A Critical Role for the Putative NCS2 Nucleobase Permease YjcD in the Sensitivity of *Escherichia coli* to Cytotoxic and Mutagenic Purine Analogs

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ABSTRACT The base analogs 6-*N*-hydroxylaminopurine (HAP) and 2-amino-HAP (AHAP) are potent mutagens in bacteria and eukaryotic organisms. Previously, we demonstrated that a defect in the *Escherichia coli ycbX* gene, encoding a molybdenum cofactor-dependent oxidoreductase, dramatically enhances sensitivity to the toxic and mutagenic action of these agents. In the present study, we describe the discovery and properties of a novel suppressor locus, *yjcD*, that strongly reduces the HAP sensitivity of the *ycbX* strain. Suppressor effects are also observed for other purine analogs, like AHAP, 6-mercaptopurine, 6-thioguanine, and 2-aminopurine. In contrast, the *yjcD* defect did not affect the sensitivity to the pyrimidine analog 5-fluorouracil. Homology searches have predicted that *yjcD* encodes a putative permease of the NCS2 family of nucleobase transporters. We further investigated the effects of inactivation of all other members of the NCS2 family, XanQ, XanP, PurP, UacT, UraA, RutG, YgfQ, YicO, and YbbY, and of the NCS1 family nucleobase permeases CodB and YbbW. None of these other defects significantly affected sensitivity to either HAP or AHAP. The combined data strongly suggest that YjcD is the primary importer for modified purine bases. We also present data showing that this protein may, in fact, also be a principal permease involved in transport of the normal purines guanine, hypoxanthine, and/or xanthine.

IMPORTANCE Nucleotide metabolism is a critical aspect of the overall metabolism of the cell, as it is central to the core processes of RNA and DNA synthesis. At the same time, nucleotide metabolism can be subverted by analogs of the normal DNA or RNA bases, leading to highly toxic and mutagenic effects. Thus, understanding how cells process both normal and modified bases is of fundamental importance. This work describes a novel suppressor of the toxicity of certain modified purine bases in the bacterium *Escherichia coli*. This suppressor encodes a putative high-affinity nucleobase transporter that mediates the import of the modified purine bases. It is also a likely candidate for the long-sought high-affinity importer for the normal purines, like guanine and hypoxanthine.

The synthetic purine base analogs 6-*N*-hydroxylaminopurine (HAP) and 2-amino-HAP (AHAP) are powerful mutagens in bacteria, as well as in eukaryotes (1–3). Structurally, HAP is similar to adenine but carries a 6-hydroxylamino group instead of the 6-amino group, whereas AHAP is similar to guanine but contains a 6-hydroxylamino group instead of the 6-oxo group (4). It is believed that these analogs enter the cell by active transport and then get converted to (deoxy)nucleoside triphosphates and serve as precursors for enzymatic RNA and DNA synthesis, producing mutations due to their ambivalent coding capacity (3). Several studies suggest that HAP can also be generated *in vivo* either by hepatic microsomal *N*-hydroxylation of adenine (5) or from the accidental use of hydroxylamine in purine biosynthesis (6). It has also been shown that HAP is a major product of the exposure of adenine to peroxyl radicals (7). Studies in the yeast *Saccharomyces*

cerevisiae demonstrated a pivotal role of adenine phosphoribosyltransferase (Apt1) in the activation of mutagenic and cytotoxic properties of HAP, whereas the purine salvage or interconversion enzymes adenine aminohydrolase (Aah1) and (d)ITP/(d)XTP triphosphatase (Ham1) were characterized as key activities protecting yeast cells against the toxic and mutagenic action of HAP (3, 8, 9). In *Escherichia coli*, a limited role of the Ham1 homolog RdgB in HAP detoxification was reported (10). However, the major protective system against *N*-hydroxylated bases in *E. coli* involves two molybdenum-cofactor (molybdopterin)-dependent oxidoreductases, YcbX and YiiM, which detoxify the *N*-hydroxylated compounds by reduction to the corresponding amines (11).

In a series of experiments aimed at understanding the additional factors that govern the sensitivity of *E. coli* to these
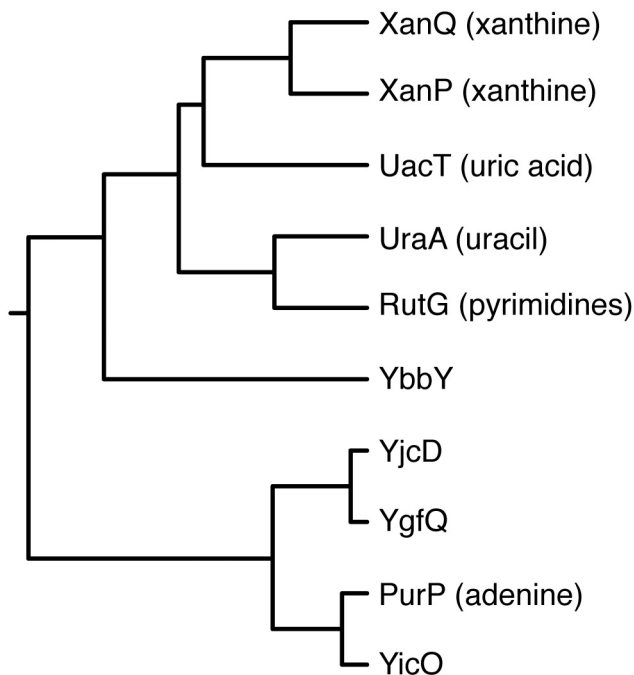
FIG 1 Phylogenetic tree of *E. coli* NCS2 family proteins based on their amino acid sequences. The dendrogram was generated using the ClustalW program, available at http://www.genome.jp/tools/clustalw/. The substrate specificities of the experimentally characterized members are represented in parentheses following the protein names.

N-hydroxylated agents, including the mechanisms of entry into the cell, we found that a transposon insertion in the *yjcD* locus strongly suppressed the HAP sensitivity of a *ycbX* mutant. YjcD encodes a hypothetical protein belonging to the nucleobase-cation symporter-2 (NCS2) family of permeases that are involved in high-affinity transport of nucleobases (see http://www.tcdb.org). As shown in Fig. 1, the *E. coli* genome contains 10 related paralogous members of the NCS2 family: the uracil permease UraA (12), the xanthine-specific transporters XanQ and XanP (13), the putative adenine permease PurP (14, 15), the uric acid transporter UacT (16), the putative uracil/thymine permease RutG (17), and four additional hypothetical transporters, YjcD, YbbY, YicO, and YgfQ. *E. coli* also contains two members of the NCS1 family of permeases, among which CodB was characterized as a cytosine-specific transporter (18) and YbbW remains a hypothetical permease possibly involved in allantoin metabolism (see http://www.tcdb.org) (19).

In the present study, we describe the properties of the *yjcD* strain with regard to its resistance to various base analogs. We also construct a set of strains carrying defined deletions of each of the members of the NCS2 and NCS1 families for an examination of any influence that these mutations may have on base-analog sensitivity. Our results suggest a pivotal role of YjcD in the uptake of HAP and related purine base analogs in *E. coli*. These results are discussed with regard to the possible physiological function of the YjcD transporter.

RESULTS AND DISCUSSION

A *yjcD* defect suppresses the cytotoxic effect of purine base analogs. A mutant containing a defect in *yjcD* was originally isolated

in a genome-wide search using random transposon insertion mutagenesis for mutations that could suppress the HAP hypersensitivity of an *ycbX* mutant defective in base analog detoxification (E. I. Stepchenkova, S. G. Kozmin, and R. M. Schaaper, unpublished data). Here, we demonstrate that a Δ*ycbX* strain carrying a defined deletion of the *yjcD* gene displays a strong reduction in sensitivity to the toxic effects of HAP or AHAP: for HAP, the zone of inhibition decreased from 39 mm to 18 mm, while for AHAP, the inhibition zone decreased from 36 mm to 0 (see Fig. 2). The Δ*yjcD* defect also suppressed the sensitivity of a wild-type strain toward the toxic action of the purine analogs 6-mercaptopurine (MP) (from a clear 50-mm zone to a 25- to 30-mm diffuse zone of inhibition) and 6-thioguanine (TG) (50 versus 0 mm) (Fig. 2). The effect of Δ*yjcD* on the sensitivity to the purine analog 2-aminopurine (AP) was tested in a *dam* strain background, which is particularly sensitive to this agent (20). The results in Fig. 2 show that the Δ*yjcD* mutation also suppressed this effect. In contrast, no effect on the cytotoxicity of 5-fluorouracil (FU) was seen for the Δ*yjcD* defect (Fig. 2). As the *yjcD* gene encodes a hypothetical protein belonging to the NCS2 family of nucleobase transporters (Fig. 1), the suppressor effect of the *yjcD* deletion is most plausibly ascribed to defective transport of the tested purine analogs into the cell.

Other members of the NCS2 and NCS1 families do not affect HAP and AHAP sensitivity. As shown in Fig. 1, *E. coli* possesses 10 members of the NCS2 protein family, including YjcD. Four of these proteins have been characterized as purine-specific transporters (XanQ, XanP, PurP, and UacT), while two are implicated in the transport of pyrimidines (UraA and RutG) (see the introduction). Four other members of NCS2 family, YjcD, YgfQ, YicO, and YbbY, are hypothetical proteins. In addition, two members of the NCS1 family of nucleobase transporters, CodB and YbbW, may also be involved in base analog uptake (see the introduction).

We investigated the role of each of these genes in sensitization to HAP and AHAP by analyzing the sensitivity of the *ycbX* strain carrying the corresponding gene deletions (see Table 1). As shown in Fig. 3, none of the deletions tested significantly affected sensitivity to HAP (36- to 39-mm inhibition zones for all mutants except Δ*yjcD*) and AHAP (36- to 38-mm inhibition zones for all mutants except Δ*yjcD*). In the Δ*ycbX* Δ*yicO* mutant, a weak background growth was noted within the HAP-induced inhibition zone (Fig. 3A). To more carefully investigate the possible operation of transporters other than YjcD, we further combined defects in all NCS2 and NCS1 family proteins with the Δ*yjcD* deletion. As shown in Fig. 4, none of the deletions tested were capable of eliminating the residual HAP sensitivity of the *yjcD* mutant; again, a weak *yicO*-associated suppression of the HAP sensitivity was observed.

While the results in Fig. 3 and 4 regarding the role of YicO may be taken to suggest that this gene has a limited involvement in HAP uptake, an alternative explanation, which we favor, is that the reduced HAP sensitivity of the Δ*yicO*::*kan* strain that we used (Table 1) is due to activation of the adjacent *yicP* (*ade*) gene, encoding an adenine deaminase (21, 22) which is capable of HAP detoxification by conversion of HAP to hypoxanthine (11). See footnote *b* of Table 1 for details. This explanation is further consistent with the lack of effect of the Δ*yicO* mutation on sensitivity to AHAP (Fig. 3B) or MP and TG (see Fig. S1 in the supplemental material). Thus, our results demonstrate a major role of, specifically, YjcD in the uptake of a series of modified purines and, fur-
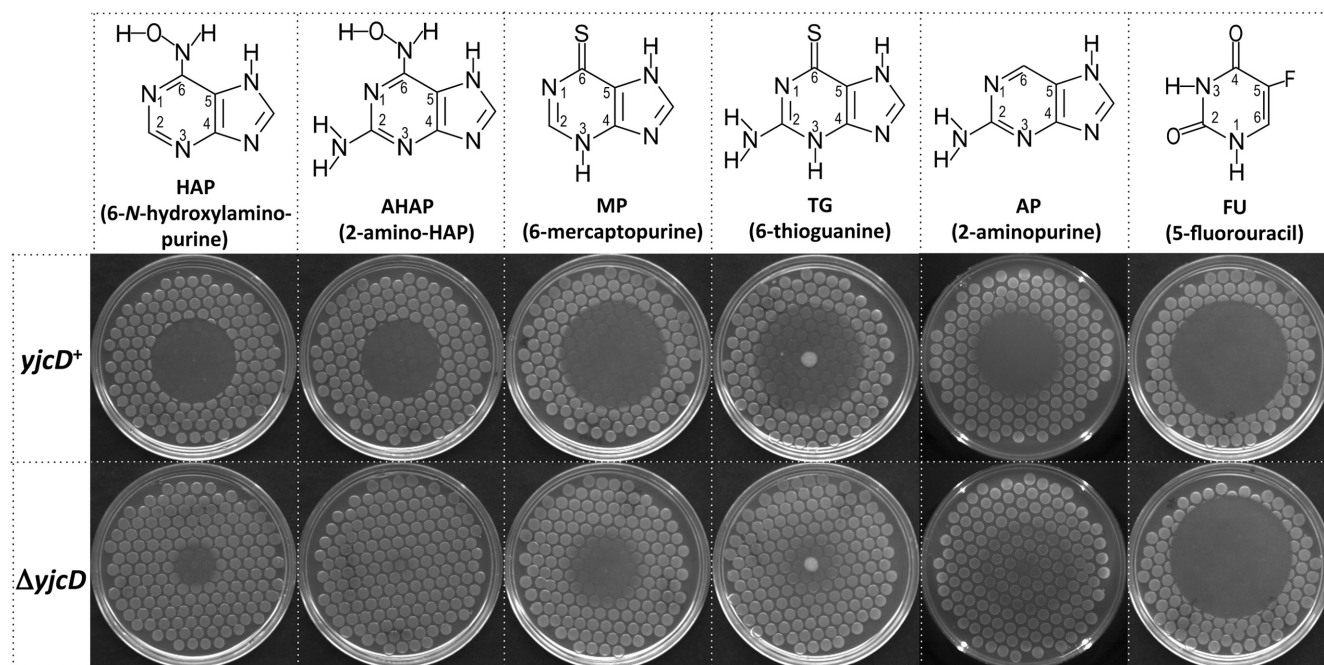
FIG 2 Sensitivities of *yjcD*⁺ and Δ*yjcD* strains to the toxic effect of various base analogs. Cell suspensions were transferred using a multiprong replicator to VB plates, and 100 µg of HAP, 50 µg of AHAP or 200 µg of MP, TG, AP, or FU (in DMSO solutions) was spotted onto the center of each plate. The plates were incubated overnight at 37°C and inspected the next day for zones of inhibition. For HAP and AHAP sensitivity tests, NR16262 (Δ*ycbX*) and its Δ*yjcD*::*tet* derivative were used. For MP, TG, and FU sensitivity tests, NR10836 (wild type [wt]) and its Δ*yjcD*::*tet* derivative were used. For the AP sensitivity test, we used strain NR15719 (*dam*) and its Δ*yjcD*::*kan* derivative.

thermore, that none of the other NCS2 and NCS1 family members play any significant role in the uptake of these analogs.

The effect of Δ*yjcD* on uptake of natural purines. In addition to playing a major role in the uptake of modified purines, YjcD might also play a role in the uptake of normal purines, like adenine, guanine, hypoxanthine, and xanthine. To test this possibility, we first used a *purC* strain, defective in *de novo* purine biosynthesis, and assayed its growth with either adenine or hypoxanthine as the purine source. The results in Fig. 5A and B show that growth on adenine is entirely unaffected by the Δ*yjcD* mutation, consistent with the existence of the high-affinity PurP adenine transporter (14, 15), but growth on hypoxanthine is significantly delayed. Second, we tested the growth of a Δ*guaB* strain, which lacks inosine monophosphate (IMP) dehydrogenase activity and, hence, requires the presence of either xanthine or guanine for growth (23). The results in Fig. 5C show strongly impaired growth of the *yjcD* derivative when guanine serves as the purine source, while a modest growth defect was seen in the presence of xanthine (Fig. 5D).

Possible physiological role of YjcD. The membership of YjcD in the NCS2 family clearly classifies the protein as a nucleobase transporter. No direct measurements of transport activities by this protein have been reported at this time, but several observations support this particular role for YjcD. Using C-terminal tagging with reporter proteins, YjcD was shown to be localized in the bacterial inner membrane (24). It was also shown that the *yjcD* promoter contains a PurR purine repressor binding motif, which is present in various genes involved in purine and pyrimidine metabolism, including several transporters (25). In agreement with the latter, PurR-dependent repression of *yjcD* expression by

exogenous adenine was reported in an independent study (26). We, likewise, observed a 4-fold down-regulation of *yjcD* gene expression in a microarray experiment upon the addition of 50 µg/ml of HAP to minimal growth medium (S. G. Kozmin and R. M. Schaaper, unpublished data). This is an important result because, in wild-type strains, HAP is readily converted to adenine by the YcbX/YiiM-mediated reduction reaction (in this manner, HAP can serve as a ready purine source for purine-requiring mutants), but this reaction and the subsequent repression of the *purR* regulon require HAP entry into the cell.

What may be the natural substrate(s) for YjcD permease? The protein is clearly important for the uptake of the tested purine analogs HAP, AHAP, MP, TG, and AP, as illustrated here. As one other example, in molybdenum-cofactor-deficient strains, HAP is already highly toxic at very low nanomolar concentrations in the growth medium, while mutagenesis by HAP, reflecting incorporation into the DNA, can be observed at concentrations as low as 0.05 nM (27). Indeed, the incorporation of HAP into both the RNA and DNA of the cell is rapid and observable within minutes (Z. Nguyen and R. M. Schaaper, unpublished data), consistent with efficient uptake of HAP from the medium. Thus, YjcD enables efficient scavenging of very low concentrations of purine analogs from the medium. It might thus be argued that YjcD represents a broad-specificity transporter for a variety of modified purines, including both adenine analogs like HAP and 6-mercaptopurine and guanine analogs like AHAP, 6-thioguanine, and 2-aminopurine.

However, our other results, as described for the experiments whose results are shown in Fig. 5, also suggest that YjcD may represent a transporter for the natural purines guanine, hypoxan-

TABLE 1 Gene deletions generated in this study

Deletion	Affected gene(s)	PCR primer pairs used to generate corresponding deletion[a]
Δ*yjcD*::*tet*	*yjcD*	5′ ttt tct tat gtc tac gcc atc agc gcg tac cgg cgg ttc act cga cgc ctA AGA GGG TCA TTA TAT TTC G 3′
Δ*yicO*::*kan*[b]	*yicO*	5′ aac ggg cag cca ttg cca ccc gtt ttc aga atc cga tga aag att aga taA CTC GAC ATC TTG GTT ACC G 3′
		5′ gcg taa tca gaa ctc atg ata tct gga aac cct cgc cag tta cta atc caG TGT AGG CTG GAG CTG CTT CG 3′
Δ*purP*::*kan*	*purP*	5′ tta tta aag aat atc cat taa tgt gca att gaa atg tga taa tta tca ctA TTC CGG GGA TCC GTC GAC C 3′
		5′ gcc ggt ttt acc acc ttc ctg acg atg gtt tac atc gtt ttt gtt aac ccG TGT AGG CTG GAG CTG CTT CG 3′
Δ(*xanQ-uacT*)::*kan*	*xanQ guaD ygfQ ygfS ygfT uacT*	5′ gca gta gga gat aaa gcc cag cgc gat acc ttc ggt aat cga gaa gct gaA TTC CGG GGA TCC GTC GAC C 3′
		5′ agc cgt tct caa ggc gtc ctg cgc ccc aaa ttc gtg gga att tat ttt acT GTG TAG GCT GGA GCT GCT TCG 3′
Δ*xanP*::*kan*	*xanP*	5′ aca gac tga ata tag cgc gca tta aaa aat tca tat tat tct cca tgc tcA TTC CGG GGA TCC GTC GAC C 3′
		5′ gtc agc taa aat gcc cgc ttt gct acc acg gga ttg ttt tcg atg tct gtG TGT AGG CTG GAG CTG CTT CG 3′
Δ*rutG*::*kan*	*rutG*	5′ gag act gcg gcg gta att atc tta ccg ccg cca gtg aat tac tgt ttt caA TTC CGG GGA TCC GTC GAC C 3′
		5′ tct gca ccg ccg gta acc ccg gcg gtt ttc tgt tta tgg ctc ctg atg aaG TGT AGG CTG GAG CTG CTT CG 3′
Δ*uraA*::*kan*	*uraA*	5′ tcc tta agg aga cag ctg atg gca atg ttc ggt ttt cct cac tgg cag ttA TTC CGG GGA TCC GTC GAC C 3′
		5′ gat gtt tga acc ggg cag caa cac tgc ccg tcg gta aca tta ttt gtc tgG TGT AGG CTG GAG CTG CTT CG 3′
Δ(*ybbW-ybbY*)::*kan*	*ybbW allB ybbY*	5′ tga gta aag cgc cta taa cac ata ata cag agg ata ata cta tga cgc gcA TTC CGG GGA TCC GTC GAC C 3′
		5′ att tta cct tat gga tgg gtt cgg ttc ata acg ttc cca att atg tga tgG TGT AGG CTG GAG CTG CTT CG 3′
Δ*codB*::*kan*	*codB*	5′ atg aca atc ttc att atc aat cct ttt tca aca aat tat tcg atg cgt tcA TTC CGG GGA TCC GTC GAC C 3′
		5′ caa aca gca aaa agg ggg aat ttc gtg tcg caa gat aac aac ttt agc caG TGT AGG CTG GAG CTG CTT CG 3′
		5′ ctg gta acc ggg cgt taa taa ttg ttt gta aag cgt tat tcg aca ctg ttA TTC CGG GGA TCC GTC GAC C 3′

[a] Lowercase letters indicate sequences corresponding to the deletion endpoints, whereas upper case letters indicate the sequences of pKD13 or Tn*10*. The orientation of antibiotic resistance markers in all deletion-insertion mutants is clockwise.
[b] The *yicO* gene is located directly adjacent to the *yicP* (*ade*) gene (http://www.ecocyc.org/). Hence, the insertion of the *kan* gene in the clockwise orientation, generating the Δ*yicO*::*kan* mutation, putatively places the *yicP* gene under the control of the P*kan* promoter, which may account for the slightly increased HAP resistance observed from the results shown in Fig. 3 (see the text). When using the Δ*yicO796*::*kan* allele from the Keio collection (33), in which the *kan* allele is inserted in the opposite orientation, the effect of the *yicO* deletion is no longer observed (see Fig. S1).

thine, and possibly, xanthine. The existence of several purine-specific nucleobase transport systems was first described many years ago (14, 28). Genetically, these studies could only identify PurP as a putative adenine-specific transporter (14, 15). A PurP-defective mutant showed diminished adenine uptake but no reductions in the uptake of hypoxanthine or guanine (14). The uptake of hypoxanthine was inhibited by guanine and vice versa, whereas adenine did not affect either uptake (28). A strong inhibitory effect of 6-thioguanine on the uptake of hypoxanthine (but not of adenine) was also reported (14). These data clearly indicate the existence of a distinct import system for guanine/hypoxanthine and related analogs. Our present data make YjcD an attractive candidate for this principal high-affinity transporter system. This possibility should be validated by further biochemical or genetic experiments, including direct transport measurements.

We note that in our growth experiments whose results are shown in Fig. 5, the inactivation of *yjcD* caused a clear growth delay but did not abolish growth in presence of guanine, hypoxanthine, or xanthine. Passive diffusion of bases through the membrane might account for this effect. Alternatively, these results may suggest the existence of YjcD-independent uptake systems for the respective bases. Based on a high level of protein sequence similarity between YjcD and YgfQ (16), the latter may be thought to be a candidate for such a system. However, our experiments with a *ygfQ* deletion did not reveal increased resistance to HAP or AHAP, either singly or in combination with Δ*yjcD* (Fig. 3 and 4; Fig. S1), or to the analogs MP or TG (Fig. S1). With regard to growth supported by xanthine, XanQ and XanP have recently been described as xanthine-specific transporters (13), and their action may account for the limited effect of the Δ*yjcD* mutation on xanthine utilization (Fig. 5D). Nevertheless, our data imply some positive role of YjcD in xanthine utilization.

It is of interest to note that the proteins implicated in the transport of adenine (PurP) or guanine and hypoxanthine (YjcD) belong to a separate cluster of orthologous groups (COG2252 at the NCBI database), represented by the lower four proteins in Fig. 1. This group is distinct from that formed by the upper six proteins (COG2233), which represent permeases involved in the transport of 2-oxopurines (xanthine and uric acid) or pyrimidines. The COG2252 proteins of *E. coli* display high sequence conservation in certain consensus sites of the TM1, TM3, TM8, TM9, TM10, and TM14 transmembrane segments, which contain amino acids that are functionally important but differ from those of the COG2233 members (16, 29). Thus, the distinct conservation pattern of
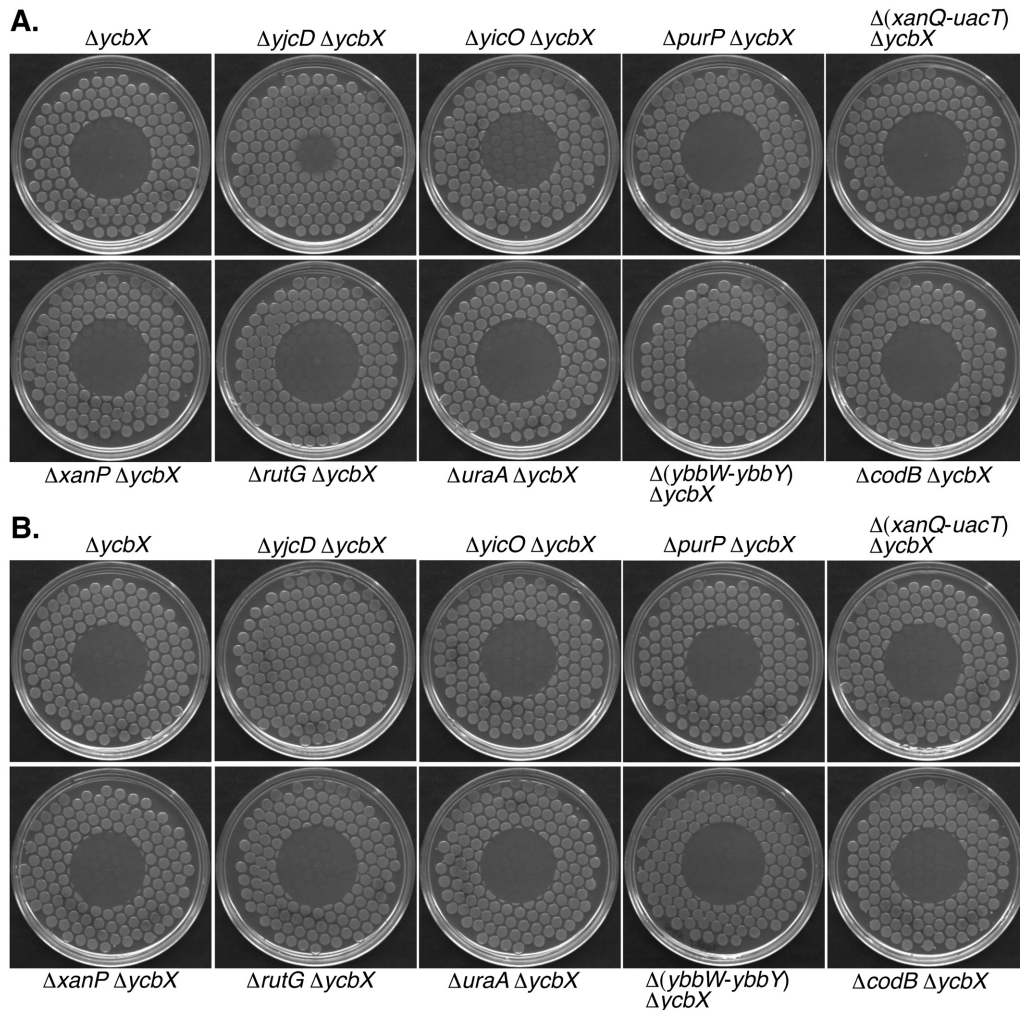
FIG 3 HAP-induced killing (A) and AHAP-induced killing (B) of *E. coli* strains lacking various NCS2 and NCS1 family proteins. The strains used were NR16262 (Δ*ycbX*) and its derivatives carrying the indicated deletions. Note that the Δ(*xanQ-uacT*) deletion also includes the *ygfQ* gene (Table 1). Spot tests were performed as described in the legend to Fig. 2 and in Materials and Methods. An amount of 100 μg of HAP or 50 μg of AHAP was applied to the center of each plate.

COG2252 genes at these sites may reflect their specificity toward 2-nonoxidized purines (adenine, guanine, and hypoxanthine).

MATERIALS AND METHODS

Media and chemicals. Bacteria were cultivated in LB broth (30) or minimal Vogel-Bonner medium (VB) (31) containing 0.2% glucose as the carbon source and supplemented with 1 μg/ml of thiamine. Solid medium contained 1.5% agar. For selection of antibiotic-resistant clones, media were supplemented with 35 μg/ml of kanamycin or 15 μg/ml of tetracycline. HAP was purchased from Midwest Research Institute (Kansas City, MO). AHAP was obtained from Ilya Kuchuk (Indiana University). 2-Aminopurine was purchased from Monomer-Polymer and Dajac Laboratories, Inc. (Trevose, United States). All other chemicals were from Sigma-Aldrich.

Bacterial strains. All gene deletions (Table 1) were initially generated in *E. coli* strain BW25113 (*lacI*q *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$) by the PCR-based gene-replacement method of Datsenko and Wanner (32), using either the Kan^r module of plasmid pKD13 (32) or the *tetA tetR* tetracycline resistance (Tet^r) module of transposon Tn*10* as a template. The resulting deletion-insertions were then transferred into *E. coli* strain NR10836 [*ara thi* Δ(*pro-lac*) F′CC106] (27) or its Δ*ycbX* derivative NR16262 (11) by P1 transduction using P1*virA*. To combine the *yjcD*

deficiency with mutations in the genes encoding other NCS2 and NCS1 family proteins, the corresponding gene deletion-insertions were further transferred into the NR16262-Δ*yjcD*::*tet* derivative by P1 transduction. To generate the *dam yjcD* double mutant, the Δ*yjcD758*::*kan* allele from Keio collection strain JW4025 (33) (National BioResource Project [NIG, Japan]: *E. coli*) was introduced by P1 transduction into strain NR15719, an NR10836 derivative carrying a *dam*::mini-Tn*10cam* insertion (our strain collection). To generate the *purC yjcD* and *guaB yjcD* double mutants, the Δ*yjcD758*::*kan* allele from Keio collection strain JW4025 was introduced by P1 transduction into strain NR15791, an NR10836 derivative carrying the *purC80*::Tn*10* transposon insertion (34), or into strain NR17097, an NR10836 derivative carrying a precise in-frame Δ*guaB* deletion (i.e., not affecting *guaA* expression) created by the method of Datsenko and Wanner (32).

Spot test for HAP sensitivity. Saturated *E. coli* cultures grown in LB were diluted 30-fold in 0.9% NaCl and transferred to VB plates using a multiprong replicator device (approximately 0.1 ml total per plate). After the spots had dried, an appropriate volume of a 10- to 40-mg/ml solution of HAP, AHAP, MP, TG, AP, or FU in dimethyl sulfoxide (DMSO) or DMSO only was spotted onto the center of the plate. The plates were incubated overnight at 37°C and inspected the next day for zones of inhibition.
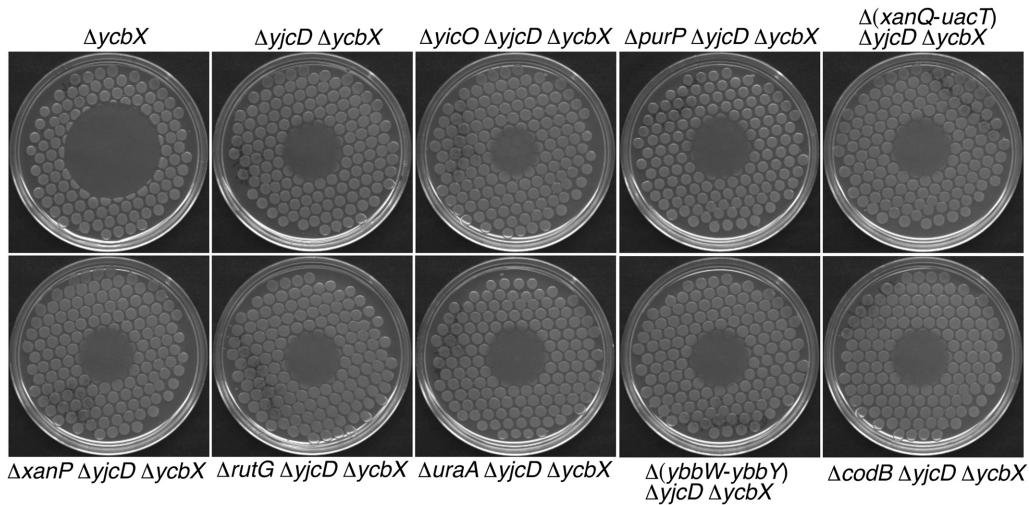
FIG 4 HAP sensitivities of *E. coli* strains carrying a *yjcD* defect combined with defects in other NCS2 and NCS1 family proteins. Note that the Δ(*xanQ-uacT*) deletion also includes the *ygfQ* gene (Table 1). The experiments were performed in the NR16262 (Δ*ycbX*) genetic background. Spot tests were performed as described in the legend to Fig. 2 and in Materials and Methods. An amount of 200 μg of HAP was applied to each plate.

Growth kinetics of *E. coli* cultures in presence of purine bases. Derivatives of strain NR10836 containing the *purC*, *purC yjcD*, *guaB*, or *guaB yjcD* defects (see above) were grown in liquid LB medium at 37°C to saturation. For each culture, 100-μl aliquots were collected by centrifu-gation, the supernatants removed, and the pellets resuspended in 1 ml of 0.9% NaCl. Five microliters of these suspensions were inoculated into 250 μl of VB medium supplemented with one of the purine bases tested (adenine, hypoxanthine, guanine, or xanthine) at a 0.1 mM concentration
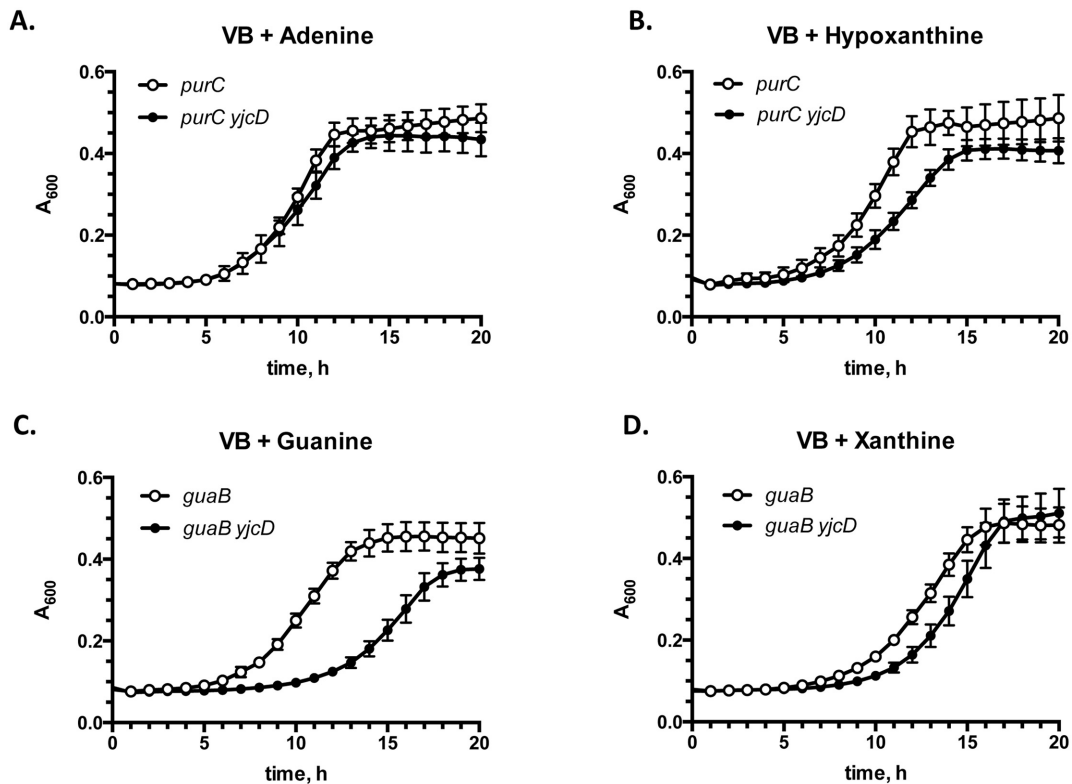

FIG 5 Effect of the *yjcD* defect on growth of the *purC* or *guaB* purine auxotrophs in the presence of various purine sources. (A and B) Growth of a *purC* purine auxotroph in the presence of adenine (A) or hypoxanthine (B). (C and D) Growth of a *guaB* purine auxotroph in the presence of guanine (C) or xanthine (D). For each time point, the average A_{600} value obtained for 12 independent cultures is presented, with the standard deviation indicated by the error bar. The background A_{600} absorbance, equal to 0.07, was not subtracted from the measurements. VB indicates the Vogel-Bonner minimal medium used in the experiment along with the noted purine supplement. See Materials and Methods for more details.

in a 96-well microplate. Growth curves were recorded at 37°C without shaking, with A_{600} readings taken every 1 h using a Tecan microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

SUPPLEMENTAL MATERIAL

ACKNOWLEDGMENTS

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