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Diverse Roles of PhoBR Two-Component Regulatory System in Gram-Negative Bacteria and *pho* Regulon Genes in *E. coli* O157:H7

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Phosphorus is one of essential nutrients for living organisms. Under conditions of inorganic phosphate (Pi) limitation, microorganisms induce *pho* regulon genes to utilize actively phosphorus sources such as Pi, organic phosphate, and phosphonate (Pn). The phosphate (*pho*) regulon of *Escherichia coli* includes at least 31 genes whose expression is induced under conditions of phosphate starvation and whose products have roles in the transport or degradation of various phosphorus sources.

The *pho* regulon genes are controlled by PhoBR two-component regulatory system, responding to the phosphate limitation. PhoB is the transcriptional activator, which acts by binding *pho* box [CTGTCATAT(A)AT(A)CTGTCAT(C)] sites immediately upstream of all known *pho* regulon promoters. These *pho* boxes are comprised of two or more well-conserved 7-bp direct repeats which are separated by poorly conserved 4-bp spacer sequences, suggesting that PhoB molecules always bind on the same face of DNA helix (7). PhoR is the histidine protein kinase that activates PhoB by phosphorylation and inactivates phospho-PhoB by dephosphorylation in response to the extracellular phosphate concentration. Transcriptional activation by phospho-PhoB involves protein-protein interaction with the σ^{70} subunit of RNA polymerase holoenzyme.

The genes in the *pho* regulon were reviewed (6). *phoA* encodes periplasmic alkaline phosphatase and *phoE* encodes an outer membrane protein, porin e. The *phn* operon that consists of 14 genes encodes proteins related to the transport and utilization of phosphonates. *psiE* is under direct positive and negative control by the PhoB protein and the cAMP-CRP complex as unknown function. The *pst-phoU* operon encodes proteins involved in the phosphate specific transport system and signal transduction in the *pho* regulon. The *ugp* operon encodes proteins involved in the transport and utilization of *sn*-glycerol-3-phosphate. *phoH* encodes a protein that contains the ATP-binding motif and actually binds to ATP, but its function in vivo is not known. The *iciA* as a member of the *pho* regulon inhibits the in vitro initiation of chromosomal DNA replication (4). The acid-inducible RNA designated *asr* is under transcriptional control by the *phoBR* operon (11). In *Serratia* sp., disruption of *pstS* caused

hyper-production of Pig (prodigiosin) and Car (1-carbapen-2-em-3-carboxylic acid). PhoB contributes towards LuxI homologue SmaI activation (9). The novel *pho* genes *of amn* (AMP nucleosidase), *tktB* (transketolase), *xasA* (acid sensitivity protein, putative transporter), *yibD* (metal ion stress response gene), and *aytfK* (hypothetical protein) were also verified (1). The *eda* (Entner-Doudoroff aldolase) gene is directly controlled by PhoB (3). *Pseudomonas stutzeri* use hypophosphite (P valence, +1) and phosphite (P valence, +3) as sole P sources. The *htx* and *ptx* operons allow for use the phosphates and their expression is *phoBR* dependent (13).

Recently, PhoB transcriptional activator has also shown to be involved in pathogenesis, indirectly. The *pstC* mutation in *Escherichia coli* O115 reduced the serum resistance and also the pathogenicity, making it unable to cause septicemia in pigs (2). In *Shigella flexneri*, the expression of *pstS* and *phoA* genes were increased in Henle cell and smaller plaques were formed on Henle cell monolayers (8). In *Edwardsiella tarda* which causes hemorrhagic septicemia in fish and gastro-intestinal infection in human, the *pstS* and *pstC* mutants showed significant decreases in virulence, smaller colonies and growth deficiency (10). In *S. enterica* serovar Typhimurium, Lucas *et al*. reported the *hilA* and invasion genes were repressed by *phoB* in the absence of *pst* system (5). The *phoB* mutation in *Vibrio cholerae* affects intestinal colonization and pathogenesis in the rabbit (12).

To identify the novel *pho* genes and its diverse roles in pathogenic *E. coli* O157:H7, we are studying for *pho* regulon by both 2-D analysis and *lacZ*-transcriptional fusion The genome library of *E. coli* O157:H7 was integrated to BW22933 (*Prha-phoB*) by using CRIM plasmid. By using PIX cloning, *pho* regulon genes (*phoE*, *phoA*, *phnC*, *phoH*, and *yibD*) were screened with other candidates. The 14 proteins were detected by phosphate limitation on 2-D gel analysis.

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