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Phenotype MicroArray Analysis of *Escherichia coli* K-12 Mutants with Deletions of All Two-Component Systems

Lu Zhou,[1] Xiang-He Lei,[2] Barry R. Bochner,[2] and Barry L. Wanner[1]*

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907,[1] *and Biolog, Inc., Hayward, California 94545*[2]

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Two-component systems are the most common mechanism of transmembrane signal transduction in bacteria. A typical system consists of a histidine kinase and a partner response regulator. The histidine kinase senses an environmental signal, which it transmits to its partner response regulator via a series of autophosphorylation, phosphotransfer, and dephosphorylation reactions. Much work has been done on particular systems, including several systems with regulatory roles in cellular physiology, communication, development, and, in the case of bacterial pathogens, the expression of genes important for virulence. We used two methods to investigate two-component regulatory systems in *Escherichia coli* K-12. First, we systematically constructed mutants with deletions of all two-component systems by using a now-standard technique of gene disruption (K. A. Datsenko and B. L. Wanner, Proc. Natl. Acad. Sci. USA 97:6640-6645, 2000). We then analyzed these deletion mutants with a new technology called Phenotype MicroArrays, which permits assays of nearly 2,000 growth phenotypes simultaneously. In this study we tested 100 mutants, including mutants with individual deletions of all two-component systems and several related genes, including *creBC*-regulated genes (*cbrA* and *cbrBC*), *phoBR*-regulated genes (*phoA*, *phoH*, *phnCDEFGHIJKLMNOP*, *psiE*, and *ugpBAECQ*), *csgD*, *luxS*, and *rpoS*. The results of this battery of nearly 200,000 tests provided a wealth of new information concerning many of these systems. Of 37 different two-component mutants, 22 showed altered phenotypes. Many phenotypes were expected, and several new phenotypes were also revealed. The results are discussed in terms of the biological roles and other information concerning these systems, including DNA microarray data for a large number of the same mutants. Other mutational effects are also discussed.

Many gene families have now been identified by DNA sequencing. The so-called two-component regulatory genes of bacteria were among the earliest genes recognized in this manner (53). Computational analysis revealed that the NtrB and NtrC proteins of *Bradyrhizobium* sp. exhibit sequence similarities at the amino acid level with several other regulatory proteins. Extensive similarity was observed for the C-terminal domain of the NtrB protein and the C-terminal domains of the CpxA, EnvZ, and PhoR proteins and, to a lesser extent, the CheA protein of *Escherichia coli* (and *Salmonella enterica* serovar Typhimurium). Extensive similarity was also observed for the N-terminal domain of the NtrC protein and the N-terminal domains of the ArcA (then called SfrA and thought to interact with CpxA), OmpR, PhoB, CheB, and CheY proteins. Accordingly, Nixon et al. (53) proposed that the genes encode two-component regulatory systems and that these systems are involved in transduction of information about the environment from the C-terminal domain of a protein belonging to the first group of proteins to the N-terminal domain of the corresponding partner protein belonging to the second group.

Two-component regulatory systems are widespread in nature. Nearly all bacteria (mycoplasmas are exceptions) encode multiple systems of this type for diverse signaling processes. A typical two-component regulatory system is comprised of a signaling histidine kinase (HK) (also called a sensor kinase) that is usually membrane associated and a cytoplasmic response regulator (RR) that is usually a transcription factor (an activator or repressor). Similar systems control the expression of genes for nutrient acquisition, virulence, antibiotic resistance, and numerous other pathways in diverse bacteria. Due to the involvement of these two-component systems in so many cellular processes, several reviews of them have now been published. A monograph on two-component signal transduction has also been written (26). There are also analogous signaling systems in cells of lower eukaryotes, including fungi, amoebae, and plants (27, 42, 73, 78, 85).

Much work has been done on particular two-component systems, especially those with known roles in cell physiology, communication, development, and, in the case of bacterial pathogens, the expression of virulence genes. For example, the NtrB/NtrC and PhoR/PhoB systems were among the first such systems recognized; these systems control catabolic genes for nitrogen (N) and phosphorus acquisition, respectively (81, 89). Several two-component HKs were initially recognized because they can replace an HK of a nonpartner RR and thereby complement defects in the corresponding two-component HK mutants. For example, the HK CreC (originally called PhoM [83]) was originally discovered because it was found to replace the HK PhoR in activation of the response regulator PhoB in a *phoR* mutant. Such examples have led to the suggestion that cross-regulation (79) may be important for the integration of cellular processes involving multiple two-component systems (56).

E. coli is thought to encode 31 different two-component regulatory systems, based on experimental evidence and protein sequence similarities. The functions of many of these

* Corresponding author. Mailing address: Department of Biological Sciences, Purdue University, West Lafayette, IN 47907. Phone: (765) 494-8034. Fax: (765) 494-0876. E-mail: BLW@bilbo.bio.purdue.edu.

systems remain undefined. Also, even the most thoroughly studied systems may have functions other than those that are now known. Furthermore, if cross-regulation among different two-component systems has a fundamental biological role, then particular systems should have functions in common. In order to identify new roles of individual two-component systems and to uncover new interactions that can occur among them, we carried out an extensive, systematic phenotype analysis of *E. coli* mutants with deletions of all two-component systems and several related genes. Most mutants were constructed by using a recently developed gene disruption technique (10). The mutants included mutants with individual deletions of all two-component systems (L. Zhou, K. A. Datsenko, H. Aiba, K. Zhang, J. L. Masella, T. Mizuno, and B. L. Wanner, unpublished data).

The phenotype analysis was carried out by using a new tool, Phenotype MicroArrays (PMs). This technology can be used to find new functions of genes by testing mutants for a large number of phenotypes simultaneously (4a, 5, 61, 75). PM tests are performed in 96-well microplates containing different nutrients or inhibitors in which cell respiration is measured with a redox indicator. Here we report the results of PM tests performed with a large collection of mutants in which we examined nearly 2,000 cellular phenotypes by using a sensitive, highly controlled, reproducible format. Mutants were expected to display one or more altered phenotypes if the mutated gene has a role under the condition(s) tested. Of 37 different two-component mutants, 22 exhibited one or more phenotypic differences. Other mutants (including *luxS* and *rpoS* mutants) also exhibited altered phenotypes. Accordingly, PM technology provides a simple way to determine mutational effects on a genome-wide scale and adds to our understanding of intricate control systems.

MATERIALS AND METHODS

Bacteria. All of the mutants used are derivatives of *E. coli* K-12 strain BW25113 (10) [*lacIp4000*(*lacI*^{q}) *rrnB3* Δ*lacZ4787* *hsdR514* Δ(*araBAD*)*567* Δ(*rhaBAD*)*568* *rph-1*] or its *rph*$^+$ derivatives BW28357 (86) and BW30383 (unpublished data). Most mutants were initially constructed by using a standard chromosomal gene disruption protocol (10). Briefly, PCR products were generated by using a pair of long (usually 56- to 60-nucleotide [nt]) primers and special template plasmids bearing a resistance marker flanked by FLP recombinase target sites. These oligonucleotides included 20 nt for priming on template plasmids and 36- to 40-nt homologous extensions for targeting recombination events to the corresponding loci on the *E. coli* chromosome. The PCR products were then introduced into cells expressing the phage λ Red recombinase, which permitted recombination in short homologous regions. Recombinants were then verified in a series of PCR tests in which locus-specific test primers were used, as described elsewhere (10). The test primers flanked the deleted region and usually were 200 nt or more from the homologous extension regions.

Two or more mutants of each type were constructed independently (Tables 1 and 2). Additional mutants were constructed by using long primers as described above or as follows. The simplest alternative method involved generating PCR products by using the corresponding flanking test primers for amplification and the mutants described above as templates. Accordingly, these PCR products had much longer regions of homologous sequences in common with the chromosome. As expected, they yielded huge numbers of recombinants when they were introduced into a new strain by using the Red system. Alternatively, the mutation was transferred by P1 transduction with P1*kc* lysates prepared with an initial mutant. All mutants were subsequently made antibiotic sensitive by using the FLP helper plasmid pCP20 to excise the resistance marker, as described elsewhere (10). Details concerning the construction and verification of these mutants will be described elsewhere (Zhou et al., unpublished). Some mutants were made in previous studies by using a standard two-step chromosomal gene deletion method (45).

Storage and handling of bacteria. Bacteria are stored at −70°C in Luria-Bertani medium containing 8% dimethyl sulfoxide as described elsewhere (80). Cells were revived without thawing by scraping the surface with a toothpick and streaking the cells onto an appropriate agar medium. To minimize inadvertent selection of suppressor mutants, fresh colonies were routinely used to inoculate culture tubes.

PM tests. Two or more independent mutants with mutations in each gene were examined. Mutants were compared pairwise with the otherwise isogenic control strain. In a few cases phenotype analysis of two independent mutants unexpectedly revealed substantial differences. Since such inconsistencies may have resulted from acquisition of unknown secondary mutations, additional mutants were sometimes constructed and assayed similarly. The results obtained with atypical strains are not described here.

With five exceptions, the mutants examined included mutants for which DNA microarray experiment results were recently reported (54). Even though all of these organisms are null mutants, it is still possible that some may contain unlinked secondary mutations. For example, during this study we found that the *cusRS* and *rssB* mutants (BW28077 and BW27555, respectively) used in DNA microarray experiments differed from other independently constructed *cusRS* mutants (BW30048, BW30049) and *rssB* mutants (BW29745, BW30416, BW30419, BW30420, BW30421), all of which were similar to other mutants of the same type. Also, as previously reported (54), the *uvrY* deletion mutant BW27874 that was used in the DNA microarray experiments has a growth defect. However, new *uvrY* mutants with in-frame (and presumably nonpolar) deletions did not have a growth defect. Accordingly, we report PM data for these new *uvrY* mutants (BW29475, BW29476) below. No data are reported for the *atoSC* and *yfhA* mutants (BW28878 and BW29430, respectively) that were used in DNA microarray experiments. However, this was due to an oversight. BW28878 and BW29430 were by chance not subjected to PM tests. Rather, two other independent *atoSC* and *yfhA* mutants were examined instead.

PM tests were performed essentially as described elsewhere (5), except that IF-0 inoculating fluid was used for PM1 to PM8 and IF-10 was used for PM9 to PM20. PM1 and PM2 are similar to ES. PM3 to PM8 are similar to EN, EPS, and EA, which used a defined medium containing 100 mM NaCl, 30 mM triethanolamine HCl (pH 7.1), 5.0 mM NH_4Cl, 2.0 mM NaH_2PO_4, 0.25 mM Na_2SO_4, 0.05 mM $MgCl_2$, 1.0 mM KCl, and 0.01% tetrazolium violet. PM3, PM4, and PM6 to PM8 contain various N, P, or S sources which are omitted from the defined medium. PM9 to PM20 are similar to ES1, ES2, and ES3, which used a rich medium containing 2.0 g of tryptone, 1.0 g of yeast extract, and 1.0 g of NaCl per liter. Strains were grown overnight at 35°C on BUG+B agar instead of R2A agar (64). All fluids, agar media, and PMs are commercially available from Biolog, Inc. (Hayward, Calif.), and all PMs were inoculated with cell suspensions at 100 μl per well. Cells were picked from the agar surface with a sterile cotton swab and suspended in 15 ml of IF-0, and the cell density was adjusted to 42% transmittance (T) on a Biolog turbidimeter. These suspensions were diluted sixfold by combining with 75 ml of IF-0 to give a density of 85% T (approximately an A_{420} of 0.12). PM1 and PM2, which measure C utilization phenotypes, were directly inoculated with 22 ml of the 85% T suspension. Six hundred sixty microliters of 2 M disodium succinate and 0.2 mM ferric citrate was then added to 66 ml of the 85% T suspension and used to inoculate PM3 to PM8, which measure N, P, and S utilization and auxotrophic phenotypes. Six hundred microliters of the 85% T suspension was diluted 200-fold into 120 ml of IF-10 and used to inoculate PM9 to PM20, which measure sensitivity to salt and pH stress, and to a wide variety of antibiotics, antimetabolites, and other inhibitors. All PMs were incubated at 36°C in an OmniLog and monitored for color change in the wells. Readings were recorded for 24 h for all PMs except PM3, PM4, and PM6 to PM8, for which readings were recorded for 48 h. Kinetic data were analyzed with OmniLog-PM software, release OL_PM_109M Jan. 14, 2002.

Additional tests were done to substantiate a subset of the PM data. To confirm phenotypes detected with the metabolic arrays (PM1 to PM8), tests were repeated once. To confirm phenotypes detected with the inhibitor sensitivity arrays (PM9 to PM20), 1.33-fold serial dilutions of some chemicals were used in 96-well microplates to retest particular mutants. BBL Sensi-Disc antimicrobial susceptibility test disks (Becton Dickinson and Co., Sparks, Md.) were also used to test some drug sensitivity phenotypes with the agar disk diffusion test procedure recommended by the manufacturer. These tests were carried out with cells which were grown in Difco Bacto Tryptic Soy broth (Becton Dickinson and Co.) and tested for antibiotic susceptibility on Difco Mueller-Hinton agar (Becton Dickinson and Co.), as recommended by the manufacturer.

TABLE 1. Sources of two-component mutants

Mutation[a]	Strain	Parent or control[b]	Method[c]	Mutation	Strain	Parent or control	Method
Δ*arcA43*	BW27422	BW25113	a	Δ(*envZ ompR*)*520*	BW29655	BW28357	c
Δ*arcA45*	BW29409	BW28357	a	Δ*phoBR580*	BW24476	BW25113[e]	d
Δ*arcB41*	BW26422	BW25113	a	Δ*phoBR758*	BW29134	BW25113	a
Δ*arcB41*	BW29859	BW28357	b	Δ*phoBR758*	BW30046	BW28357	c
Δ*atoSC571*	BW29009	BW25113	a	Δ*phoQP1244*	BW27558	BW25113	b
Δ*atoSC573*	BW29010	BW25113	a	Δ*phoQP1244*	BW30007	BW28357	c
Δ*baeSR579*	BW27553	BW25113	b	Δ*qseBC*(*ygiXY*)*1302*	BW27551	BW25113	b
Δ*baeSR610*	BW29744	BW28357	b	Δ*qseBC*(*ygiXY*)*1304*	BW29747	BW28357	b
Δ*barA614*	BW27563	BW25113	b	Δ*rcsB1320*	BW27870	BW25113	a
Δ*barA1358*	BW29434	BW25113	a	Δ*rcsB1320*	BW30009	BW28357	c
Δ*basSR616*	BW27549	BW25113	b	Δ*rssB1275*	BW29745	BW28357	b
Δ*basSR1287*	BW27848	BW25113	a	Δ*rssB1273*	BW30418	BW25113	c
Δ*basSR620*	BW29371	BW28357	a	Δ*rssB1273*	BW30419	BW25113	c
Δ*cheZYBA1218*[d]	BW28079	BW25113	a	Δ*rssB1273*	BW30420	BW30383	c
Δ*cheZYBA1218*	BW29661	BW28357	c	Δ*rssB1273*	BW30421	BW30383	c
Δ*cpxAR623*	BW27559	BW25113	b	Δ*rstAB1278*	BW27552	BW25113	b
Δ*cpxAR623*	BW29849	BW28357	c	Δ*rstAB1280*	BW29806	BW28357	b
Δ*creABCD154*	BW26983	BW25113[e]	d	Δ*torSTRCAD518*	BW26423	BW25113	a
Δ*creABCD176*	BW29135	BW25113	a	Δ*torSTRCAD518*	BW29855	BW28357	c
Δ*cusSR1204*	BW30048	BW28357	c	Δ*uhpBA1322*	BW27871	BW25113	a
Δ*cusSR1204*	BW30049	BW28357	c	Δ*uhpBA1322*	BW29852	BW28357	c
Δ*dcuRS*(*yjdGH*)*1330*	BW27878	BW25113	a	Δ*uvrY1310*	BW29475	BW25113	a
Δ*dcuRS*(*yjdGH*)*1330*	BW29657	BW28357	c	Δ*uvrY1362*	BW29476	BW25113	a
Δ*dpiBA*(*citAB*)*1289*	BW27876	BW25113	a	Δ*yedVW1298*	BW27550	BW25113	b
Δ*dpiBA*(*citAB*)*1289*	BW29656	BW28357	c	Δ*yedVW1300*	BW29746	BW28357	b
Δ*evgAS1291*	BW27869	BW25113	a	Δ*yehTU1324*	BW27877	BW25113	a
Δ*evgAS1291*	BW29663	BW28357	c	Δ*yehTU1324*	BW29858	BW28357	c
Δ*fimZ1214*	BW28078	BW25113	a	Δ*yfhA1326*	BW27868	BW25113	a
Δ*fimZ1214*	BW29659	BW28357	c	Δ*yfhA1326*	BW29851	BW28357	c
Δ*kdpEDCBAF1224*	BW27564	BW25113	b	Δ*yfhK1328*	BW27872	BW25113	a
Δ*kdpEDCBAF1224*	BW30166	BW28357	c	Δ*yfhK1328*	BW29857	BW28357	c
Δ*narLX1316*	BW27864	BW25113	a	Δ*yojN1332*	BW27866	BW25113	a
Δ*narLX1316*	BW29658	BW28357	c	Δ*yojN1332*	BW29856	BW28357	c
Δ*narP1312*	BW27873	BW25113	a	Δ(*yojN rcsBC*)*1308*	BW27557	BW25113	b
Δ*narP1312*	BW30265	BW28357	c	Δ(*yojN rcsBC*)*1308*	BW29861	BW28357	c
Δ*narQ1314*	BW27865	BW25113	a	Δ*ypdAB1334*	BW27875	BW25113	a
Δ*narQ1314*	BW30008	BW28357	c	Δ*ypdAB1334*	BW29860	BW28357	c
Δ*ntrCB*(*glnGL*)*1318*	BW27880	BW25113	a	Δ*zraSR*(*hydHG*)1336	BW27867	BW25113	a
Δ*ntrCB*(*glnGL*)*1318*	BW30011	BW28357	c	Δ*zraSR*(*hydHG*)1336	BW29660	BW28357	c
Δ(*envZ ompR*)*520*	BW26424	BW25113	a				

[a] Common alternative nomenclature is indicated in parentheses. Different alleles are used to identify mutations that differ with regard to the template or primer used for generation. The allele assignments for multiple gene deletions are in clockwise order in accordance with the K-12 map. Mutants are available from the Coli Genetic Stock Center (http://cgsc.biology.yale.edu/). Genes are designated according to their order within an operon.

[b] *E. coli* K-12 strains BW28357 and BW30383 are independent *rph*$^+$ derivatives of BW25113 that were made by using the Red system and P1 transduction, respectively (K. A. Datsenko and B. L. Wanner, unpublished data).

[c] Mutants were made in one of four ways. In method a, mutants were isolated as kanamycin-resistant derivatives of the parent carrying pKD46 following introduction of a PCR fragment generated with long (56- to 60-nt) primers and pKD13 as the template, after which the resistance marker was eliminated with pCP20 as described elsewhere (10). In method b, mutants were isolated as kanamycin-resistant transductants by using P1*kc* grown on the corresponding kanamycin-resistant mutant, after which the resistance marker was eliminated with pCP20. In method c, mutants were isolated as kanamycin-resistant derivatives of the parent carrying pKD46 following introduction of a PCR fragment generated with locus-specific test primers and the corresponding kanamycin-resistant mutant as the template, after which the resistance marker was eliminated with pCP20. In method d, the mutation was constructed on a conditionally replicative plasmid by using standard cloning techniques and then recombined onto the chromosome by using our standard two-step allele replacement method (45), after which the mutation was transferred to a parent by cotransduction with nearby *proC*$^+$ and *thr*$^+$ markers for the *phoBR* and *creABCD* loci, respectively.

[d] The Δ*cheZYBA1218* mutation has a deletion of several nearby genes and corresponds to the Δ(*flhEAB cheZYBR tap tar cheWA motBA flhCD* IS*1*)*1218* mutation.

[e] BW24476 and BW26983 are descendents of strains like BW25113 and served as controls.

RESULTS

Overview. All phenotypic differences exhibited by two or more independent mutants of each type are briefly described below. Many results confirmed or expanded our knowledge of the two-component systems. Other results were unexpected and not easily understood in light of current knowledge. For clarity, two-component partner proteins are designated HK/RR below when the corresponding genes were deleted simultaneously. Otherwise, they are identified parenthetically as an HK, Hpt (histidine phosphotransfer domain only), hybrid (containing both HK and RR domains), or RR protein. Several mutants also had nearby related genes deleted (Tables 1 and 2). In general, no attempt was made to ascertain whether phenotypic differences were attributable to the loss of the two-component system per se or to the loss of the function of a nearby gene(s).

We first describe results for the two-component systems (ArcB/ArcA, PhoR/PhoB, UhpB/UhpA, and NtrB/NtrC) that

TABLE 2. Sources of other mutants

Mutation[a]	Strain	Parent	Method[b]
Δ*cbrA179*	BW29846	BW28357	a
Δ*cbrA181*	BW29847	BW28357	a
Δ*cbrBC183*	BW29869	BW28357	a
Δ*cbrBC183*	BW30003	BW28357	a
Δ*cbrBC185*	BW29848	BW28357	a
Δ*csgD1202*	BW28106	BW25113	a
Δ*csgD1202*	BW29850	BW28357	c
Δ*luxS1368*	BW30044	BW28357	a
Δ*luxS1368*	BW30045	BW28357	a
Δ*phnP-C75*[c]	BW29687	BW28357	a
Δ*phnP-C75*	BW29688	BW28357	a
Δ*phoA760*	BW29256	BW28357	a
Δ*phoA760*	BW30047	BW28357	a
Δ*phoH764*	BW29689	BW28357	a
Δ*phoH766*	BW29690	BW28357	a
Δ*psiE768*	BW29844	BW28357	a
Δ*psiE770*	BW29845	BW28357	a
Δ*rpoS1271*	BW28465	BW28357	a
Δ*rpoS1271*	BW29923	BW28357	a
Δ*ugpQCEAB772*	BW29685	BW28357	a
Δ*ugpQCEAB774*	BW29686	BW28357	a

[a] The *creB*-regulated *cbrA* and *cbrBC* genes correspond to the *yidS* and *yieIJ* genes, respectively (S.-K. Kim and B. L. Wanner, unpublished data). The *psiE* gene is the same as *yjbA* (46).

[b] See Table 1, footnote *c*.

[c] The Δ*phnP-C75* allele corresponds to the Δ*phnCDEFGHIJKLMNOP75* mutation.

gave phenotypes which were largely in agreement with expectations. These results are especially valuable for validation purposes. We then discuss other systems alphabetically. No significant differences were observed for 15 different two-component mutants [BasS/BasR, CheA (HK)/CheB (RR); CheY (RR), CreC/CreB, DpiB/DpiA, EvgS/EvgA, FimZ (RR), NarX/NarL, NarQ (HK), TorS/TorR, YedV/YedW, YehU/YehT, YfhK (HK), YfhA (RR), YpdA/YpdB, and ZraS/ZraR]. The CsgD mutants also exhibited no phenotypic difference. The effects of mutations in *phoBR*- and *creBC*-regulated genes are described in the context of the PhoR/PhoB and CreC/CreB systems, respectively. Data for the *luxS* and *rpoS* mutants are also summarized below.

ArcB/ArcA. The two-component system consisting of ArcB (HK) and ArcA (RR) modulates expression of numerous operons and regulons involved in respiratory and fermentative metabolism in response to oxygen deficiency or redox potential (28, 29). The oxidized forms of quinones are thought to be ArcB-specific signals that silence, rather than stimulate, ArcB kinase activity during aerobiosis (19), thus showing that there is a direct connection between the control of gene expression by the Arc system and the electron transport chain. The Arc system has also been shown to inhibit *E. coli* chromosomal initiation in vitro (40).

The *arcA* and *arcB* genes are not linked, so each gene was deleted individually and the corresponding mutants were tested independently. The *arcA* and *arcB* mutants were both highly pleiotropic, displaying more than 50 phenotypes (Table 3). There was a high degree of overlap of the phenotypic changes, confirming their relationship. Both *arcA* and *arcB* mutants exhibited increased resistance to β-chloro-L-alanine and hypersensitivity to more than 40 chemicals, most of which affect membrane or membrane-associated functions, such as respiration. Phospho-ArcA acts as a transcriptional activator of several genes and as a repressor of other genes. Accordingly, the discordance for phenotypes not in common may have been due to different effects resulting from the loss of ArcA or phospho-ArcA on gene expression. Alternatively, they may have been due to an additional specific role(s) for one partner protein. For example, ArcB controls not only ArcA-dependent gene expression but also OmpR-dependent gene expression in vivo (44). While the *arcA* and *arcB* mutants had two phenotypes (thioridazine and cobalt chloride sensitivity) in common with EnvZ/OmpR mutants, neither phenotype was specific for *arcA* or *arcB* mutants.

PhoR/PhoB. The PhoR/PhoB two-component system controls genes of the phosphate (Pho) regulon for assimilation of alternative P sources (81). Representative PM results for a *phoBR* mutant showed that the principal phenotypic changes were in the top five rows of PM4 (Fig. 1), which measured P metabolism. All three *phoBR* mutants showed decreased use of several organophosphates as P sources, which was expected.

We also tested the effects of mutations of Pho regulon genes (Table 2), including deletions of the *phoA* gene (which encodes the bacterial alkaline phosphatase Bap), the *ugpBAECQ* operon (which encodes an ABC transporter for uptake of *sn*-glycerol-3-phosphate and a glycerophosphoryl diester hydrolase), the *phnC-phnP* operon (which codes for breakdown of phosphonates), and two genes of unknown function (*phoH* and *psiE*). The *phoA* and *phoBR* mutants showed decreased use of the same six organophosphates (Table 4). Hence, the phenotypic changes for the *phoBR* mutants in PM4 were due to loss of Bap, a nonspecific phosphohydrolase. Accordingly, Bap is required for use of these six organophosphates as sole phosphorus sources. The failure to detect changes in the *ugpBAECQ* mutants is also understandable. An effect on use of glycerol 3-phosphate as a P source is likely to be masked in *ugpBAECQ* mutants that are both *phoA*$^+$ and *glpT*$^+$. Bap can hydrolyze glycerol 3-phosphate, whereas GlpT is an alternative glycerol 3-phosphate transporter.

The *phn* mutants showed defective growth at pH 10 and hypersensitivity to a few inhibitors, as did the *psiE* and *phoH* mutants (Table 4). It is interesting, although not surprising, that mutants in which genes activated by the PhoR/PhoB system were deleted displayed phenotypes that were not observed in *phoBR* mutants. Basal-level expression of individual Pho regulon genes or operons may be sufficient for functioning of the corresponding gene products. Other controls also act on these genes (81). The *phn* operon encodes 14 gene products, including an ABC transporter, a C-P lyase, and two apparent transcriptional regulators. No tests were done to determine which *phn* gene(s) is responsible for these phenotypes. The *phoH* gene product is an ATP-binding protein, and the *psiE* product is a highly conserved, putative membrane protein. New studies are needed to define more precisely the genetic basis, as well as the biochemical and physiological basis, of these phenotypic changes.

UhpB/UhpA. The UhpB/UhpA system controls synthesis of the hexose phosphate transporter UhpT (84). As expected, we found that the *uhpAB* mutants were specifically defective in the use of glucose 6-phosphate and fructose 6-phosphate as carbon sources. Since the *uhpAB* mutants were *phoA*$^+$, they were able to use these compounds as P sources.

TABLE 3. *arcA* and *arcB* mutant phenotypes

Test[a]	Difference[b]		Mode of action
	arcA	*arcB*	
β-Methyl-D-glucuronate	60	—[d]	C source
β-Chloro-L-alanine	120	88	Amino acid analog
Potassium tellurite	—	78	Transport, toxic anion
Atropine	−150	−180	Acetylcholine antagonist
pH 8 to pH 10[c]	−64	−68	Alkaline pH
Myricetin	−110	−170	Antimicrobial
Sanguinarine	−130	—	ATPase inhibitor
Aztreonam	−140	−200	Cell wall, lactam
EGTA	—	−210	Chelator, Ca^{2+}
EDTA	−180	−250	Chelator, hydrophilic
Sodium pyrophosphate	−210	−140	Chelator, hydrophilic
1,10-Phenanthroline	−120	−83	Chelator, lipophilic
2,2′-Dipyridyl	—	−110	Chelator, lipophilic
5-Chloro-7-iodo-8-hydroxyquinoline	—	−130	Chelator, lipophilic
8-Hydroxyquinoline	—	−140	Chelator, lipophilic
Fusaric acid	−71	−94	Chelator, lipophilic
Caffeine	—	−71	Cyclic AMP phosphodiesterase
Benserazide	−72	−62	Fungicide
Dichlofluanid	−240	−220	Fungicide
Nordihydroguaiaretate	—	−94	Fungicide, lipoxygenase
Harmane	—	−76	Imidazoline binding sites, agonist
Trifluoperazine	−120	−110	Ion channel, Ca^{2+}
Dequalinium	−200	−220	Ion channel, K^+
2-Phenylphenol	−110	—	Membrane agent
m-Cresol	—	−100	Membrane agent
o-Cresol	−70	—	Membrane agent
p-Cresol	−150	−150	Membrane agent
Phenylethanol	−120	−160	Membrane agent
Alexidine	−90	—	Membrane, biguanide, electron transport
Benzethonium chloride	—	−63	Membrane, cationic detergent
Methyltrioctylammonium chloride	−150	−91	Membrane, cationic detergent
Guanidine hydrochloride	−85	−75	Membrane, chaotropic agent
Lauryl sulfobetaine	—	−120	Membrane, zwitterionic detergent
Amitriptyline	—	−94	Membrane, transport
Glycyl-L-cysteine	−120	−57	N source
5% Sodium sulfate	−320	−400	Osmolarity
Lawsone	−360	−340	Oxidizing agent
Plumbagin	−160	−220	Oxidizing agent
Potassium superoxide	−92	−100	Oxidizing agent
Chlorpromazine	—	−79	Phenothiazine
Compound 48/80	−110	—	Phospholipase C, ADP ribosylation
Chloramphenicol	—	−120	Protein synthesis
Puromycin	—	−160	Protein synthesis
Tylosin	—	−110	Protein synthesis
Geneticin (G418)	−110	—	Aminoglycoside
Paromomycin	−130	—	Aminoglycoside
Troleandomycin	—	−77	Macrolide
Thioglycerol	−210	−170	Reducing agent
Tetrazolium violet	−110	−180	Respiration
Thioridazine	−110	−170	Respiration
Pentachlorophenol	−150	−130	Respiration, H^+ ionophore
Menadione	—	−120	Respiration, uncoupler
Sodium azide	−180	—	Respiration, uncoupler
100 mM ammonium sulfate (pH 8)	−200	—	Toxicity, ammonia
200 mM sodium benzoate (pH 5.2)	—	−67	Toxicity, benzoate
100 mM sodium nitrite	−650	−840	Toxicity, nitrite
200 mM sodium phosphate (pH 7)	−430	−500	Toxicity, phosphate
Sodium cyanate	−110	−210	Transport, toxic anion
Sodium cyanide	−150	−170	Transport, toxic anion
Sodium metaborate	−61	−79	Transport, toxic anion
Sodium metasilicate	−120	−150	Transport, toxic anion
Sodium nitrite	—	−200	Transport, toxic anion
Sodium tungstate	—	−160	Transport, toxic molybdate analog
Cobalt chloride	−120	−190	Transport, toxic cation
Manganese(II) chloride	—	−160	Transport, toxic cation
Thallium(I) acetate	−170	−220	Transport, toxic cation
Zinc chloride	−250	−220	Transport, toxic cation

[a] Chemicals were tested in 96-well PMs.

[b] The OmniLog-PM software generates time course curves for respiration (tetrazolium color formation) and calculates differences in the areas for mutant and control cells. The units are arbitrary. Positive values indicate that the mutant showed greater rates of respiration than the control. Negative values indicate that the control showed greater rates of respiration than the mutant. The differences are averages of values reported for two or more mutants of each type compared with the corresponding control strains.

[c] Minimal differences are given for alkaline pH sensitivities.

[d] —, software reported no significant difference.

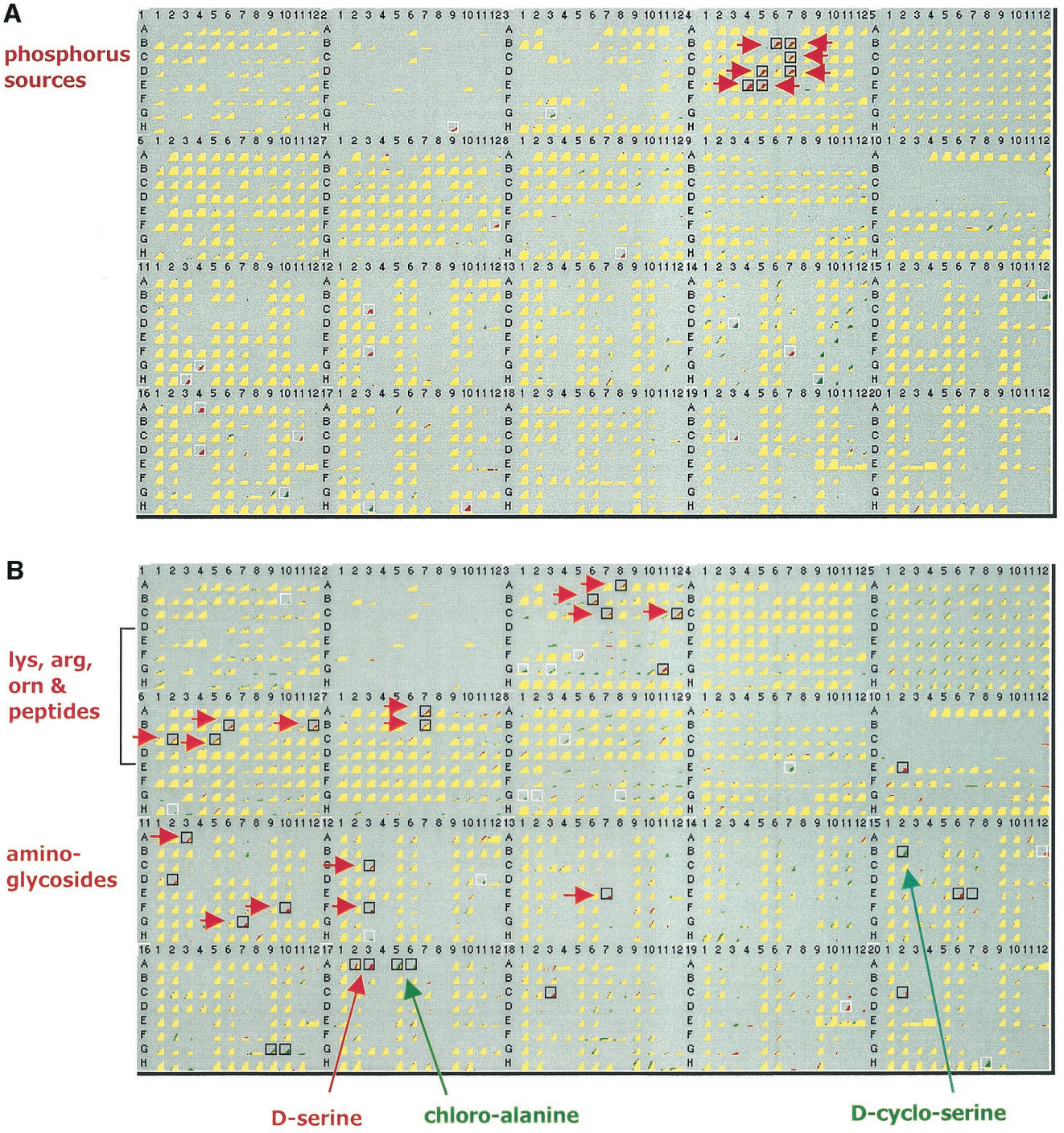

FIG. 1. Phenotypic changes in PM assays. Significant changes are enclosed in boxes and indicated by arrows. Yellow indicates that growth of the wild type and growth of the mutant were similar. Red indicates faster growth of the wild type. Green indicates faster growth of the mutant. The quantitative difference values are shown in Table 4. (A) Comparison of BW29134 (Δ*phoBR*) with BW25113. Well B6, D-2-phosphoglyceric acid; well B7, D-3-phosphoglyceric acid; well C7, 6-phosphogluconic acid; well D5, *O*-phospho-D-serine; well D7, *O*-phospho-L-threonine; well E4, phosphorylcholine; well E5, o-phosphorylethanolamine. (B) Comparison of BW27880 (Δ*ntrBC*) with BW25113.

NtrB/NtrC. The NtrB/NtrC system controls the expression of genes for N assimilation. These include the *glnALG* operon (*glnA* encodes glutamine synthetase, *glnL* encodes NtrB, and *glnG* encodes NtrC), the *glnHPQ* operon (which encodes glutamine permease), the *glnK-amtB* operon (*glnK* encodes a PII homolog, and *amtB* encodes an ammonium uptake protein), the *nac* gene (which encodes a nitrogen assimilation control protein), the *astCADBE* operon (which encodes an arginine catabolic system), and other transporters and enzymes important in N assimilation (89). Approximately 2% of the *E. coli*

TABLE 4. Mutant phenotypes

Mutants	Test[a]	Difference[b]	Mode of action
Two-component mutants			
atoSC	Glucuronamide	64	C source
	Polymyxin B	−180[c]	Membrane agent, outer
	Methyltrioctylammonium chloride	−150[c]	Membrane, detergent, cationic
	6% NaCl	−51	Osmolarity
	Dihydrostreptomycin	−140[c]	Aminoglycoside
	Iodonitrotetrazolium violet	−130[c]	Respiration
baeSR	Myricetin	−140	Antimicrobial
	Gallic acid	−220	Antimicrobial
	Nickel chloride	−76	Transport, toxic cation
	Sodium tungstate	−390[d]	Transport, toxic molybdate analog
barA	Melibionic acid	59	C source
	D-Melibiose	77[e]	C source
cpxRA	pH 9.5 + anthranilate, glycine, L-alanine, L-arginine, L-asparagine, L-aspartate, L-glutamate, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, or L-threonine	−70 to −110[e]	Alkaline pH, deaminase
	Leu-Trp	−87	N source
	15 or 20% Ethylene glycol	−230	Osmolarity
	Amikacin	−140[d,f]	Aminoglycoside
	Dihydrostreptomycin	−150[d]	Aminoglycoside
	Geneticin (G418)	−170[d]	Aminoglycoside
	Hygromycin B	−84[d]	Aminoglycoside
	Kanamycin	−110[d,f]	Aminoglycoside
	Tobramycin	−140[d,f]	Aminoglycoside
	Methane sulfonate	−72	S source
	Lithium chloride	−79	Transport, toxic cation
cusRS	1,10-Phenanthroline	−110[d]	Chelator, lipophilic
dcuSR[g]	Bromosuccinate	−65	C source
	DL-Malate or D-Malate	−130	C source
	Fumarate	−110	C source
	L-Asparagine	−71	C source
	L-Aspartate	−140	C source
	L-Malate	−130	C source
	m-Tartarate	−73	C source
	Succinate	−57	C source
kdpFABCDE	Novobiocin	95[d]	DNA topoisomerase
	Hygromycin B	−75[c]	Aminoglycoside
narP	5-Chloro-7-iodo-8-hydroxyquinoline	−110[d]	Chelator, lipophilic
ntrBC	β-Chloro-L-alanine	120	Amino acid analog
	D-Cycloserine	85[d]	Cell wall, sphingolipid synthesis
	pH 9.5 + L-alanine	−76	Alkaline pH, deaminase
	Nitrofurazone	−150	DNA synthesis
	D-Serine	−240[d]	Inhibitor, 3-phosphoglycerate dehydrogenase
	Poly-L-lysine	−110	Membrane, detergent, cationic
	Arg-Arg	−75	N source
	Arg-Lys	−66	N source
	Arg-Phe	−39	N source
	Arg-Tyr	−43	N source
	D-Lysine	−36	N source
	L-Argininc	−50	N source
	L-Lysine	−44	N source
	L-Ornithine	−45	N source
	Lys-Arg	−68	N source
	Met-Arg	−32	N source
	δ-Amino-*N*-valerate	−82	N source
	Amikacin	−100[d,f]	Aminoglycoside
	Geneticin (G418)	−120[d]	Aminoglycoside
	Gentamicin	−120[f]	Aminoglycoside
	Neomycin	−100[d]	Aminoglycoside
	Paromomycin	−110[d]	Aminoglycoside
	Tobramycin	−120[d,f]	Aminoglycoside
	Thioridazine	−102	Respiration
	Capreomycin	−62	Respiration, Na^+-K^+ ATPase
ompR-envZ	β-D-Allose	56	C source
	D-Fructose	64[e]	C source
	D-Mannitol	65	C source

Continued on following page

TABLE 4—*Continued*

Mutants	Test[a]	Difference[b]	Mode of action
	N-Acetyl-D-glucosamine	87	C source
	α-D-Glucose	100	C source
	Cefamandole	250[d]	Cell wall, cephalosporin
	Cephalothin	260[d,f]	Cell wall, cephalosporin
	Aztreonam	140	Cell wall, lactam
	Norfloxacin	120	DNA topoisomerase
	Sulfachloropyridazine	89	Folate antagonist
	Ata-Ser	61	N source
	Lys-Ser	46	N source
	Oxacillin	−110	Cell wall, lactam
	15% Ethylene glycol	−97	Osmolarity
	Thioridazine	−130	Respiration
	Cobalt chloride	−120	Transport, toxic cation
	Sodium selenite	−253	Toxic anion
	Potassium chromate	−74	Transport, toxic anion
	Lithium chloride	−71	Transport, toxic cation
	Sodium dichromate	−83	Transport, toxic SO_4 analog
phoBR[h]	D-2-Phosphoglycerate, D-3-phosphoglycerate, *O*-phospho-D-serine, *O*-phospho-L-threonine, *O*-phosphorylethanolamine, phosphorylcholine	−53 to −82	P source
	Tobramycin	−140[c,i]	Aminoglycoside
	Paromomycin	−130[c]	Aminoglycoside
phoPQ	D-Fructose	54[e]	C source
	D-Mannitol	52	C source
qseBC (*ygiXY*)	Ruthenium red	−73	Respiration, mitochondrial Ca^{2+} porter
	Cesium chloride	−75	Transport, toxic cation
	Cobalt chloride	−75	Transport, toxic cation
	Cupric chloride	−150[c]	Transport, toxic cation
	Nickel chloride	−74	Transport, toxic cation
rcsB	Nitrofurazone	−68[d]	DNA synthesis
	Trimethoprim	−110[i]	Folate antagonist
	5 to 6% NaCl	−25 to −62[d]	Osmolarity
	Iodonitrotetrazolium violet	−210[d]	Respiration
rssB[g]	pH 4.5 + L-lysine	−85	Acidic pH, decarboxylase
	α-Ketoglutarate	−110	C source
	α-Methyl-D-galactoside	−91	C source
	β-Hydroxypyruvate	−62	C source
	Bromosuccinate	−84	C source
	DL-α-Glycerol phosphate	−94	C source
	DL-Malate	−100	C source
	D-Alanine	−120	C source
	D-Glucuronate	−69	C source
	D-Malate	−68	C source
	Fumarate	−90	C source
	Glyoxylate	−98	C source
	L-Asparagine	−100	C source
	L-Aspartate	−130	C source
	L-Malate	−89	C source
	m-Tartarate	−90	C source
	Succinate	−88	C source
	Ala-Asn	−62	N source
	Ala-Leu	−65	N source
	Ala-Ser	−87	N source
	Arg-Tyr	−60	N source
	Asp-Leu	−92	N source
	Asp-Phe	−60	N source
	D-Ala-Gly	−95	N source
	D-Ala-Gly-Gly	−66	N source
	δ-Amino-*N*-valerate	−130	N source
	D-Asparagine	−77	N source
	Glu-Trp	−60	N source
	Gly-D-Ala	−68	N source
	Gly-Gly-D-Leu	−78	N source
	Gly-Gly-Leu	−60	N source
	Gly-Phe-Phe	−73	N source
	Gly-Tyr	−79	N source
	His-Trp	−74	N source
	L-Cysteine	−160	N source
	Leu-Ala	−86	N source
	Leu-Glu	−82	N source

Continued on following page

TABLE 4—*Continued*

Mutants	Test[a]	Difference[b]	Mode of action
	Leu-Gly	−85	N source
	Leu-Trp	−89	N source
	L-Proline	−89	N source
	Met-Trp	−67	N source
	Phe-Trp	−130	N source
	Pro-Leu	−71	N source
	Putrescine	−75	N source
	Ser-Leu	−75	N source
	Trp-Leu	−120	N source
	Trp-Phe	−90	N source
	Trp-Trp	−92	N source
	Trp-Tyr	−100	N source
	L-Cysteate	−80	S source
	Methane sulfonate	−77	S source
	Sodium nitrite	−76	Transport, toxic anion
	Sodium tungstate	−76	Transport, toxic molybdate analog
rstAB	Ketoprofen	−100	Anticapsule, anti-inflammatory
	Pridinol	−72	Cholinergic antagonist
	Troleandomycin	−140	Macrolide
uhpAB	D-Glucose 6-phosphate	−69	C source
	D-Fructose 6-phosphate	−73	C source
uvrY	D-Melibiose	73[e]	C source
	Hydroxylamine	89	DNA damage, antifolate
	Nitrofurazone	−70[d]	DNA synthesis
	Methyltrioctylammonium chloride	−130[c]	Membrane, cationic detergent
	Polymyxin B	−150[c]	Membrane agent, outer
	Dihydrostreptomycin	−120[c]	Aminoglycoside
	Iodonitrotetrazolium violet	−67[c]	Respiration
yojN	20% Ethylene glycol	−130	Osmolarity
	3 to 6% NaCl	−13 to −83	Osmolarity
yoJN rcsBC	Trimethoprim	−110[i]	Folate antagonist
	3 to 6% NaCl	−21 to −100	Osmolarity
Other mutants			
cbrA (*yidS*)	Hydroxylamine	94	DNA damage, mutagen, antifolate
	Ofloxacin	−81	DNA topoisomerase, quinolone
	5,7-Dichloro-8-hydroxyquinaldine	−86	Chelator, lipophilic
	18-Crown-6 ether	−87	Respiration, ionophore
cbrBC (*yieIJ*)[j]	Nitrofurazone	−68	DNA synthesis
luxS	Caffeine	110	Cyclic AMP phosphodiesterase
	Oxycarboxin	75	Fungicide, respiratory enzymes
	pH 10	−57	Alkaline pH
	pH 9.5 + L-alanine, L-histidine, or L-lysine	−39 to −47	Alkaline pH, deaminase
	1,10-Phenanthroline	−110	Chelator, lipophilic
	5,7-Dichloro-8-hydroxyquinaldine	−98	Chelator, lipophilic
	Sulfamethoxazole	−92	Folate antagonist
	Sulfadiazine	−94	Folate antagonist
	Sulfanilamide	−74	Folate antagonist
	Sulfathiazole	−70	Folate antagonist
	D-Valine	−41	N source
	L-Homoserine	−21	N source
phoA	Phosphorylcholine, D-2-phosphoglycerate, *O*-phospho-L-threonine, *O*-phosphorylethanolamine, *O*-phospho-D-serine, D-3-phosphoglycerate	−54 to −90	P source
phoH	Pridinol	73	Cholinergic antagonist
	Troleandomycin	94	Macrolide
	Cefoxitin	−100	Cell wall, cephalosporin
	Spiramycin	−61	Macrolide
phn	Troleandomycin	95	Macrolide
	pH 10	−70	Alkaline pH
	Cefoxitin	−69	Cell wall, cephalosporin
	5,7-Dichloro-8-hydroxyquinaldine	−79	Chelator, lipophilic
psiE	8-Hydroxyquinoline	−90	Chelator, lipophilic
rpoS	L-Threonine	68	C source
	β-Methyl-D-glucuronate	60	C source

Continued on following page

TABLE 4—*Continued*

Mutants	Test[a]	Difference[b]	Mode of action
	L-Threonine	75	N source
	Tyr-Tyr	65	N source
	Guanosine	75	N source
	Tyr-Phe	62	N source
	L-Tyrosine	67	N source
	Ile-Tyr	74	N source
	Methylene diphosphonate	58	P source
	α-Hydroxybutyrate	−98	C source
	Glycyl-L-aspartate	−86	C source
	α-Ketobutyrate	−80	C source
	Hygromycin B	−64	Aminoglycoside

[a] See Table 3, footnote *a*. All di- and tripeptides are L isomers unless indicated otherwise.
[b] See Table 3, footnote *b*.
[c] Not confirmed by serial dilution (see Materials and Methods).
[d] Confirmed qualitatively by serial dilution (see Materials and Methods).
[e] Confirmed by repeating the PM tests (see Materials and Methods).
[f] Confirmed qualitatively with BBL Sensi-Disc tests (see Materials and Methods).
[g] The *dcuSR* and *rssB* mutants were defective in the use of succinate as a C source and showed many defects with the normal PM3 to PM8 plates. These mutants were therefore reassayed by using PM3 to PM8 with glycerol as the C source, on which the *dcuSR* mutants showed no phenotypic differences. Results obtained for PM3 to PM8 with glycerol as the C source are shown for the *rssB* mutants. See text.
[h] Unlike BW29134 and BW30046, BW24476 did not show increased aminoglycoside sensitivity. See text.
[i] Not confirmed with BBL Sensi-Disc tests (see Materials and Methods).
[j] Unlike BW29869 and BW30003, BW29848 did not show increased nitrofurazone sensitivity. See text.

genome (~75 genes) appears to be under NtrC control, and nearly two-thirds of the genes encode systems for scavenging N-containing compounds, including those released into the periplasmic space during cell growth and division (e.g., D-alanine and the D-alanyl-D-alanine dipeptide).

PM analysis of *ntrBC* mutants provided the best example for comparing PM results (Fig. 1B and Table 4) directly with a detailed gene expression analysis (89). In general, the agreement was very good. As expected, the mutants grew normally on minimal medium and did not require glutamine (Fig. 1B, PM5, well A1) (68). Defects were clearly detected in N catabolic pathways (Fig. 1B, PM3, PM6, and PM7), but these defects were specific for utilization of arginine, lysine, and ornithine or peptides of these amino acids. This was presumably due to Ntr regulation of the *ast* operon (arginine catabolism) and/or the *argT* (basic amino acid transport) and *ygjG* (probable ornithine aminotransferase) genes (89).

The *ntrBC* mutants were resistant to β-chloro-L-alanine and D-cycloserine and hypersensitive to D-serine, which suggests that there was altered regulation of the *nac*, *cycA*, *dadA*, and/or *metC* genes (38). The *cycA* gene (uptake of D-alanine, D-serine, and glycine) and the *nac* gene (nitrogen assimilation control) are already known to be involved in Ntr control (89). The *cycA* protein is controlled by the Nac protein (89), which is controlled by NtrC and also connects control by NtrC to σ^{70}-dependent genes (50). The toxicity of D-serine is elevated if it is not efficiently deaminated, leading to inhibition of 3-phosphoglycerate dehydrogenase (9).

In addition to being resistant to the L-alanine analog β-chloro-L-alanine, the *ntrBC* mutants were also defective in L-alanine deamination (PM10, well E2). This could have resulted from an effect on expression of an unknown gene(s), a gene(s) encoding a shared Ntr-regulated alanine transport system, or a gene encoding a deaminase (or transaminase) required for detoxifying the analog. A defect was also seen in the use of δ-amino-*N*-valeric acid as an N source. This is a novel phenotype, and no genes are known to be involved. All of these phenotypic changes observed in the *ntrBC* mutants are reasonably attributable to the NtrB/NtrC function in the regulation of amino acid catabolism as an N source.

Unexpectedly, the *ntrBC* mutants were hypersensitive to several aminoglycosides, including tobramycin, paromomycin, gentamicin, Geneticin, neomycin, and amikacin, and to nitrofurazone, thioridazine, and capreomycin. Aminoglycoside sensitivity was verified by additional tests (Table 4). The biochemical or physiological basis of these susceptibilities is unknown and remains to be explained. Some possible candidate genes are *ubiF*, *topA*, *rplF*, and especially *metC*, which also mediates β-chloro-L-alanine resistance (38).

AtoS/AtoC. The AtoS/AtoC system regulates expression of the *atoDAEB* operon for acetoacetate metabolism (31). The only metabolic phenotype detected in the *atoSC* mutants was increased use of glucuronamide as a carbon source. The *atoSC* mutants also showed increased sensitivity to sodium chloride but not to potassium chloride. In addition, these mutants showed greater susceptibilities to membrane agents (polymyxin B and methyltrioctylammonium chloride), the aminoglycoside dihydrostreptomycin, and the respiration inhibitor iodonitrotetrazolium violet (Table 4). However, we were unable to confirm these susceptibilities by using serial dilution tests as described in Materials and Methods. The basis of these phenotypes is not known.

BaeS/BaeR. The BaeS/BaeR system is now thought to control genes for an efflux pump (*mdtABC*) (2, 51) and a third envelope stress pathway (62). This system was originally revealed by the ability of BaeS to suppress the loss of the HKs CreC, EnvZ, and PhoR in the corresponding mutants (52). The *baeSR* mutants showed increased sensitivity to myricetin, gallic acid, and nickel chloride and especially to sodium tungstate (Table 4). Because tungstate is a molybdate analog, the BaeS/BaeR system may also regulate a gene(s) whose product has a molybdate cofactor. The increased susceptibilities of the *baeSR* mutants support the hypothesis that the BaeS/BaeR system controls an efflux pump.

BarA/UvrY. The *barA* and *uvrY* genes are not linked. BarA and UvrY were shown to be two-component partner proteins

by finding that *barA* and *uvrY* mutants are both hypersensitive to hydrogen peroxide and that phospho-BarA efficiently transfers its phosphoryl group to UvrY (58). The hydrogen peroxide sensitivity is attributed to an effect on RpoS, which in turn controls synthesis of the major catalase (KatE) in *E. coli*. BarA (also called AirS) is a hybrid HK. Homologous BarA/UvrY systems have been widely studied due to their roles in animal and plant pathogenesis. BarA homologs include ExpS in *Erwinia* and GacS (also called LemA or PheN) in *Pseudomonas* species; UvrY homologs include ExpA in *Erwinia*, GacA in *Pseudomonas*, SirA in *Salmonella*, and VarA in *Vibrio* species. Many effects attributed to these systems are likely to be indirect. So far, the only direct target of UvrY is *csrB*, which encodes a small regulatory RNA and whose homologs include *rsmZ* in *Pseudomonas* and *rsmB* in *Erwinia* species (72). The BarA/UvrY system has a role in biofilm formation (30).

PM analysis revealed that both *barA* and *uvrY* mutants gave a stronger carbon utilization response with D-melibiose, providing further evidence that there is an association between BarA and UvrY. Recently, this system was found to be required for efficient switching between glycolytic and gluconeogenic carbon sources in uropathogenic *E. coli* (57). It is therefore a bit surprising that PM tests failed to reveal additional carbon source effects for these mutants. The *uvrY* mutant, but not the *barA* mutant, also showed increased resistance to hydroxylamine and increased sensitivity to several unrelated inhibitors (Table 4).

CpxA/CpxR. The CpxA/CpxR system controls expression of genes involved in relieving envelope protein stress, biofilm formation, motility and chemotaxis, cell proliferation, adaptation to or recovery from the stationary phase, and pathogenesis. The Cpx system also plays a critical role in the regulation of adhesion-induced gene expression (55). Based on a combination of computational and experimental approaches, it has been estimated that about 100 promoters (including the *ompC* promoter) are controlled by the CpxA/CpxR system in *E. coli* (14).

The *cpxRA* mutants had two dominant phenotypes. They were hypersensitive to several amino acids at an alkaline pH and to aminoglycosides (Table 4). These results are in agreement with accounts describing, among several other seemingly unrelated phenotypes, effects of a *cpxA** mutation on resistance to amikacin and growth at high pH, which were shown to be CpxR dependent (13, 63). The *cpxRA* mutants also showed defects in the use of Leu-Trp as an N source, the use of methane sulfonate as an S source, and sensitivity to lithium chloride and the nonionic osmolyte ethylene glycol.

CreC/CreB. Neither the role nor the signal for the CreC/CreB system is known, although this system appears to be connected to carbon and energy metabolism (82). Elsewhere, genes controlled by this system that were identified by searching for promoter-*lacZ* fusions regulated by CreB have been described (86). This search revealed open reading frames of unknown function, which were designated *cbrA*, *cbrB*, and *cbrC* (*creB*-regulated genes A, B, and C, respectively). No phenotypic changes were observed for the *creABCD* mutants, yet differences were apparent in the *cbrA* and *cbrBC* mutants. The *cbrA* mutants displayed greater resistance to hydroxylamine and hypersensitivity to ofloxacin, a lipophilic chelator, and an ionophore (Table 4). Two *cbrBC* mutants showed hypersensitivity to nitrofurzone; however, a third mutant did not (Table 4). The identification of phenotypes for the *cbrA* and *cbrBC* mutants but no differences for the *creABCD* mutants is consistent with the hypothesis that the *cbrA* and *cbrBC* genes are subject to an additional control(s). New studies are needed to understand the basis of these phenotypes.

CusS/CusR. The CusS/CusR system is required for expression of at least one chromosomal gene, *cusC*, which probably encodes a copper ion efflux system (49). The CusS/CusR system also controls copper-induced expression of the promoter for *pcoE* in the plasmid-borne copper resistance *pco* operon.

The *cusRS* mutants displayed a single phenotypic change, hypersensitivity to 1,10-phenanthroline, which was also displayed by *arcA*, *arcB*, and *luxS* mutants (Table 4). 1,10-Phenanthroline is a chelator with high affinities for copper and other metals, including iron and zinc (69). These data support the hypothesis that the CusS/CusR system plays a role in control of a copper efflux system.

DcuS/DcuR. The DcuS/DcuR system is closely related to a subgroup of two-component systems, including the citrate-responsive CitA/CitB system of *Klebsiella* (47). These systems activate expression of *dcuB* (which encodes the anaerobic fumarate-succinate antiporter), *frdABCD* (which encodes fumarate reductase), and *dctA* (which encodes the aerobic succinate carrier) in response to the C_4 dicarboxylates fumarate, succinate, malate, aspartate, tartrate, and maleate (20, 88).

The *dcuSR* mutants gave many expected findings. In particular, defects in the use of D- and L-malic acids, fumaric acid, *m*-tartaric acid, bromosuccinic acid, L-asparagine, and L-aspartic acid as C sources were expected. The *dcuSR* mutants also had a slight defect in the use of succinate, in agreement with a previous report (20). Many additional defects were also seen for use of various N, P, and S sources. These results are not shown in Table 4 because they were secondary to the defect in metabolism of succinate, which was used as the C source in the assays. When the tests were repeated with glycerol as the C source, no phenotypic differences were seen.

EnvZ/OmpR. The EnvZ/OmpR system controls expression of the *ompF* and *ompC* porin genes and many other genes in response to the medium osmolarity (1). The amounts of OmpF and OmpC vary differently. OmpC synthesis is favored under high-osmolarity conditions, and OmpF synthesis is dominant under low-osmolarity conditions (59). OmpF and OmpC synthesis is also subject to other controls, which involve both OmpR-dependent and OmpR-independent effects of adenylate cyclase, RpoS, and acetyl phosphate synthesis, pH, nutrient limitation, and other factors (41, 59, 60).

The *ompR-envZ* mutants showed diverse phenotypic changes (Table 4). Their greater resistance to several antibiotics (including cephalosporins, β-lactam, topoisomerase inhibitor, and folate antagonist) was likely a consequence of defects in porin synthesis that prevented access through the outer membrane. Membrane defects were also probably the cause of hypersensitivity to other agents, including thioridazine, cobalt chloride, and sodium dichromate. These mutants showed increased use of several hexoses as C sources (allose, fructose, mannitol, *N*-acetyl-D-glucosamine, and glucose), which was unexpected. They also displayed increased use of two serine-containing dipeptides as N sources. The *ompR-envZ* mutants were hypersensitive to ethylene glycol (an osmotic agent), but not to other

osmotic agents, such as sodium chloride, sodium sulfate, and sodium lactate. These results support the hypothesis that the EnvZ/OmpR system plays a role in the control of porin synthesis and provide new, unexpected data concerning phenotypic differences as well.

KdpD/KdpE. The KdpD/KdpE system controls expression of the *kdpFABCDE* operon, which encodes the high-affinity K^+ transporter, as well as KdpD and KdpE (18, 77). The expression of this operon is inducible by both NaCl and CsCl via different mechanisms (33). The entire *kdpFABCDE* operon was deleted in the KdpD/KdpE mutants. These mutants displayed increased resistance to novobiocin and increased sensitivity to hygromycin (Table 4). Whether these differences resulted from a direct role of the Kdp transporter in uptake or efflux of these antibiotics or were an effect of the KdpD/KdpE system on expression of unlinked genes is not known. Our failure to detect phenotypic changes directly attributable to a K^+ transport defect was probably a consequence of the presence of multiple K^+ transporters in *E. coli* (16).

NarP (RR). The Nar system is comprised of two HKs (NarQ and NarX) and two RRs (NarL and NarP), which control genes for anaerobic respiration and fermentation in response to the electron acceptors nitrate and nitrite (3, 39, 71). The genes regulated by the Nar system include *adhE* (which encodes alcohol dehydrogenase), *dmsABC* (which encodes dimethyl sulfoxide/trimethylamine-*N*-oxide reductase), *fdnGHI* (which encodes formate dehydrogenase), *frdABCD* (which encodes fumarate reductase), *modABCD* (which encodes components of a molybdate uptake system), *narK* (which encodes a nitrite exporter), *narGHJI* and *napA* (which encode cellular nitrate reductases), *nirBDC* and *nrfABCDEFG* (which encode nitrite reductases), and *pfl* (which encodes pyruvate-formate lyase).

Three different *nar* mutants were examined, as *narL* and *narX* are adjacent while *narQ* and *narP* are at different loci. No phenotypic differences were observed for *narQ* and *narXL* mutants. The *narP* mutants displayed a single difference, increased sensitivity to the lipophilic chelator 5-chloro-7-iodo-8-hydroxyquinoline (Table 4). Such sensitivity could be indicative of an essential metal cofactor. It is reasonable to suppose that finding additional phenotypic differences would require testing of the *nar* mutants under anaerobic or microaerophilic conditions. This has not been done, however.

PhoQ/PhoP. The PhoQ/PhoP system has been studied primarily in *S. enterica*, in which it was first recognized as a regulatory system that controls synthesis of an acid phosphatase (36) and was later shown to be required for pathogenesis (17, 22, 48). The finding that the PhoQ/PhoP system controls synthesis of an Mg^{2+} transporter led to the discovery that PhoQ senses Mg^{2+} in several gram-negative bacteria (21). Many genes are activated or repressed by this system in *S. enterica* (23). The genes regulated by the PhoQ/PhoP system in response to Mg^{2+} in *E. coli* include *phoPQ*, *mgtA*, and *mgrB* (35). No growth phenotype has ever been reported for *phoPQ* mutants.

The *phoPQ* mutants showed increased use of fructose and mannitol as C sources. Curiously, these phenotypes were also seen in *ompR-envZ* mutants (Table 4). The PM assays were done under conditions of excess Mg^{2+}. Other phenotypes may be specific to low-magnesium conditions.

QseC/QseB. The QseC/QseB system has recently been shown to be involved in quorum sensing and transcriptional regulation of *flhDC*, the master regulator operon for the flagellar and motility genes in *E. coli* (70). Quorum sensing in *E. coli* is thought to respond to a universal signal (called autoinducer-2) which is produced by LuxS in a large number of bacteria and which has been shown to be a furanosyl borate diester (7). Whether QseC is the only sensor kinase that responds to autoinducer-2 is unclear.

The *qseBC* mutants displayed hypersensitivity to several toxic cations (cesium, cobalt, copper, nickel, and ruthenium) (Table 4). Accordingly, the QseC/QseB system may have an undetermined role in metal metabolism.

RcsC (HK)/YojN (Hpt)/RcsB (RR). The RcsC/YojN/RcsB system is a three-component phosphorelay system, in which the Hpt protein YojN accepts a phosphoryl group from phospho-RcsC and then transfers it to RcsB (74). This system regulates expression of the capsular polysaccharide synthesis *cps* genes, the cell division *ftsAZ* genes, an osmoregulated *osmC* gene, and the small RNA gene *rprA*, as well as genes involved in motility and chemotaxis (11, 43). The RcsC sensor appears to respond to stress that affects the cell membrane; however, the precise signal is unknown (8).

The *rcsB*, *yojN*, and *rcsC* genes are adjacent, but they are not in an operon. Three kinds of mutants were examined: mutants with individual deletions of *rcsB* and *yojN* and mutants with all three genes deleted. The *rcsB* mutants showed hypersensitivity to sodium chloride as an osmotic agent and to nitrofurazone, iodonitrotetrazolium violet, and trimethoprim. Likewise, the *yojN* mutant was hypersensitive to sodium chloride. However, it was also hypersensitive to ethylene glycol as an osmotic agent. The *yojN* mutant did not show greater sensitivity to other inhibitors. The triple *yojN-rcsBC* mutant showed hypersensitivity to sodium chloride as an osmotic agent and to trimethoprim but no other phenotypic differences. Although variations were also apparent, all RcsC/YojN/RcsB system mutants were hypersensitive to sodium chloride. Accordingly, this system may have a role in osmotic protection.

RssB (RR). RssB is an orphan RR for which no partner HK has been identified. Unlike most RRs, RssB is also not a transcription factor. Rather, RssB regulates the stability of the sigma factor RpoS and is essential for RpoS proteolysis (25). Phospho-RssB catalyzes the delivery of RpoS to the protease ClpXP for degradation (87).

PM analysis showed that the *rssB* mutants were highly pleiotropic. The *rssB* mutants also resembled the *dcuSR* mutants in that both types of mutants showed defects in the use of several carboxylic acids, including succinic acid, L- and D-malic acids, fumaric acid, L-aspartic acid, L-asparagine, D-alanine, and other acids. Accordingly, like the *dcuSR* mutants, the *rssB* mutants were retested for effects on the use of N, P, and S sources with glycerol as a C source. However, whereas defects in the use of N, P, and S sources were eliminated in the *dcuSR* mutants under these conditions (see above), the *rssB* mutants still showed defects in the use of several N sources (including leucine dipeptides, aspartic acid dipeptides, tryptophan dipeptides, and others) (Table 4). The *rssB* mutants also showed defects in the use of L-cysteate and methane sulfonate as S sources and hypersensitivity to sodium nitrite and tungstate. It is reasonable to suspect that many of these phenotypic changes

TABLE 5. Summary of PM data for two-component mutants[a]

System (HK/RR)	Function(s)	No. of phenotypes[b]		New phenotype(s) or function(s)
		Gained	Lost	
ArcA (RR)	Respiration control	2	47	None[c]
ArcB (hybrid)	Respiration control	2	59	None[c]
AtoS/AtoC	Acetoacetate metabolism	1	5	Glucuronamide use and NaCl sensitivity
BaeS/BaeR	Unknown	0	4	Sodium tungstate sensitivity
BarA (hybrid)	Hydrogen peroxide sensitivity	2	0	Melibiose metabolism
UvrY (RR)	Hydrogen peroxide sensitivity	2	5	Melibiose metabolism
CpxA/CpxR	Cell envelope stress	0	23	Ethylene glycol sensitivity
CusS/CusR	Response to copper	0	1	1,10-Phenanthroline sensitivity
DcuS/DcuR	C_4 dicarboxylate utilization	0	9	None
EnvZ/OmpR	Osmotic regulation	12	8	Increased use of carbohydrates, cephalosporin resistance
KdpD/KdpE	Potassium transport	1	1	Novobiocin resistance
NarP (RR)	Nitrate regulation	0	1	Chelator sensitivity
NtrB/NtrC	Nitrogen regulation	2	23	Aminoglycoside sensitivity
PhoQ/PhoP	Response to magnesium	2	0	Increased use of fructose and mannitol
PhoR/PhoB	Phosphate regulation	0	8	None[d]
QseC/QseB	Quorum sensing	0	5	Metal sensitivity
RcsB (RR)	Capsule synthesis	0	6	NaCl and trimethoprim sensitivity
RcsC/YojN(Hpt)/RcsB	Capsule synthesis	0	6	NaCl and trimethoprim sensitivity
YojN(Hpt)		0	6	NaCl sensitivity
RssB (RR)	σ^S Stability	0	53	Decreased use of C_4 di- and monocarboxylates and amino acid N sources
RstB/RstA	Unknown	0	3	Ketoprofen, pridinol, and troleandomycin sensitivity
UhpB/UhpA	Hexose phosphate uptake	0	2	None

[a] The following 15 other two-component mutants did not have altered phenotypes: BasS/BasR, CheA (HK)/CheB (RR)/CheY (RR), DpiB/DpiA, CreC/CreB, EvgS/EvgA, FimZ (RR), ZraS/ZraR, NarX/NarL, NarQ (HK), TorS/TorR, YedV/YedW, YehU/YehT, YfhK (HK), YfhA (RR), and YpdA/YpdB.
[b] Phenotypes gained means PM assays showing increased growth or respiration (positive values in Table 4). Phenotypes lost means PM assays showing decreased growth or respiration (negative values in Table 4).
[c] The *arcA* and *arcB* mutants had many phenotypes that were attributed to membrane-associated functions, which may be considered new.
[d] As mentioned in the text, two of the three *phoBR* mutants showed increased sensitivity to aminoglycoside.

are attributable to the role of RssB in proteolysis; however, this cannot be ascertained.

RstB/RstA. No role has yet been reported for the RstB/RstA system. The *rstAB* mutants were hypersensitive to ketoprofen, pridinol, and troleandomycin. The basis for these sensitivities is unknown.

LuxS. As noted above, LuxS synthesizes a furanosyl borate diester that is believed to be a universal autoinducer for cell-to-cell communication among diverse bacteria (7, 12). We examined *luxS* mutants because autoinducer-2 has been proposed to act as a signaling molecule for a two-component system(s), including the QseC/QseB system (70).

The *luxS* mutants showed hypersensitivity to two chelators, 1,10-phenanthroline and 5,7-dichloro-8-hydroxyquinaldine (Table 4). 1,10-Phenanthroline has a high affinity for copper, as well as other metals, such as iron and zinc (15). Hypersensitivity to 1,10-phenanthroline was also observed for the *cusRS* mutants, which affect copper transport (49). The *luxS* mutants were also more sensitive to several folate antagonists, including sulfamethoxazole, sulfadiazine, sulfathiazole, and sulfanilamide, and were more resistant to oxycarboxin and caffeine. In addition, the *luxS* mutants showed defects in the use of D-valine or L-homoserine as an N source, a growth defect at pH 10, and sensitivities to L-alanine, L-histidine, and L-lysine at basic pH values (which are indicators of a deaminase defect[s]). Further studies are required to unravel the basis of these phenotypes.

RpoS. RpoS is the stationary-phase sigma factor (also called σ^{38}). We examined *rpoS* mutants because the RR RssB is involved in its turnover (25). The *rpoS* mutants were pleiotropic. They showed increased use of L-threonine and β-methyl-D-glucuronic acid as C sources, increased use of L-threonine, guanosine, L-tyrosine, and dipeptides such as Tyr-Tyr, Tyr-Phe, and Ile-Tyr as N sources, and increased use of methylene diphosphonic acid as a P source. The *rpoS* mutants also exhibited defects in the use of α-hydroxybutyric acid, glycyl-L-aspartic acid, and α-ketobutyrate as C sources and sensitivity to hygromycin B (Table 4). None of these phenotypic differences was exhibited by the *rssB* mutants. Both *rpoS* and *rssB* mutations affected L-threonine metabolism, but they did so in opposite ways. Two *rssB* mutants were defective in the use of L-threonine as an N source, while the *rpoS* mutants showed improved growth.

DISCUSSION

We surveyed mutants with mutations in all two-component systems and several related genes in *E. coli* by determining the phenotypes of a large set of well-defined deletion mutants that are otherwise isogenic. We found phenotypic changes for 22 different two-component-system mutants and not for 15 other systems (Tables 3 and 4). Several two-component system mutants (*arcA*, *arcB*, *cpxRA*, *ompR-envZ*, *ntrBC*, *rssB*) were highly pleiotropic. We discovered new phenotypes or functions for 14 systems, including the AtoS/AtoC, BaeS/BaeR, BarA/UvrY, CpxA/CpxR, CusS/CusR, EnvZ/OmpR, KdpD/KdpE, NarP, NtrB/NtrC, PhoQ/PhoP, QseC/QseB, RcsC/YojN/RcsB, RssB, and RstB/RstA systems (Table 5). We also identified phenotypic changes for 8 of 10 related non-two-component mutants surveyed (Table 4).

This study was enabled by two technologies. One was a method for one-step inactivation of chromosomal genes in *E. coli* with PCR products (10), which permits easy deletion or modification of a target gene(s). The other was the PM technology (5), which permits examination of cellular phenotypes in a high-throughput format.

Altogether, we constructed over 100 deletion mutants, including two or more independent mutants with mutations in each gene or gene cluster. The mutations included 65 different deletions (50 distinguishable two-component mutations and 15 different other function mutations) (Tables 1 and 2). Many of these mutations were obtained by using new special template plasmids for creation of multiple mutations in the same strain. Details on the methodology, construction, and verification of these mutants will be described elsewhere (Zhou et al., unpublished).

The PM technology used in this study is an extension of previous work (5). Previously, assays were developed for measuring about 700 different phenotypes in seven 96-well microplates. This system was validated by using a small collection of *E. coli* mutants having lesions in genes whose functions are known. While most results were confirmatory, surprises were also encountered. For example, *xylA* and *ynjB* mutants, which had been thought to have single mutations, were shown to carry secondary lesions, including a linked lesion (5). Accordingly, phenotypic effects can be uncovered by detailed comparisons of mutant and parental strains by using standardized PM assays. High-throughput assays based on 300 growth phenotypes have also been developed for *Saccharomyces cerevisiae* (65–67).

PMs have now been expanded to include nearly 2,000 assays in a set of 20 96-well microplates. One-half of these assays measure basic cellular metabolism and stress functions. The others measure susceptibility to about 240 inhibitors at four different concentrations. Because the PM technology is new, it was important to evaluate and validate the data. We did this in three ways: (i) we carefully compared mutants with mutations in several systems for which many phenotypic changes were predictable; (ii) we conducted additional phenotypic tests to confirm numerous changes; and (iii) we always examined two or more independent mutants and looked for phenotypic changes that they had in common in order to reduce the chance of finding effects of secondary mutations.

We detected most, if not all, of the expected phenotypes for mutants with mutations in five systems (ArcB/ArcA, DcuS/DcuR, NtrB/NtrC, PhoR/PhoB, and UhpB/UhpA). We also found many expected phenotypes, as well as new phenotypes for several systems and, in particular, for the *rssB* and *rpoS* mutants (Table 5). Many mutants had phenotypes that are also reasonable based on current knowledge. For example, we found that the *baeSR* mutants exhibited antibiotic and toxic metabolite susceptibilities. This finding supports the recent discovery that the BaeS/BaeR system controls synthesis of an efflux pump (2, 51). We found that the *cusRS* mutants are hypersensitive to 1,10-phenanthroline, a high-affinity copper chelator (69). This provides further evidence that this system plays an important role in copper homeostasis (49). These results therefore provide confidence in the validity of the PM assays.

In a few cases we were unable to confirm phenotypic changes by additional tests. Since it was not feasible to conduct additional tests for all mutants, we are not certain about the cause of such discrepancies. It is notable that we were unable to confirm most changes for the *atoSC* and *uvrY* mutants by serial dilution tests (Table 4). We do not know whether this reflects variability with regard to these mutants or whether particular PM tests are less reliable. In contrast, most other confirmation tests substantiated the PM assays. Perhaps, these mutants are phenotypically less stable than other mutants. Without more detailed knowledge of the basis of these phenotypes, it is difficult to be certain about the validity of the results of particular PM assays, such as these assays.

PM analysis permits a "big picture" perspective at the biological level. Not only does it reveal phenotypes that change, but in revealing phenotypes that do not change, it eliminates possibilities and helps focus the direction of future investigations. However, it is important to also consider the limitations of the technology. There are a number of reasons why PM technology does not uncover all phenotypes and all members of a regulon. First, the phenotyping set is, of course, not all-inclusive. Certainly, it is likely to miss phenotypes involving surface structures and functions such as flagella, attachment, biofilm formation, motility, and chemotaxis, as well as functions turned on only under anaerobic conditions. In this study, we were unable to detect motility and chemotaxis phenotypes (e.g., in *cheABYZ* mutants) and anaerobic phenotypes (e.g., in *narXL*, *narP*, *narQ*, and *torRS* mutants). Second, deletion of genes requiring other special conditions for expression would also not result in a mutant phenotype. And third, deletion of genes may not reveal mutant phenotypes if they involve redundant cellular functions.

Conclusions about gene function drawn from this study rely on the validity of comparing isogenic strains. Even genetically identical strains can rapidly acquire unknown secondary mutations (32, 76), especially if the strain carries a mutation that impairs growth or creates a condition which favors a compensatory mutation. The *E. coli* K-12 genome (4) contains a large number of transposable insertion elements which are known to give rise to frequent mutations (6, 37) and which may be especially problematic for genes near their insertion sites (34). We therefore examined at least two independent mutants for each system and considered only those phenotypes that the mutants had in common. In two cases (the *phoBR* and *cbrBC* mutants) we found agreement between two independent mutants but not with a third similar mutant. The basis of these anomalies is not understood. Nevertheless, the finding that two independent mutants had phenotypes in common provides confidence that the majority of the PM assay results are correct.

We also detected common phenotypes for mutants with mutations in individual genes belonging to the same regulatory system. While the *arcA* and *arcB* mutants each displayed about 50 phenotypic changes (Table 3), the majority of these changes were in common (Table 5). The *barA* and *uvrY* mutants had a new phenotype in common, enhanced utilization of melibiose (Table 5). The change in melibiose metabolism could be a secondary manifestation of a change in sodium metabolism, since this sugar is uniquely metabolized by a sodium cotransport system (75a). We also detected a dominant phenotype

TABLE 6. Phenotypes that two-component and other mutants have in common[a]

Mode of action	Phenotype	Genes deleted
Alanine deamination	Increased sensitivity to L-alanine at pH 9.5	*cpxRA*, *ntrBC*, *luxS*
Alkaline pH sensitivity	Increased sensitivity to pH 10	*arcA*, *arcB*, *luxS*, *phnC-P*
Amino acid analog	Increased resistance to β-chloro-L-alanine	*arcA*, *arcB*, *ntrBC*
Anion transport	Increased sensitivity to sodium nitrite	*arcB*, *rssB*
Anion transport	Increased sensitivity to sodium tungstate	*arcB*, *baeSR*, *rssB*
Antimicrobial	Increased sensitivity to myricetin	*arcA*, *arcB*, *baeSR*
C source	Decreased utilization of C_4 acids	*dcuRS*, *rssB*
C source	Increased utilization of D-fructose and D-mannitol	*ompR-envZ*, *phoPQ*
C source	Increased utilization of D-melibiose	*barA*, *uvrY*
C source	Increased utilization of β-methyl-D-glucuronic acid	*arcA*, *rpoS*
Cation transport	Increased sensitivity to cobalt chloride	*arcA*, *arcB*, *ompR-envZ*, *qseCB*
Cation transport	Increased sensitivity to nickel chloride	*baeSR*, *qseCB*
Cell wall inhibitor	Increased sensitivity to cefoxitin	*phnC-P*, *phoH*
Cholinergic antagonist	Increased sensitivity to pridinol	*phoH*, *rstAB*
DNA damage, antifolate	Increased resistance to hydroxylamine	*cbrA*, *uvrY*
DNA synthesis inhibitor	Increased sensitivity to nitrofurazone	*cbrBC*, *ntrBC*, *rcsB*, *uvrY*
Folate antagonist	Increased sensitivity to trimethoprim	*rcsB*, *yojN-rcsB*
Lipophilic chelator	Increased sensitivity to 1,10-phenanthroline	*arcA*, *arcB*, *cusRS*, *luxS*
Lipophilic chelator	Increased sensitivity to 5,7-dichloro-8-hydroxyquinaldine	*cbrA*, *luxS*, *phn*
Lipophilic chelator	Increased sensitivity to 5-chloro-7-iodo-8-hydroxyquinoline	*arcB*, *narP*
Lipophilic chelator	Increased sensitivity to 8-hydroxyquinoline	*arcB*, *psiE*
Membrane agent, outer	Increased sensitivity to polymyxin B	*atoSC*, *uvrY*
Membrane perturbant	Increased sensitivity to membrane-active agents	*arcA*, *arcB*
Membrane, cationic detergent	Increased sensitivity to methyltrioctylammonium chloride	*arcA*, *arcB*, *atoSC*, *uvrY*
N source	Decreased utilization of Leu-Trp	*cpxRA*, *rssB*
N source	Decreased utilization of δ-amino-*N*-valeric acid	*ntrBC*, *rssB*
Osmotic sensitivity	Increased sensitivity to ethylene glycol	*cpxRA*, *ompR-envZ*, *yojN*
Osmotic sensitivity	Increased sensitivity to NaCl	*atoSC*, *rcsB*, *yojN*, *yojN-rcsB*
P sources	Decreased utilization of organophosphates as P sources	*phoA*, *phoBR*
Protein synthesis inhibitor	Increased resistance to troleandomycin	*phn*, *phoH*
Protein synthesis inhibitor	Increased sensitivity to amikacin	*cpxRA*, *ntrBC*
Protein synthesis inhibitor	Increased sensitivity to dihydrostreptomycin	*atoSC*, *cpxRA*, *uvrY*
Protein synthesis inhibitor	Increased sensitivity to Geneticin (G418)	*arcA*, *cpxRA*, *ntrBC*
Protein synthesis inhibitor	Increased sensitivity to hygromycin B	*cpxRA*, *kdpFABCDE*, *rpoS*
Protein synthesis inhibitor	Increased sensitivity to paromomycin	*arcA*, *ntrBC*, *phoBR*
Protein synthesis inhibitor	Increased sensitivity to tobramycin	*cpxRA*, *ntrBC*, *phoBR*
Protein synthesis inhibitor	Increased sensitivity to troleandomycin	*arcB*, *rstAB*
Respiration inhibitor	Increased sensitivity to iodonitrotetrazolium violet	*atoSC*, *rcsB*, *uvrY*
Respiration inhibitor	Increased sensitivity to thioridazine	*arcA*, *arcB*, *ntrBC*, *ompR-envZ*
S source	Decreased utilization of methane sulfonic acid	*cpxRA*, *rssB*
Transport, toxic cation	Increased sensitivity to lithium chloride	*cpxRA*, *ompR-envZ*

[a] See text.

that the *rcsB*, *yojN*, and *yojN-rcsBC* mutants had in common, sodium chloride sensitivity (Table 4).

Besides the *arcA* and *arcB* mutants, four other mutants showed more than 10 phenotypic differences (Table 5). The *cpxRA* mutants displayed 23 changes with varied features that are coherent with the many gene regulatory targets of the *cpxRA* system (14). Mutants with mutations in the EnvZ/OmpR and NtrB/NtrC systems exhibited both expected and unexpected phenotypes. Curiously, the *dcuSR* and *rssB* mutants had many phenotypes in common (Table 4). The *rssB* mutants also had a large number of defects in the use of amino acids and peptides as N sources. Whether these phenotypes are related to a role in protein turnover (24) remains to be determined.

The mutants examined in this study included most of the mutants that were studied for global effects on gene expression (54). Mutations in the ArcA, AtoS/AtoC, DpiB/DpiA (CitA/CitB), EnvZ/OmpR, RcsB, UvrY, and YpdA/YpdB systems were shown to affect flagellar synthesis. Since PM assays do not measure motility, effects on flagellar synthesis would go unnoticed. It was also found that *arcB*, *cpxRA*, *fimZ*, *ompR-envZ*, *rstAB*, and *yfhA* mutations led to up-regulation of the *ent* operon (which encodes enzymes for enterochelin biosynthesis). Such phenotypes would also probably not be detected in PM assays. The maltose transport system was up-regulated in the *arcB*, *dpiBA* (*citAB*), *rcsB*, and *uvrY* mutants. Although we did see increased use of other C sources, we found no effects on maltose use in these or other mutants.

Several studies have now shown that there are regulatory connections among two-component systems and with other global regulators (25, 72). For example, the HK CreC can activate PhoB under certain conditions both in the absence of PhoR (82) and in the presence of PhoR (J. L. Masella and B. L. Wanner, unpublished results). The HK ArcB has also been shown to be involved in porin gene regulation in a manner requiring OmpR (44). In addition, results of DNA microarray studies provided evidence of regulatory interactions that are indicative of cross-regulation or overlapping regulons between the EnvZ/OmpR and AtoS/AtoC systems and among ArcB, RssB, UvrY, and the RpoS regulon (54).

We also found evidence which supports the hypothesis that there are regulatory networks, as many mutants had phenotypes in common with each other (Table 6). Here we found osmotic sensitivity of the *ompR-envZ* and *atoSC* mutants, except that they are sensitive to different agents. The *cpxAR*, *ntrBC*, and *luxS* mutants showed susceptibility to alanine at an elevated pH, which is indicative of a deamination defect. The *arcA*, *arcB*, *luxS*, and *phnC-P* mutants showed alkaline pH sensitivity. The *arcB* and *rssB* mutants were both sensitive to sodium nitrite. Is ArcB therefore the partner HK for RssB? The *arcB*, *baeSR*, and *rssB* mutants had sodium tungstate sensitivity in common. Clearly, many potentially interesting connections are suggested by the results summarized in Table 6. An understanding of the biochemical and physiological basis of these phenotypes requires further investigation.

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