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Molecular cloning, Mapping, and regulation of PHO regulon genes for phosphonate break down by the phosphonatase pathway of *Salmonella typhimurium* LT2

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Two pathways exist for cleavage of the carbon-phosphorus(C-P) bond of phosphonates: the C-P lyase and the "phosphonatase" pathways. It was previously demonstrated that *Escherichia coli* carries genes(named *phn*) only for the C-P lyase pathway and that *Ent. aerogenes* carries genes for both pathway(K.-S.Lee, W.W. Metcalf, and B.L. Wanner, J. Bacteriol. 174:2501-2510, 1992). In contrast, here it is shown that *Salmonella typhimurium* LT2 carries gene only for the phosphonatase pathway. Genes for the *S. typhimurium* phosphonatase pathway were cloned by complementation of *E. coli* Δ *phn* mutants. Genes for these pathway were proven not to be homologous and to lie in different chromosomal regions. The *S. Typhimurium phn locus* lies near 10min; the *E. coli phn locus* lies 93 min. The *S. typhimurium phn gene cluster* is about 7.2-kb in length and, on the basis of gene fusion analysis, appears to be comprised of two(or more) genes or operons that are divergently transcribed. Like the *E. coli phn locus*, the expression of the *S. typhimurium phn locus* is activated under conditions P_i limitation and is subject to Pho regulon control. This was shown both by complementation of the appropriate *E. coli* mutants and by the construction of *S. typhimurium* mutants with lesions in *phoB* and *pst*, which are required for activation and inhibition of Pho regulon gene expression, respectively. Complementation studies indicate that the *S. typhimurium phn locus* probably include genes both for phosphonate transport and for catalysis of C-P bond cleavage.

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PCR을 이용한 *Bradyrhizobium* sp.(*Cassia*)의 *nodD* 유전자의 크로닝과
염기서열 분석

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실거리나무 아과(Caesalpinioideae)에 속하는 콩과식물 차풀(*Cassia nomame*)의 공생세균인 *Bradyrhizobium* sp.로부터 숙주식물의 flavonoid 존재하에서 다른 *nod* 유전자들의 activator로 작용하는 NodD의 유전자를 PCR을 사용하여 크로닝하고 그 염기서열을 밝혔다. *Bradyrhizobium japonicum* USDA110의 common *nod* 유전자의 *nod*-box 부위의 22mer(TGGTAAATCGATTGTTTCGAT)와 *nodD2*의 5' coding region 부위의 20mer(GCGACCAGAAGATTTAGATC)를 primer로 사용, PCR을 수행한 바, 1.8kb 크기의 단일 band 만 나타났으며, 이 DNA fragment를 pDK101에 크로닝하고, 그 염기서열을 분석한 결과 *B. japonicum* USDA110의 *nodD1* 유전자의 염기서열과는 90%이상 상동성을 나타냈으나 *nodD1*과 *nodD2* 유전자 사이의 intervening sequence와의 상동성은 크지 않았다.