The major facilitator superfamily (MFS) is the main class of proteins that utilize protons as symporters or antiporters to transport a variety of molecules across the cytoplasmic membrane of bacteria and eukaryotes. Some MFS members, such as TetA, responsible for resistance to tetracycline, have a narrow substrate specificity. Others, such as EmrB or Bmr, can exclude from the cells a wider range of organic molecules (10, 13). The complete sequencing of bacterial genomes has revealed a large number of genes belonging to the MFS. In *Escherichia coli*, 64 such genes can be identified on the basis of sequence similarities (14), and the exact number may be somewhat higher (13). A majority of these genes were identified in the course of sequence annotation, and their transport capabilities have not been established yet. Here we report that an MFS transporter encoded by a gene in the terminus region of the *Escherichia coli* chromosome, ydeA, excludes l-arabinose (l-Ara) and isopropyl-β-D-thiogalactopyranoside (IPTG).

The bacterial strains used in this study are listed in Table 1. The ydeA gene codes for an essential GTPase (1). We have constructed a strain, JS1855 (Table 1), which expresses ydeA from the l-Ara-regulated PBAD promoter on a low-copy-number plasmid. Since JS1855 is deleted for the ara-leu region, ydeA expression can be regulated by adjusting the extracellular l-Ara concentration. Strain JS1855 grew normally with 1 mg of l-Ara/ml or more but very slowly with 20 μg of l-Ara/ml. DNA from appropriate clones was digested with restriction enzymes and ligated, and subjected to reverse PCR by using primers MTL (GATAAATCCAAATCCAGCCATCCC) and MTR (GATAAATAATTCCCAATTTGGTCAACC). Then, the chromosomal disruption was achieved by infection with phage λI52. The disruption might be explained by a higher intracellular l-Ara content resulting from ydeA disruption. To test this possibility, we determined the effect of the mutation on the sensitivity of PBAD to added l-Ara. The ydeA::mini-tet no. 4 mutation was transduced into the Δlac strain JS219, yielding JS1910. Then JS219 and JS1910 were transformed with pDAG92, a pBAD18 (7) derivative carrying lacZ under PBAD control. Steady-state levels of β-galactosidase were measured in cultures grown in Luria broth containing high or low levels of l-Ara. In the presence of 2 mg of l-Ara/ml, β-galactosidase specific activity (in units/optical density at 600 nm [OD600]) was essentially the same for both strains (21,000 and 23,000 U of JS219 and JS1910, respectively). In sharp contrast, the disruption strain expressed PBAD-lacZ at a much higher rate (15,000 U) than the parent strain (2,400 U) in the presence of 5 μg (33 μM) of l-Ara/ml. This suggested that the disruption strain retained more intracellular arabinose than the wild-type parent.

To establish directly that YdeA expels l-arabinose from the cells, accumulation and efflux of l-Ara were determined in strains JS219 (ydeA1) and JS1910 (ydeA::mini-tet) (Fig. 2). The concentration of l-Ara (5 μM) used in these experiments was such that only the ATP-dependent high-affinity AraFGH system could contribute significantly to l-Ara uptake (9). As expected, accumulation of l-Ara was higher in the YdeA mutant than in the wild-type parent. Since the strains used lacked araC and are therefore not l-Ara inducible, an indirect interference of YdeA with l-Ara uptake capabilities can be ruled out. In addition, the initial rate of efflux, 14.3%/min at 25°C, was higher in the wild type than in the mutant (6.2%/min) (Fig. 2). The efflux data strongly favor the hypothesis that YdeA promotes l-Ara export.

We have previously reported the inhibitory effect on cell division of a Φ(malE-miniE) fusion gene overexpressed from the PBAD promoter (15) and the isolation of multicopy suppressors of this effect (15, 16). Two suppressor plasmids, pMesBA5 and pMesBA24, were localized with respect to the Kohara library of ordered phage recombinants (8), as described for pMesJE11 (16), and aligned to Kohara’s map by

---

* Corresponding author. Mailing address: Laboratoire de Microbiologie et de Génétique Moléculaire du CNRS, 31062 Toulouse, France. Phone: (33)-561-335-961. Fax: (33)-061-335-886. E-mail: gbpouche@ibcg.biotoul.fr.

† Present address: Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS 66160-7420.
A BLAST search with YdeA as the query sequence indicated of yicM, a subfamily including the products of Slr0616 gene products. Lolkema and Slotboom (11) proposed pMESBA24 insert is 7,460 bp.

Bacillus subtilis ywfA, Synechocystis, and E. coli proteins. Paulsen et al. (13) defined it as subgroup d, which in among the 12-transmembrane segment (12-TMS) efflux proteins. Only a few proteins of this group have a known function. arad, is positively regulated by araC and arabinose. Reeder and Schlief proposed that AraJ might be a transporter for arabinose-containing oligosaccharides (17). The resemblance of the ydeA gene product to AraJ suggested that the actual substrates for YdeA might be arabinose-containing antibiotics. To examine this possibility, strains JS1910, JS219, and JS1921 were transformed with plasmid pUT-DCK, a pBR322 derivative containing the gene for mid pUT-DCK, a pBR322 derivative containing the gene for.

Restriction analysis (Fig. 1). A PstI deletion derivative of pMESBA5, pJPB274 (Fig. 1), still suppressed the filamentation phenotype of overexpressed Φ(malE-minE). The only complete gene contained in the plasmid insert was ydeA. YdeA is located distally in an operon also containing yneJ (a member of the lysR family) and yneK, of unknown function. In the pMESBA plasmids, transcription of ydeA may occur from the S' end of the operon and from a weak promoter in the yneK-ydeA intergenic region (3). In pJPB274, transcription can take place from the ydeA promoter and from resistance gene aadA in the vector.

The results indicating that ydeA excludes L-Ara prompted us to determine whether the suppression of lac-dependent filamentation by Φ(malE-minE) could be due to inducer exclusion. To test this possibility, strain JS219 was transformed with pJPB274, resulting in JS1921. Then, strains JS219 and JS1921, which express ydeA at normal and elevated levels, respectively, as well as JS1910 (ydeA::mini-tet) were transformed with pMBL1115, a pBR322 derivative carrying lacP4, the lac regulatory region, and lacZ (5). The strains were compared for lacZ induction in the presence of different extracellular concentrations of IPTG (Table 2). The presence of the ydeA-overexpressing plasmid led to a significant reduction in lac activity at low or intermediate concentrations of inducer. These data suggest that ydeA is capable of also excluding IPTG and that this accounts for the MalE-MinE suppression phenotype. However, this activity seems to be weak compared to L-Ara exclusion, since no IPTG exclusion effect was observed with the wild-type strain compared to the ydeA deletion strain (Table 2).

Comparisons of sequences (13) and of hydropathy profiles (11) have revealed that YdeA belongs to a defined subfamily among the 12-transmembrane segment (12-TMS) efflux proteins. Paulsen et al. (13) defined it as subgroup d, which includes S. lividans cml, Pseudomonas aeruginosa cmlB and opdE, Bacillus subtilis ywfA, and Synechocystis sp. open reading frame Sllr0616 gene products. Lolkema and Slotboom (11) proposed a subfamily including the products of arad, ydhP, ydeA, and yicM of E. coli and of ybcL, ydhL, ybdD, and yfbI of B. subtilis. A BLAST search with YdeA as the query sequence indicated that except for Slr0616, all these proteins have Expect values (number of expected matches by chance) of <10−20.

Only a few proteins of this group have a known function. cm1 and cm1A confer resistance to chloramphenicol. We compared the wild-type, deletion, and YdeA-overproducing strains for their resistance to chloramphenicol. No difference was observed. Another gene of the subfamily, arad, is positively regulated by araC and arabinose. Reeder and Schlief proposed that AraJ might be a transporter for arabinose-containing oligosaccharides (17). The resemblance of the ydeA gene product to AraJ suggested that the actual substrates for YdeA might be arabinose-containing antibiotics. To examine this possibility, strains JS1910, JS219, and JS1921 were transformed with plasmid pUT-DCK, a pBR322 derivative containing the gene for

![FIG. 1. Map of the chromosomal region carried by suppressing plasmids and location of mini-tet insertions. Gene b numbers (1a) are indicated below four-letter designations (18). Relevant putative promoters are shown above intergenic spaces. Mini-tet insertions in ydeA are indicated by vertical arrows. The size of the pMESBA24 insert is 7,460 bp.](http://jb.asm.org/)

![FIG. 2. Arabinose efflux in ydeAΔ− and ydeAΔ::mini-tet strains. Strains JS219 (ydeAΔ−) and JS1910 (ydeAΔ::mini-tet) were grown in M9 medium supplemented with 1 μg of thiamine/ml, 0.2% Casamino Acids, and 1% glycerol until the OD600 was nearly 0.6. Cultures (5 ml) were transferred to 25°C, and 3H-labeled L-Ara was added for 15 min. The cells were filtered through a 48-mm-diameter 0.45-μm HAWP Millipore filter and washed briefly with and resuspended into the same volume of Ara-free medium. Then, 0.2-ml samples were taken at 1-min (JS219) or 2-min (JS1910) intervals, filtered through 25-mm-diameter Millipore filters, washed, and counted. Open squares, JS219; closed squares, JS1910.](http://jb.asm.org/)

### TABLE 1. Bacterial strains

<table>
<thead>
<tr>
<th>Designation</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS219</td>
<td>araD Δ(arad) Δ(lacZΔ4)Δ7 malPc : lacPc::lacI93</td>
<td>Our collection</td>
</tr>
<tr>
<td>JS1955</td>
<td>JS219 pMesBA5::cat(paraC, F_RAD-ydeA)</td>
<td>Our collection</td>
</tr>
<tr>
<td>JS1908</td>
<td>JS1855 ydeA::mini-tet no. 4</td>
<td>This work</td>
</tr>
<tr>
<td>JS1909</td>
<td>JS1855 ydeA::mini-tet no. 11</td>
<td>This work</td>
</tr>
<tr>
<td>JS1910</td>
<td>JS219 ydeA::mini-tet no. 4</td>
<td>This work</td>
</tr>
<tr>
<td>JS1921</td>
<td>JS219/pJPB274</td>
<td>This work</td>
</tr>
</tbody>
</table>

### TABLE 2. Induction of the lac promoter in different ydeA contexts

<table>
<thead>
<tr>
<th>Strain and ydeA status</th>
<th>LacZ activity (U/OD600) with indicated IPTG concn (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>JS1910/pMBL1115 ydeAΔ::mini-tet</td>
<td>30</td>
</tr>
<tr>
<td>JS219/pMBL1115 ydeAΔ</td>
<td>37</td>
</tr>
<tr>
<td>JS219/pJPB274/pMBL1115 ydeAΔ::p(ydeAΔ−)</td>
<td>8</td>
</tr>
</tbody>
</table>

* Values are the averages of two independent experiments.
human deoxycytidine kinase constitutively expressed from a strong synthetic promoter. This allowed phosphorylation of cytidine derivatives and made the cells sensitive to cytosine arabinoside (cytarabin, Ara-C) (4). Then the three derived strains were compared for susceptibility to Ara-C in Luria broth. In each case, the transition between normal cell morphology and a mixture of normal cells and filaments appeared between 10 and 20 $\mu$M Ara-C. Therefore, ydeA does not appear to confer resistance to arabinosyl nucleoside compounds.

Recently, Bohn and Bouloc reported that another MFS gene, cmlA/mdfA, was also capable of expelling IPTG from the cells (2). cmlA was first shown to confer resistance to chloramphenicol in E. coli and then to a variety of drugs, such as ethidium bromide, tetraphenylphosphonium, rhodamine, daunomycin, and puromycin (6). These findings suggest that the substrates most efficiently transported by YdeA may have only a remote relationship with the two compounds identified in this study.

We thank David Lane (Toulouse) for a gift of pDAG92 and Jean-Paul Reynes (St. Cayla, Toulouse) for providing plasmid pUT-DCK.

ADDITIONAL

After this article was submitted for publication, Bost et al. (3) reported that YdeA interferes with the accumulation of L-Ara and thus with the induction of the PBAD promoter.

REFERENCES