The *Bacillus subtilis* AraE Protein Displays a Broad Substrate Specificity for Several Different Sugars

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***Bacillus subtilis* 168 is unable to grow on xylose and galactose as sole carbon sources, owing to the lack of specific transporters. We show that they are imported into the cell by the activity of AraE, an arabinose transporter whose synthesis is induced by L-arabinose.**

The soil bacterium *Bacillus subtilis* is able to use a wide range of carbon sources, including even unusual sugar derivatives like the β-arylglucosides salicin and arbutin (5, 6). On the other hand D-xylose and D-galactose, two sugars frequently found in nature, are unable to serve as sole carbon sources (7, 13). This is very surprising, because *B. subtilis* synthesizes all proteins necessary to degrade both sugars (3, 4). This unusual feature is due to the fact that *B. subtilis* is unable to import these two sugars (4, 12).

We were interested in the toxic effects of galactose on *galE*-negative *B. subtilis* strains (4). In the course of this work, we coincidentally obtained spontaneous mutants of *B. subtilis* 168 which were able to grow on D-galactose as their sole carbon source. The characterization of these mutants revealed that a transporter for arabinose, AraE, functions as a transporter for at least three different sugars. *B. subtilis* is able to grow on both D-galactose and D-xylose when L-arabinose, the inducer of AraE synthesis, is added to the growth medium.

***B. subtilis* Gal+ is able to import D-galactose.** By chance, we isolated mutants capable of growth on D-galactose as the sole carbon source. One of these mutants (strain Gal+ [the strains and plasmids used in this study are listed in Table 1]) was further characterized. Transport of D-galactose into the cell was determined as described previously (12). The results of this experiment are depicted in Fig. 1. No D-galactose accumulation was detected for the *B. subtilis* wild-type strain. In contrast, significant D-galactose uptake was observed for the D-galactose-positive mutant. This transport activity was not dependent on the presence of D-galactose in the growth medium. Import of D-galactose was abolished when cells were grown in the presence of glucose. We further characterized the strain by using the API50CH kit with API 50 CHB medium (bio-Mérieux, Marcy l'Etoile, France) (data not shown). As expected, *B. subtilis* Gal+ was able to use D-galactose; surprisingly, it was also able to use D-xylose. Utilization of L-arabinose was slightly improved. No effect was detected for any of the other sugars tested. We then tested our strain for growth on D-xylose as its sole carbon source. In contrast to the wild type, the strain grew on minimal medium with D-xylose as a sole carbon source (data not shown). *B. subtilis* 168 encodes all genes necessary for xylose degradation (3). These genes are tightly regulated. High-level transcription is induced in the presence of xylose. However, utilization of xylose is not possible, because xylose is not transported into the cell. Spontane-

ous mutants capable of xylose importation are easily obtained (12). We concluded that a single mutation is sufficient to convert the *B. subtilis* wild-type strain into a derivative which is able to import both D-galactose and D-xylose.

Inactivation of the *araE* gene renders Gal+ strains D-galactose negative. *B. subtilis* strains without the functional *galE* gene are unable to grow in the presence of high concentrations of D-galactose (4). We therefore inactivated the *galE* gene of *B. subtilis* Gal+ by transforming *B. subtilis* Gal+ with chromosomal DNA from *B. subtilis* EP2 (4) and selecting for neomycin-resistant clones. This mutant is not capable of growth in the presence of even low concentrations of D-galactose (data not shown). We transformed this strain with plasmid pIC333 (1). This plasmid was constructed to allow easy and random transposon mutagenesis of *B. subtilis*. Mutagenesis was done as described previously (1). Integrants were obtained and grown on NB medium containing spectinomycin and 1 mM D-galactose. While a *galE*-negative wild-type strain was able to grow on such plates, the *galE*-negative Gal+ derivative of *B. subtilis* was not (data not shown). Several hundred integrants resistant to spectinomycin and D-galactose were obtained. We screened for candidates which were resistant to 1 mM D-galactose but which lysed in the presence of 10 mM D-galactose. Chromosomal DNAs from 10 clones were isolated and used to transform *B. subtilis* Gal+. Spectinomycin-resistant clones were isolated. Eight strains were unable to grow with D-galactose as the sole carbon source. Chromosomal DNA from four of these candidates was digested with *Hind*III, an enzyme which is unable to hydrolyze the transposon DNA itself, and religated. The religated DNA was transformed into *Escherichia coli*. One spectinomycin-resistant clone was isolated for each independent *B. subtilis* strain. The resulting plasmids were named p14, p17, p35, and p75. The plasmid DNA was sequenced with primers 333L (5′-CCCACTTATAAACAAAAGATCGG-3′) and 333R (5′-GGCCGATTCATTAATGCAGGGGG-3′). Both primers anneal to internal sequences of the mini-Tn*10* transposon. The sequences were compared with the sequence of the SubtiList database (8). All transposons were integrated into the *B. subtilis araE* gene (p14, position 3483.424; p17, 3484.517; p35, 3484.349; p75, 3483.225 [nucleotide numbers are as given in reference 8]). The encoded protein is an arabinose transporter of *B. subtilis*. This indicates that AraE is directly or indirectly necessary for transport of D-galactose and D-xylose into *B. subtilis* Gal+. AraE is necessary for arabinose transport at low arabinose concentrations (11). At high arabinose concentrations, the AraNPQ transporter is sufficient to allow growth with arabinose as the sole carbon source. Our working hypothesis was that AraE is a transporter for several sugar molecules. L-arabinose, D-galactose, and D-xylose are of

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype or relevant markers	Source or reference[a]
E. coli DH5α	*hsdR17 endA1 recA1 gyrA96 thi relA1 supE44* φ80d*lacZ*ΔM15 Δ(*lacZ-argF*)*U169*	BRL
B. subtilis strains		
168	*trpC2*	BGSC
Gal+	*trpC2*; Gal+ Xyl+ mutant	This work
EP2	*trpC2 galE::aphA3*	4
Gal+ EP2	*trpC2 galE::aphA3*	This work
ΩaraR	*trpC2 araR::pΩaraR*	This work
Plasmids		
pSGMU2	*bla cat*	2
p14, p17, p35, p75	*spec*	This work
pIC333	*bla* Tn*10spec'*	1
pΩaraR	*bla cat ' araR '*	This work

[a] BRL, Bethesda Research Laboratories; BGSC, *Bacillus* Genetic Stock Center.

limited similarity. The simplest explanation for our results is that all three sugars are imported into the cell by the activity of AraE.

The D-galactose-positive phenotype is linked to *araR*. If AraE is able to transport D-galactose and D-xylose into *B. subtilis*, a galactose- and xylose-positive phenotype should be obtained when *araE*, whose transcription is under the control of AraR (11), is expressed. AraE synthesis is repressed in the presence of glucose. This is in accordance with the fact that *B. subtilis* Gal+ grown in the presence of glucose is unable to transport D-galactose (Fig. 1). L-Arabinose-independent expression of *araE* is achieved by inactivating either *araR* or the AraR-binding sequence in front of *araE* (10). The regulatory gene *araR* is located next to *araE* (10). Due to the fact that there are no *Hin*dIII sites within *araR*, the entire gene is en-
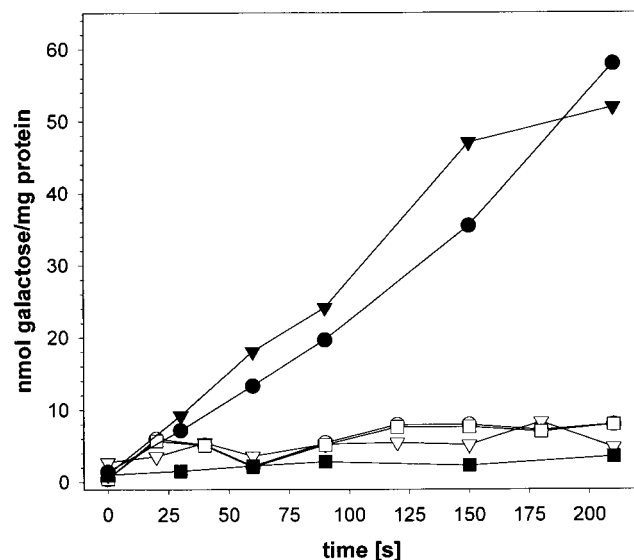

FIG. 1. Galactose uptake of *B. subtilis* 168 (open symbols) and *B. subtilis* Gal+ (filled symbols). Cells were grown either in NB (circles), in NB with 0.2% galactose (triangles), or in NB with 0.2% glucose (squares). Uptake of ¹⁴C-labelled sugar was determined as described previously (12).
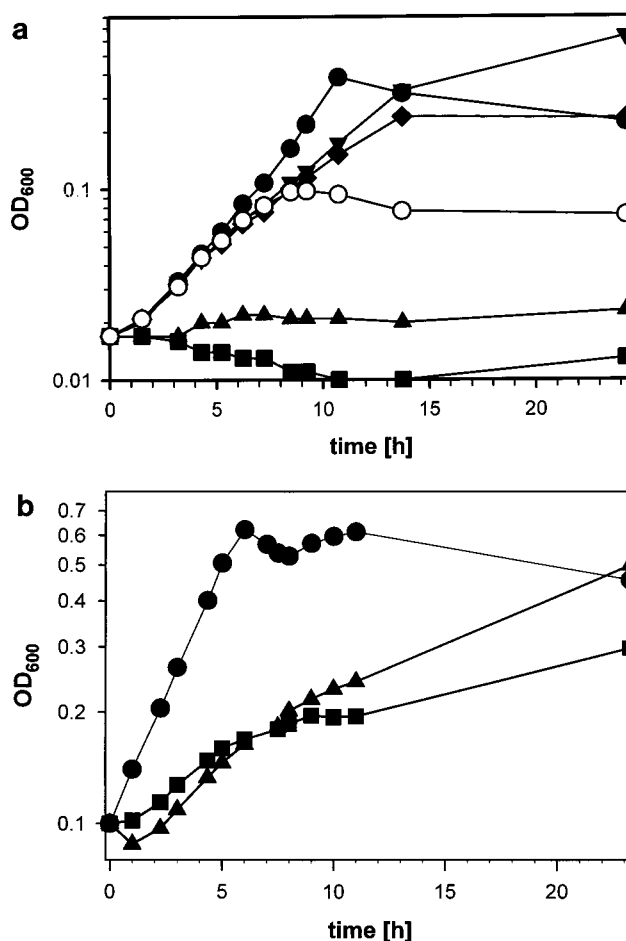

FIG. 2. Growth of *B. subtilis* 168 on different carbon sources. The optical density at 600 nm (OD₆₀₀) is plotted against time. Circles, growth on 5 mM (closed symbols) or 1 mM (open symbols) arabinose; upward-pointing triangles, growth on 5 mM xylose; squares, growth on 5 mM galactose; downward-pointing triangles, growth on 5 mM xylose plus 1 mM arabinose; diamonds, growth on 5 mM galactose plus 1 mM arabinose. Cells were pregrown in MOPSO medium (9) with succinate (a) or arabinose (b) as the carbon source, washed, and resuspended in medium with the same carbon source.

coded on parts of the plasmids which were isolated by religating chromosomal DNA from the *araE* transposon insertions. We linearized plasmid p17 with *Hin*dIII, transformed it into *B. subtilis* 168, and selected for growth on D-xylose as the sole carbon source. Clones which were able to grow on D-xylose as the sole carbon source were obtained. This proves that the mutation which enables *B. subtilis* 168 to grow on D-xylose is closely linked to *araE*. To test whether an inactivation of *araR* is sufficient to obtain a galactose- and xylose-positive *B. subtilis* strain, we constructed plasmid pΩaraR by inserting the internal *Dra*I/*Taq*I fragment of *araR* (positions 1173 to 1493 in reference 10) into *Acc*I/*Sma*I-digested plasmid pSGMU2 (2). The resulting plasmid was transformed into *B. subtilis* 168. All tested chloramphenicol-resistant clones were able to grow on D-xylose as the sole carbon source. This proves that an inactivation of *araR* is sufficient to render *B. subtilis* 168 galactose and xylose positive.

B. subtilis 168 is able to utilize D-galactose and D-xylose in the presence of L-arabinose. *B. subtilis* is able to use D-galactose and D-xylose as carbon sources when AraE is synthesized. D-Xylose, D-galactose, and L-arabinose are part of the cell wall

of plants. We therefore hypothesized that, in nature, *B. subtilis* rarely encounters a situation in which D-galactose or D-xylose is the single carbon source. The likelihood is far greater that a mixture of several sugars is utilized as a carbon source. We therefore grew *B. subtilis* 168 either with 5 mM L-arabinose, D-galactose, or D-xylose as the sole carbon source or with 5 mM D-xylose or D-galactose in the presence of 1 mM L-arabinose (Fig. 2a). *B. subtilis* was able to grow at the expense of L-arabinose, but, as described previously (7, 13), we were unable to detect any growth with D-galactose or D-xylose as the sole carbon source. In the presence of inducing amounts of L-arabinose, *B. subtilis* 168 was able to utilize both D-galactose and D-xylose as carbon sources. Both sugars were also used as a carbon source when *B. subtilis* cells which were grown with L-arabinose as the sole carbon source were resuspended in media with either D-galactose or D-xylose as the sole carbon source (Fig. 2b). Therefore, it seems clear that *B. subtilis* is able to use both D-galactose and D-xylose as single carbon sources in nature. Possibly it streamlined its sugar uptake systems by creating a transporter with broad substrate specificity, the AraE protein. It seems that this protein is able to transport at least three structurally different sugar molecules, L-arabinose, D-galactose, and D-xylose. This is quite useful under natural conditions, because plant material is a polymeric mixture of different sugars. Natural rubber (Gummi arabicum) contains L-arabinose and D-galactose; hemicellulose, a major constituent of plant cell walls, contains mainly D-xylose and L-arabinose. Therefore, the presence of arabinose is quite often an indicator of the presence of xylose or galactose. The complex composition of natural C sources was not tested under simplified laboratory conditions. This artificial situation has given rise to the opinion that *B. subtilis* is unable to use two of the most important natural sugars.

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