 처리하여 southern blotting한 결과 *E. carotovora* subsp. *carotovora*는 처리구에서 2개의 band가 검출 되었고, *E. chrysanthemi*는 1개의 band가 검출 되었으며, *X. campestris* pv. *campestris*는 *pst*I 처리구에서 2개의 band가, *Eco*RI과 *Hin*dIII 처리구에서는 각각 1개의 band가 검출 되었으나, *P. marginalis*는 band가 검출되지 않았다. *E. rhapontici*는 southern blotting한 결과 *Eco*RI 으로 처리 하였을때 4.5kb 부근에 2개의 *pel* 유전자 copy를 확인할수 있었다.

E. rhapontici 의 *pel* 유전자가 cloning 된 plasmid를 가지고있는 *E. coli*는 접종 36시간 후에 히야신스 구근을 약간 붕괴 시켰으나, *E. rhapontici*를 접종한 히야신스 구근에 비하여 병진전이 매우 완만하였다.