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In Silico and Transcriptional Analysis of Carbohydrate Uptake Systems of *Streptomyces coelicolor* A3(2)

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***Streptomyces coelicolor* is the prototype for the investigation of antibiotic-producing and differentiating actinomycetes. As soil bacteria, streptomycetes can metabolize a wide variety of carbon sources and are hence vested with various specific permeases. Their activity and regulation substantially determine the nutritional state of the cell and, therefore, influence morphogenesis and antibiotic production. We have surveyed the genome of *S. coelicolor* A3(2) to provide a thorough description of the carbohydrate uptake systems. Among 81 ATP-binding cassette (ABC) permeases that are present in the genome, we found 45 to encode a putative solute binding protein, an essential feature for carbohydrate permease function. Similarity analysis allowed the prediction of putative ABC systems for transport of cellobiose and cellotriose, α-glucosides, lactose, maltose, maltodextrins, ribose, sugar alcohols, xylose, and β-xylosides. A novel putative bifunctional protein composed of a substrate binding and a membrane-spanning moiety is likely to account for ribose or ribonucleoside uptake. Glucose may be incorporated by a proton-driven symporter of the major facilitator superfamily while a putative sodium-dependent permease of the solute-sodium symporter family may mediate uptake of galactose and a facilitator protein of the major intrinsic protein family may internalize glycerol. Of the predicted gene clusters, reverse transcriptase PCRs showed active gene expression in 8 of 11 systems. Together with the previously surveyed permeases of the phosphotransferase system that accounts for the uptake of fructose and *N*-acetylglucosamine, the genome of *S. coelicolor* encodes at least 53 potential carbohydrate uptake systems.**

Streptomycetes represent a major fraction of the bacterial soil population (20). They contribute substantially to carbon recycling by degrading a whole variety of biopolymers that stem from dead plant and animal material (16). These organic compounds, which include xylan, chitin, and cellulose, are broken down by exoenzymes. The products are funneled into the cell by specific carbohydrate importers that usually recognize mono- and disaccharides. The recent publication of the genome of the model organism *Streptomyces coelicolor* A3(2) revealed two interesting features concerning carbon utilization (7), that is, (i) a huge number of 172 genes encoding secreted proteins, such as hydrolases, chitinases, cellulases, lipases, nucleases, and proteases, and (ii) the occurrence of 81 ATP-binding cassette (ABC) permeases that are possibly used for the uptake of sugars, oligopeptides, and nucleosides as well as for drug export (46, 56). The numerousness of exoenzymes and ABC systems is 5- to 10-fold higher than that of other bacteria, underlining the broad metabolic capacity of streptomycetes (7).

Canonical carbohydrate-specific ABC systems in gram-positive bacteria are oligoprotein assemblies: a membrane-anchored substrate-binding protein is exposed to the outside of the cell, scavenging for a specific substrate molecule (10) (Fig. 1). Two membrane-integral proteins usually form the transport channel, which typically consists of 12 transmembrane segments. The energy for uptake is delivered by an intracellular ATPase, which in streptomycetes is usually not encoded within the same operon (18, 39, 42). This has been demonstrated by the characterization of the *msiK* gene, which encodes the ATPase subunit of several ABC sugar porters.

Among the carbohydrate transport systems that have been characterized at the molecular level, ABC transporters have been shown to translocate maltose and maltodextrins in *S. coelicolor*, cellobiose, cellotriose, and trehalose in *Streptomyces reticuli*, cellobiose and xylobiose in *Streptomyces lividans*, and chitobiose and *N*-acetylglucosamine in *Streptomyces olivaceoviridis* (18, 39–43, 57, 64). In *S. coelicolor*, fructose, *N*-acetylglucosamine, and possibly two further carbohydrates are transported by the phosphotransferase system (PTS) (29, 30, 33, 54). A homologous PTS specific for *N*-acetylglucosamine was also found in *S. olivaceoviridis* (61).

In this report, we provide a compilation of carbohydrate uptake systems present in *S. coelicolor*. In addition to the few already known transport systems, we identified new potential ones by similarity searches of the genome DNA-protein data bank with well-characterized prokaryotic and eukaryotic carbohydrate permeases. To define the possible substrate specificity, each system was evaluated by the analysis of the adjacent metabolic and regulatory genes. Newly identified genes were subjected to reverse transcriptase PCR (RT-PCR) to determine whether they are expressed or not.

MATERIALS AND METHODS

Computer analyses and screening strategies. All data are based upon sequence comparisons made by using the freely accessible genome data of *S. coelicolor* A3(2) (www.sanger.ac.uk/Projects/S_coelicolor). Sequences were sampled and subjected to similarity searches (BLAST) at the websites of the Na-

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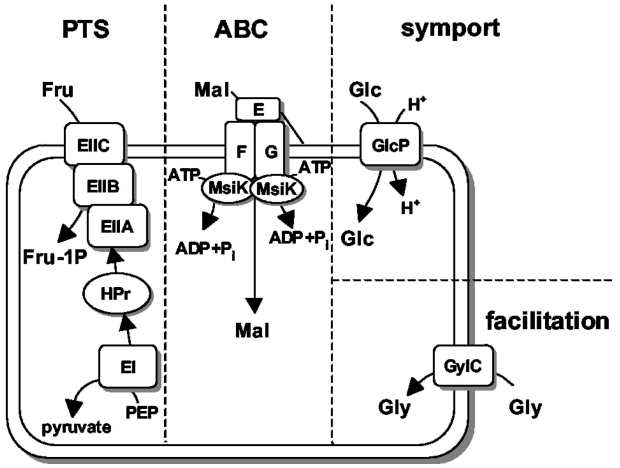

FIG. 1. Schematic overview of carbohydrate uptake systems of *S. coelicolor*. Representative sugars for the depicted transport systems are fructose (Fru) for PTS, maltose (Mal) for ABC, glucose (Glc) for symport, and glycerol (Gly) for facilitated diffusion. Abbreviations: E, MalE; F, MalF; G, MalG; EIIA, enzyme IIA; EIIB, enzyme IIB; EIIC, enzyme IIC; PEP, phosphoenolpyruvate.

tional Center for Biotechnology Information at the National Institutes of Health, Bethesda, Md., at www.ncbi.nlm.nih.gov, and of The Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom, at www.sanger.ac.uk/Projects/S_coelicolor. Default settings were used, however, without filtering low-complexity regions. Sequence alignments were conducted with CLUSTALW, applying predefined algorithms at www.ebi.ac.uk/clustalw of The European Bioinformatics Institute at The European Molecular Biology Laboratory. Protein family signature sequences were obtained from the Institute of Bioinformatics, Geneva, Switzerland, at www.expasy.org/prosite. Clone Manager 5 of Scientific & Educational Software, Durham, N.C., was applied to process and analyze DNA sequence data.

To detect ABC systems involved in carbohydrate import, regions adjacent to genes encoding paralogues of the well-characterized sugar binding protein MalE of *Escherichia coli* and MalE of *S. coelicolor* were analyzed (10, 57). In most cases, the juxtaposed genes bore high similarity to genes encoding membrane proteins of the ABC type and were thus considered a putative carbohydrate permease operon. The flanking genes were analyzed whether they encode possible metabolic enzymes, regulators, or ATP binding proteins. In the case that the gene products of such a locus correlated with the respective binding protein in terms of substrate specificity as determined by BLAST analysis, we considered the permease to be the one for the particular carbohydrate. Other types of carbohydrate transport systems, which include the major facilitator superfamily (MFS), the solute-sodium symporter (SSS) family, and the major intrinsic protein (MIP) family, were selected by similarity analysis of well-characterized proteins found in other organisms. The sequences were obtained from the recently updated transporter classification database at The University of California at San Diego (http://tcdb.ucsd.edu/tcdb) (9).

RT-PCR. Cells of *S. coelicolor* M145 A3(2) (genotype SCP1$^-$ SCP2$^-$, prototroph) were grown for 12 to 18 h under static conditions and were subsequently subjected to 24 h of vigorous shaking at 28°C. The growth medium was tryptic soy broth without dextrose (Difco) with or without 50 mM test carbon source. As an exception, cells tested for *glcP1*, *glcP2*, and *fruA* expression were grown on mineral medium with 50 mM glycerolglucose, or fructose, respectively (34).

Total RNA was prepared as described previously (30). The One-Step RT-PCR kit (Qiagen) was used in combinations with gene-specific oligonucleotides, which were as follows (forward and reverse): for *cebE1*, TTCGAGGACATGTCC AAGG and ATGAACTTGTTCCAGTCACC; for *cebE2*, AAGGAGATCACC GAGACC and TTGTCGTCGTAGTACTGC; for *bxlE1*, AAGATCGAACAG CAGATCGTCG and ACGGACGGCAGGAAGTTGTCC; for *bxlE2*, AGAAC GACGCCTACAAGACG and ACGGCATTGCGTAGATCTTGC; for *xylF*, TA CGAGAAGTTCGACAAGC and TTGTCGAACGAGGTGTAGG; for *lacG*, TTCGCCCTGGTGTTCCTCG and ATGGAGGCGAGCACCAGGTCG; for *smoE*, ATCAAGGTGAACTTCACC and AAGGTGTTCACGACCGTCG; for *rbsH*, TCCTCATCTCGTACGGGAAGCTGC and ATGGCGAGCTTCTGCC GCTTCACC; for *rbsE2*, ACACCTTCTGGGACATCGTCC and TCGAAGGT CTTCTCCACTCC; for *rbsE3*, TACTTCACCGTCGCCGACAAGG and TTG ACGTACTTGCGCATGTCG. For 16S rRNA, oligonucleotides 16SrRNA1 and 16SrRNA2 were used (30). The assay mixture contained, in a 20-μl volume, 100 ng of RNA and 5 pmol of each primer. Four microliters of the reaction mixture was taken at appropriate PCR cycles (cycles 21 to 36, depending on the appearance of signal in the linear range), and amplification products were separated and visualized on a 1% agarose gel. RT-PCR experiments without prior reverse transcription were performed to assure exclusion of DNA contamination. The quality of the RNA preparations was controlled by the presence of equal amounts of 16S rRNA, which is constitutively expressed. The functionality of oligonucleotides was checked by PCR of chromosomal DNA, giving amplification products of equal intensity for each pair. Data were verified in two independent experiments.

RESULTS AND DISCUSSION

Fifty-three carbohydrate transport systems were detected in the genome of *S. coelicolor* (Tables 1 and 2). Table 1 gives an overview of gene clusters whose functions are known from experimental investigation, for which a possible substrate could be proposed based on unambiguous similarity data, or for which homologous systems of high similarity have been studied in other organisms. These 22 systems include 14 permeases of the ABC transporter family, four PTS-specific permeases, two copies of a putative sugar porter of the MFS, one member of the SSS family, and one facilitator of the MIP family (14, 32, 33, 36, 46, 49). In the following sections, we describe these systems in detail. Table 2 lists 32 putative gene loci, which, however, were less well definable concerning the possible substrate. This table contains only genes for ABC-type porters. Thus, 87% of all found carbohydrate transporters in *S. coelicolor* appear to be ATP-driven systems.

ABC-type carbohydrate transporters. When we started our analysis, there was only one ABC-type carbohydrate permease, MalEFG, described for *S. coelicolor* that transports maltodextrins and maltose (44, 57). As a first step in the identification of further permeases, we screened the genome for the presence of such systems that were described for other *Streptomyces* species (18, 39, 41, 42, 59, 64). This led to the identification of genes for two potential cellobiose- and cellotriose-specific permeases, *ceb1* and *ceb2*, two potential β-xyloside-specific permeases, *bxl1* and *bxl2*, two putative gene clusters that could account for uptake of α-glucosides, *agl1* and *agl2*, and a gene locus containing genes for two adjacent ABC systems, one of which has been shown to translocate *N*-acetylglucosamine and chitobiose (Fig. 2 and 3).

Cellobiose and cellotriose. BLAST searches with the *ceb* genes of *S. reticuli* revealed two similar gene loci, *ceb1* and *ceb2*, within the *S. coelicolor* genome with an identical gene order, *cebREFGbglCΩshl*, and 47 to 66% identical proteins (exception, Shl1 and Shl2 with 11% identity) among each other (Fig. 2A and B) (41). Both gene clusters encode a divergently transcribed regulator, CebR of the LacI family, a sugar-binding protein, CebE, two integral membrane proteins, CebF and CebG, the β-glucosidase BglC, and a putative sugar hydrolase, Shl. The latter gene is not part of a putative operon, since it is convergently positioned. The deduced proteins share 50 to 86% and 44 to 72% amino acid identities, respectively, to an ABC transporter for cellobiose and cellotriose encoded by the well-characterized *ceb* operon of *S. reticuli*. The *S. reticuli* system has been extensively characterized. Genes *cebEFG* are

TABLE 1. List of carbohydrate transport systems of *S. coelicolor*

Family and substrate(s)	Permease gene(s)	Cosmid location(s)	Representative homologue	Source(s) or reference(s)
ABC family				
Maltodextrins	*malEFG*	SC10B7.24c to -26c	*malEFG*, *E. coli*	10, 44, 58
Cellobiose, cellotriose	*ceb1EFG*	2SCC13.03 to -05	*cebEFG*, *S. reticuli*	41, this work
	ceb2EFG	SC5F1.09 to -11		
β-Xylosides	*bxl1EFG*	SC1H10.17 to -19	*bxlEFG*, *S. lividans*	Accession no. AF043654, this work
	bxl2EFG	SCF85.18 to -20		
α-Glucosides	*agl1EFG*	SC1H10.02.c SC8F11.37c and -38c	*aglEFG*, *S. lividans*	59
α-Glucosides	*agl2EFG*	SCE50.06 to -08	*aglEFG*, *S. melilotii*	63
Xylose	*xylFGH*	SC7B7.06 to -08	*xylH*, *E. coli*	51
Chitobiose	*ngcEFG*	SC7B7.02 to -04	*ngcEFG*, *S. olivaceoviridis*	64
Lactose	*lacGFE*	SC6D11.04 to -06c	*lacEFG*, *A. radiobacter*	13, 62, this work
Sugar alcohols	*smoEFG*	SC17.16 to -18	*smoEFG*, *R. sphaeroides*	50, this work
Ribose	*rbsH*	SCC57A.16 to -19	*rbsCB*, *E. coli*	35, this work, this work
	rbsE2F2	SCAH10.22 to -24		
	rbsE3F3G	SCF43.19c to -21c		
PTS family				
Fructose	*fruA*	SCE22.13c	II^{Fru}, *E. coli*	30
N-Acetylglucosamine	*nagE2*	SCE19A.07	IIC^{Nag}, *E. coli*	29
	malX2	SCE19A.05c	IIB^{Nag}, *E. coli*	
	crr	SC1A8A.10	IIA^{Glc}, *E. coli*	
Unknown	*nagE1*	SCE19A.06	II^{Nag}, *E. coli*	33
Unknown	*malX1*	SC51A.12	II^{MalX}, *E. coli*	33
SSS family, galactose	*galP*	SCE66.18	SglT, *V. parahaemolyticus*	1, 38, this work
MFS family, glucose	*glcP1*	SC7A1.22	GlcP, *Synechocystis* sp. strain PCC6803	65, this work
	glcP2	SC9A4.15	GlcP, *Synechocystis* sp. strain PCC6803	65, this work
MIP family, glycerol	*glyC*	SC152.01	*glpF*, *E. coli*	14

induced by cellobiose, the encoded permease is a high-affinity uptake system for cellobiose and cellotriose, and since a *cebE* mutation abolishes this function, this *ceb* operon is the only system for these substrates (41). A palindromic sequence of 18 bp was found 128 bp upstream of both *cebE1* and *cebE2*. This *cis* element has been reported as the CebR binding site O_{CebR} in *S. reticuli* (40). It is perfectly conserved in system one, whereas system two shares 72% DNA sequence identity.

β-Xylosides. Two operons were found that share high similarity with the *bxl* genes of *S. lividans* (accession no. AF043654), encoding possible β-xyloside BxlEFG permeases of the ABC type (Fig. 2C and D). The metabolic enzyme β-D-xylosidase, BxlA, is encoded by the adjacent downstream gene. The gene cluster *bxl1* is almost identical to the *S. lividans* system, with 89 to 99% protein identity, whereas the *bxl2* gene cluster exhibits the same gene order but is more distantly related, with protein identity values from 34 to 71%. The putative regulators BxlR1 and BxlR2, encoded within these loci, belong, as in the case of the *ceb* operons, to the LacI family. A common palindromic element of low GC content (TTTCGAAA) was detected in the *bxlR-bxlE* intergenic regions of both systems. This sequence is situated 7 bp in front of *bxlE1*. The element occurs twice at positions bp 154 and 63 upstream of *bxlE2*. This sequence is also found in the intergenic region between *bxlR* and *bxlE* of *S. lividans*, and at the 5′ end of xylanase genes (*xln*) in various streptomycetes. A regulatory role has therefore been postulated (accession no. AF043654).

α-Glucosides. We identified two gene clusters, *agl1* and *agl2*, that encode potential ABC transporters for α-glucosides. *agl1EFG* encodes a permease that bears considerable similarity (35, 38, and 46% protein identity, respectively) to AglEFG encoded by the *aglEFGAK* operon of *Sinorhizobium meliloti* (Fig. 2E) (63). Heterologous expression of the latter genes in *Ralstonia eutrophus* promoted growth on all tested α-glucosides, which were sucrose, maltose, and trehalose. Thus, the *agl1* locus may encode a permease with a broad specificity for α-glycosidic di- and trisaccharides. No genes for a catabolic α-glucosidase or a regulator were found in the vicinity of *agl1EFG*. However, *S. coelicolor* has at least three α-glucosidases that share about 56% identical amino acids among each other. *aglA1* is associated with the *malEFG* operon (57), *aglA2* is part of the *agl2* gene cluster (see below), and the third one is encoded by SCH63.27.

A second gene cluster, *agl2REFGAX*, was found on cosmid SC8F11 (Fig. 2F). The gene products exhibit 98 to 100% identity to *aglEFGAX* of *S. lividans*, which has been proposed to encode a transporter for an α-glucoside (59). However, the authors were unable to determine the substrate, although this has been addressed thoroughly by assaying the purified AglA α-glucosidase for 24 α-glycosidic compounds (59). The *S. lividans agl* cluster is regulated by the LacI-like regulator RDR (regulator gene within the right-directed repeat of AUD1), which is located upstream of *aglE* together with the *cis* elements RIP (right imperfect palindrome) and PBS (potential binding sequence). RDR is part of the highly amplifiable

TABLE 2. Putative binding protein-dependent carbohydrate transport systems of *S. coelicolor*[a]

Cosmid location	E	F	G	C	ATPase
SC2H4.13	Mal	Mal	Mal, Mdx	β-Mannosidase	
SC3F9.04	Rib	Rib		Oxidoreductase	Ara, Rib
SC4A7.32	Unspec	Ara, Xyl		Aldose epimerase	Ara, Xyl
SC6A11.06c	Tre, Mal	Mal, Mdx	Lac, Gly3P	Phosphofructokinase	
SC7B7.06	Xyl	Xyl		Chitinase	Unspec
SC7E4.29	Mal	Unspec	Mdx	β-hexosaminidase, Glm-6P-deaminase	
SC8A6	Mal	Mal	Mal	β-Glucosidase	
SC6D10.01	Tre, Mal	Lac	Lac	Sugar hydrolase	
SC8F11.05c	Rib	Gal			Rib
SC9A4.29c	Unspec	Unspec	Unspec		
SCBAC17A6.22c	Unspec	Mdx	Unspec		
SCBAC17A6.36	Unspec	Unspec	Unspec	Hydrolase	
SCC24.05c	Unspec	Unspec	Unspec	α-L-Arabinofuranosidase	
SCD95A.19	Unspec			Tagatose-6P kinase, glucose kinase	
SCE59.05c	Unspec	Unspec	Lac	*N*-Acetylglucosaminidase, UDP-Nag transferase	
SCE65.20	Mal	Mal	Mal, Cdx	β-Galactosidase, racemase	
SCE134.06c	Tre, Mal	Unspec	Unspec	Oxidoreductase	
SCF1.15	Unspec	Unspec	Lac	α-Galactosidase	
SCF11.11	Unspec	Tre, Mal	Pal, Lac	β-*N*-acetylglucosaminidase	
SCF11.18	Unspec	Lac	Mal, Lac	α-Galactosidase	
SCF41.11	Unspec	Lac	Mal	Oxidoreductase	
SCF51A.31	Unspec	Unspec	Sbt, Mtl		
SCF91.20	Lac	Gly3P	Lac	Gly3P-dehydrogenase	
SCG22.02	Unspec	Tre, Mal, Lac	Pal, Mal	α-,β-Glucosidases	
SCG22.11c	Lac, Ara	Lac, Ceb	Ceb, Ara	β-Glucoronidase β-Galactosidase	
SC139.29	Mal, Tre	Mal, Lac	Tre, Mal	Xylanase, xylosidase	
SCJ4.46	β-Xyl	Lac	α-Glc		
SCF1.15	Unspec	Lac, Gly3P	Unspec	α-Galactosidase	
SCL2.29c	Unspec	Lac	Unspec	α-Galactosidase	
SCM10.02c	Mal				
SCM11.07	Unspec	Lac, Mdx, Gly3P	Lac, Ceb	α-Mannosidase	

[a] Abbreviations: Ara, arabinose; ATPase, ABC-type ATPase; C, catabolic enzyme; Cdx, cyclodextrin; Ceb, cellobiose; E, periplasmic sugar-binding protein; F and G, ABC-type membrane protein; Glm, glucosamine; Gly3P, glycerol-3-phosphate; α-Glc, α-glucoside; Lac, lactose; Mdx, maltodextrin; Mal, maltose; Mtl, mannitol; Nag, *N*-acetylglucosamine; Pal, palatinose; Rib, ribose; Sbt, sorbitol; Tre, Trehalose; Unspec, unspecific; Xyl, xylose; β-Xyl, β-xyloside.

11.4-kb element AUD1 that encodes two further related regulators, LDR and MDR (regulator genes within the left- and middle-directed repeat of AUD1). These regulators are involved in the massive amplification of AUD1 to more than 100 copies (59). Hence, RDR has a dual regulatory function in *S. lividans*. Since the AUD1 element also precedes the *agl2* gene cluster in *S. coelicolor*, the systems are most likely homologous and should, therefore, be subject to identical regulation.

The *ngc-xyl* locus. In *S. olivaceoviridis*, *N*-acetylglucosamine and *N*,*N*′-diacetylchitobiose are transported by the ABC permease NgcEFG (64). The system has been characterized by extensive biochemical analysis of NgcE, which binds *N*-acetylglucosamine and other chitin-degradation products. Inactivation of the gene locus demonstrated further the specificity of NgcEFG. The *S. coelicolor* gene cluster with the highest similarity was found on cosmid SC7B7 (Fig. 3). It comprises two consecutive *EFG* gene arrangements interrupted by a regulator gene of the ROK family (53). The first *EFG* gene set shares protein identities of 31 to 48% with *ngcEFG* of *S. olivaceoviridis* and was therefore designated *ngcEFG*. However, the genes do not encode a functional permease for *N*-acetylglucosamine because *S. coelicolor* exclusively uses the PTS for the uptake of this carbon source (29). Thus, *ngcEFG* may be a good candidate for a chitobiose transport system. The ROK regulator shows, with 94%, a >50% higher identity to NgcR of *S. olivaceoviridis* than the *ngcEFG*-encoded products. Thus, it can be considered a true homologue concerning the physiological role. We have tentatively designated the gene *rok7B7*, since it is not clear yet which of the adjacent operons is controlled by the encoded protein.

The second gene cluster includes genes whose products have similarity (34 to 47% protein identity) to the predicted xylose ABC permease XylFGH of *E. coli* (51). With the same gene order as found in *E. coli*, the genes encode a solute-binding protein, an ATPase, and a membrane protein. Thus, in contrast to the other ABC systems described above, this permease comprises the required ATPase gene but only one membrane-integral protein. A putative chitinase gene in the opposite orientation follows downstream. *xylFGH* may encode a xylose permease. *S. coelicolor* can readily use this carbon source, and gene homologues for the metabolic enzymes xylose isomerase, XylA, and xylulokinase, XylB, are located on the overlapping cosmids 2SCG11 and SCG11A. The putative *xylA* (2SCG11.03c) and *xylB* (SCG11A.01) genes are divergently transcribed. A possible ROK family regulator gene (SCG11A.02) is situated downstream of *xylB*, which could thus be involved in the regulation of *xylA*, *xylB*, and *xylFGH*.

ABC systems for lactose, ribose, sugar alcohols, and other carbohydrates. Further potential ABC-type porters for carbohydrates were classified under the assumption that the gene locus must encode a periplasmic binding protein (E), which is usually not found in noncarbohydrate ABC-type systems. A

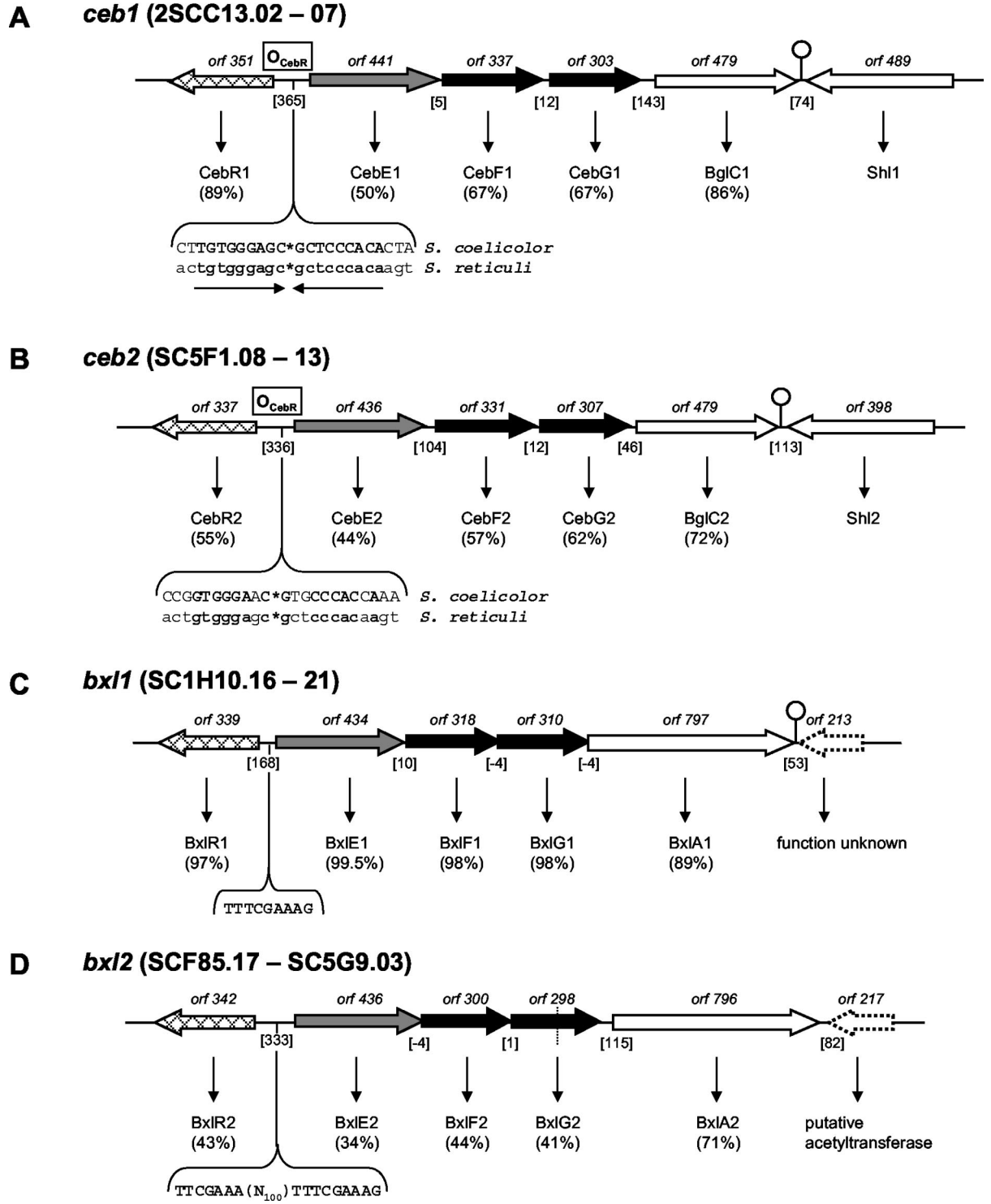

FIG. 2. (A) *ceb1* system. The depicted region includes six *orf*s, *cebE1*, *cebF1*, *cebG1*, *bglC1*, and *shl1*, comprising the ABC transport system and the first step of cellobiose utilization flanked by *cebR1* and the probable secreted sugar hydrolase-encoding *shl1*. A palindromic region of 18 bp upstream of *cebE1*, shown to be part of the *ceb* operator (O_{CebR}) in *S. reticuli*, was detected (the half-sites are separated by asterisks). ORF numbers denote the lengths of the gene products. Genes that encode membrane proteins (generally with the suffix F or G) are shown in black (membrane-spanning proteins); genes that encode substrate-binding proteins are shown in grey (with the suffix E). White arrows represent metabolic genes, probably functionally correlated to the respective system. Vertically striped arrows denote genes for ATP binding and/or hydrolyzing protein. Cross-hatched arrows indicate regulatory genes. Genes of unknown function and/or presumably not involved in the respective system are represented by dotted arrows. Putative regulatory elements are shown in uppercase type and boldface type where in consensus with comparable sequences or to emphasize direct or inverted repeats therein. Dyad repeats are drawn as a stem-loop sign. Dotted lines represent cosmid borders. The numbers of intergenic base pairs are given in brackets, the location of an ORF on the cosmid is designated by SCXX.nn, with "c" denoting complementarity. (B) *ceb2* locus. (C and D) *bxl1* and *bxl2* loci. (E and F) *agl1* and *agl2* loci. RIP, right imperfect palindrome; PBS, potential binding sequence (60). Abbreviations and designations for panels B to F are the same as for panel A. For further explanations, see the text.

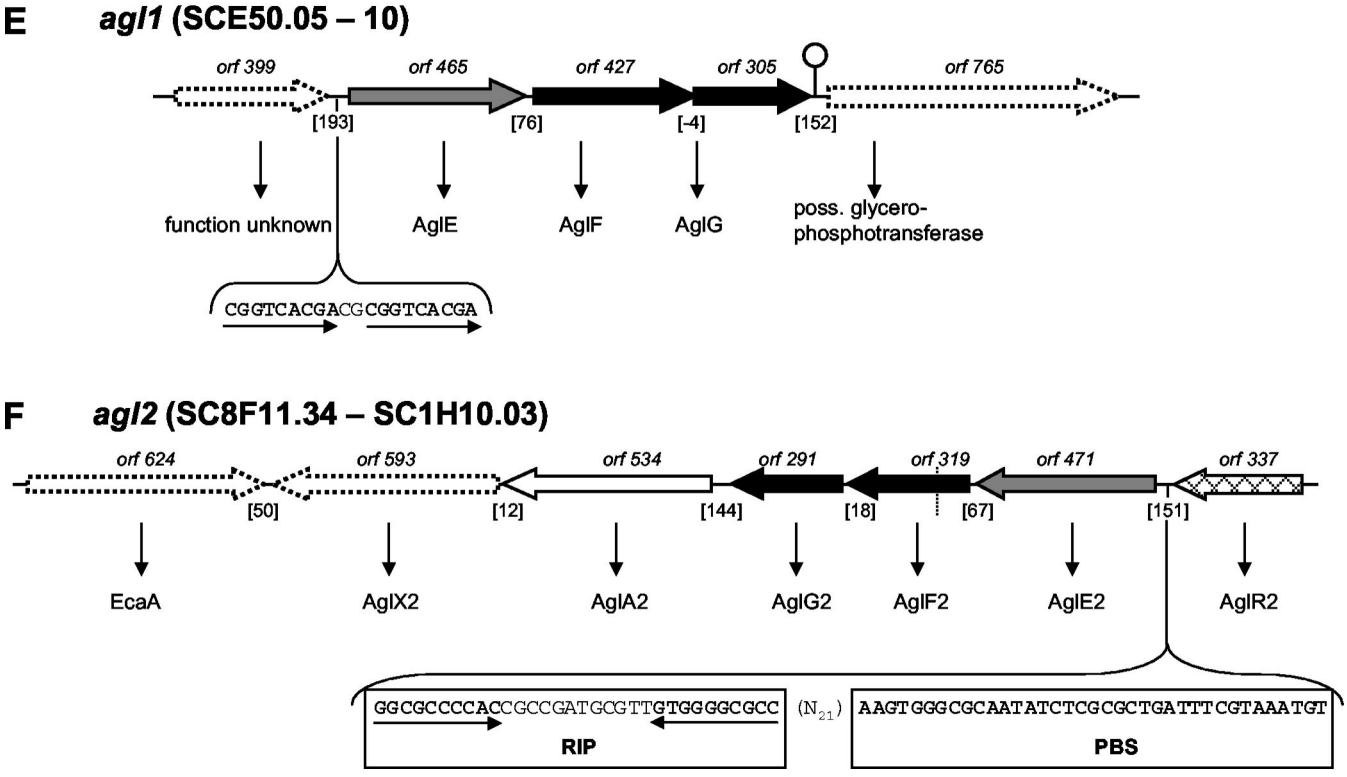

FIG. 2—*Continued.*

BLASTP query yielded around 30 paralogues of *S. coelicolor* MalE, which served as a sequence template of an ABC solute binding-protein (57). An iterative search of a far distant paralogue resulted in the nearly identical set of proteins, presumably containing all ABC carbohydrate binding proteins that are present in *S. coelicolor*. Amino acid identities ranged from 23 to 35%. A phylogenetic tree of MalE paralogues revealed a high degree of radial symmetry with no remarkable clustering (data not shown). A signature sequence of 18 amino acids sharing the consensus pattern (GAP)-(LIVMFA)-(STAVDN)-X_4-(GSAV)-$(LIVMFY)_2$-Y-(ND)-X_3-(LIVMF)-X-(KNDE) could be observed with fairly good agreement in the N-terminal third of the proteins (52). In particular, MalE and the products of open reading frame (ORF) SCD95A.19 and ORF SC6D11.04c matched exactly to this signature sequence.

Lactose. An assembly of genes around ORF SC6D11.04c may encode a lactose-specific ABC system because the products of SC6D11.04c to SC6D11.06c exhibit 24, 29, and 31% protein identity to LacEFG of *Agrobacterium radiobacter*, respectively (13, 62) (Fig. 4A). Interestingly, the system has an unusual reversed gene order, GFE. The permease gene cluster is flanked by a putative β-galactosidase (SC6D11.03c) that shows 43% protein identity to the β-galactosidase BgaB of *Bacillus stearothermophilus* (15). A probable regulator of the LacI family with an identity of 35% to LacI of *E. coli* was found to precede *lacG* and was thus designated LacI (12). Between these two ORFs, two identical copies of a perfect 16-bp palindrome are located as possible *cis* regulatory elements. They are spaced by 29 bp, with the closer one positioned 38 bp upstream of *lacG*.

Sugar alcohols. Sugar alcohols are reduced forms of aldoses or ketoses. While mannitol is a very good carbon source for *S. coelicolor*, growth on sorbitol is barely detectable. A gene cluster on cosmid SCI7 was found that could encode a permease

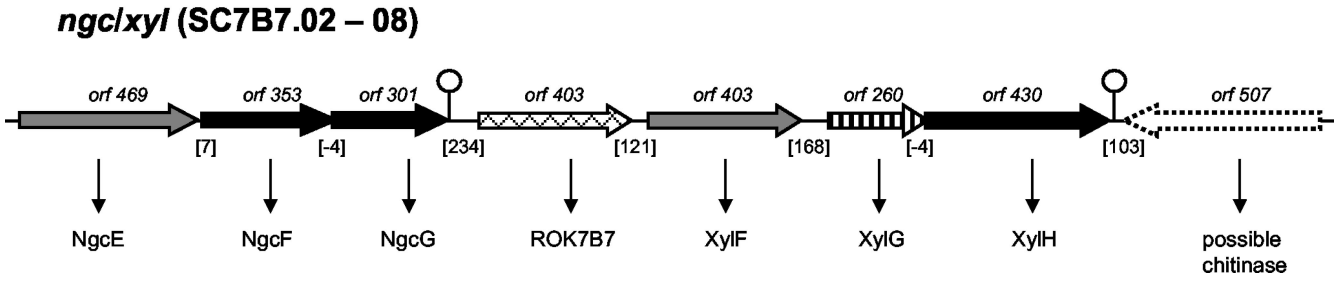

FIG. 3. Two *EFG*-like gene arrangements are shown that represent the *ngc/rok7B7/xyl* gene locus. The conventions of presentation and designations are described in the legend to Fig. 2A.

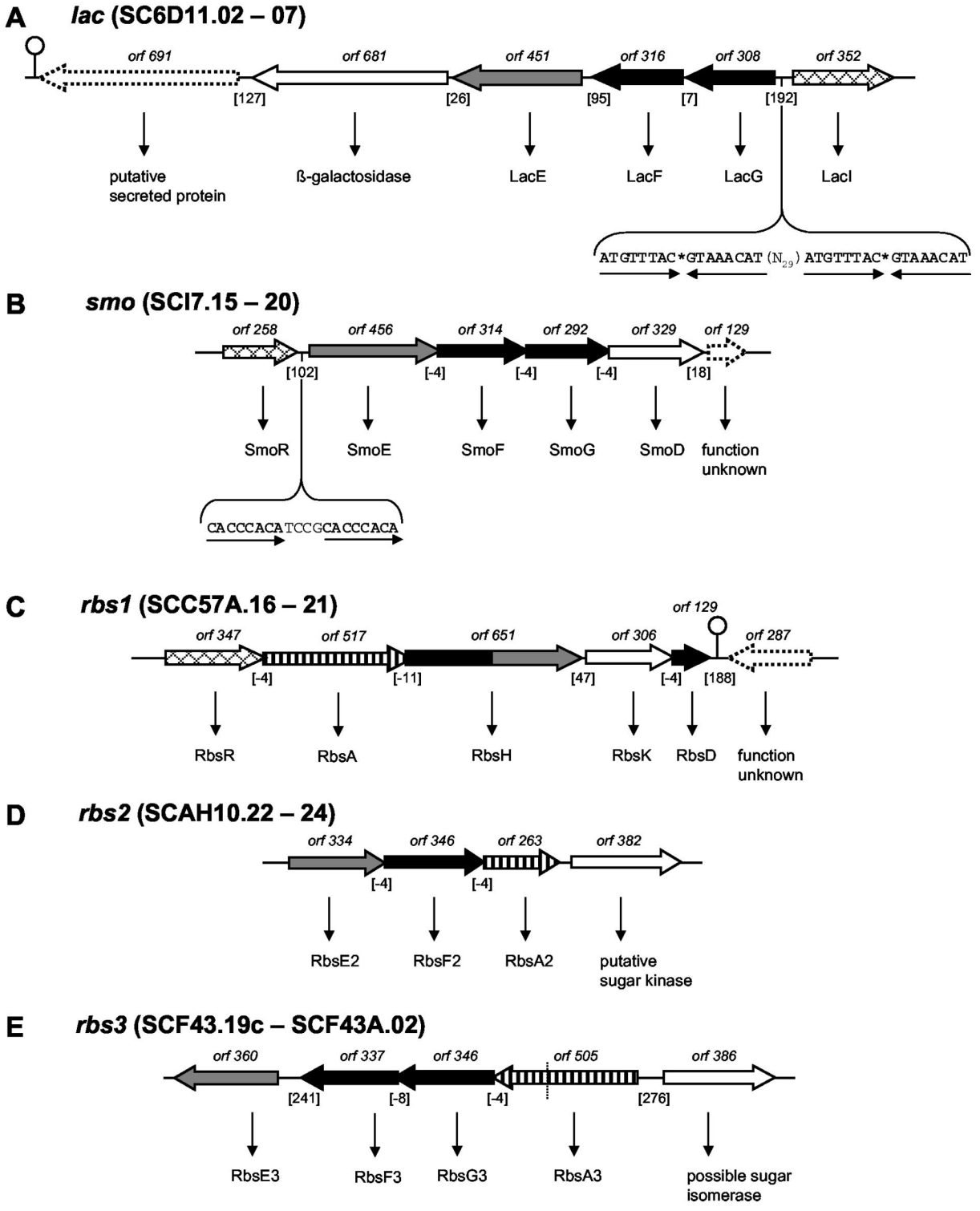

FIG. 4. The *lac*, *smo*, *rbs1*, *rbs2*, and *rbs3* loci are shown. The conventions of presentation and designations are described in the legend to Fig. 2A.

for mannitol or another sugar alcohol (Fig. 4B). Designated *smoREFGD*, this cluster contains *smoR*, a putative regulator gene of the operon, *smoE*, the solute binding protein-encoding gene, *smoF* and *smoG*, genes coding for membrane translocators, and *smoD*, a putative alcohol dehydrogenase-encoding gene. The deduced protein product of *smoR* is 39% identical to the galactitol regulator GatR from *Klebsiella oxytoca* and is categorized as a member of the DeoR family (7a). Identities of the other deduced proteins to the corresponding system in *Rhodobacter sphaeroides* are 43% for the periplasmic sorbitol

binding protein SmoE and 41% for sorbitol-mannitol transport inner membrane proteins SmoF and SmoG (accession no. AFO18073) (50). SmoD exhibits 30% identity to GutB, a sorbitol dehydrogenase present in *Bacillus subtilis* (27). Two direct repeats of 8 bp, spaced by four nucleotides and 54 bp upstream of *smoE*, might be the *cis* sites for SmoR.

Putative ribose permease with novel domain structure. A putative ABC system for ribose uptake was discovered on cosmid SCC57A (Fig. 4C). Most remarkably, the permease gene *rbsH* encodes a unique fusion protein composed of a membrane-spanning moiety and an extracellular solute-binding moiety, which both exhibit 47% protein identity to the ribose permease RbsC and to the ribose-binding protein RbsB of *E. coli* (35). Another noteworthy feature is the presence of an ATPase gene (*rbsA*) (8). The gene cluster further comprises a ribokinase gene (*rbsK*), whose product bears 41% protein identity to the ribokinase of *E. coli*, and *rbsD*, which encodes a small protein (129 amino acids) showing 33 and 44% identity to the *E. coli* and *B. subtilis* high-affinity RbsD ribose transport proteins (6, 17, 31). A probable LacI family-type regulator, RbsR, is encoded in the beginning of the gene cluster. RbsR shares 37% identity to its *E. coli* counterpart (24).

Two further gene clusters were found that potentially could encode a ribose ABC permease (Table 1). The assumed gene products of SCAH10.22 to -24 (*rbs2EFA*) are a substrate-binding lipoprotein, an integral membrane protein, and an ATPase, each with remarkable similarities to ribose ABC systems of other bacteria. SCF43.19 to -22 (*rbs3EFGA*) may encode a third ribose-like permease. Despite the primary similarity to ribose systems, these loci might also be involved in the transport of ribose-containing substrates, such as nucleoribosides, that streptomycetes are estimated to feed on.

The presence of ATPase genes in all three putative ribose uptake systems is in obvious contrast to the majority of the other ABC systems described here.

Less-well-definable ABC porters for carbohydrate. Thirty-one gene clusters were detected that could encode further ABC-type porters for carbohydrates (Table 2). All but two contain genes for a solute-binding protein with at least one adjacent permease gene. Four of them comprise a gene for an ABC-type ATPase, which implies that most ABC porters must share a common ATPase. It was not possible to suggest a probable substrate for the encoded permeases because similarity analyses revealed a substrate heterogeneity among each gene cluster. Yet, their presence underlines the fact that *S. coelicolor* has an astonishing number of carbohydrate transporters at its disposal, predominantly of the ABC type. The system to which SC7E4.29 belongs has been described as the *dasRABC* regulon of *Streptomyces griseus*, which encodes an ABC transporter that is involved in a glucose-dependent differentiation process (48). The transporter is expressed towards the commencement of aerial hypha formation and during sporulation. However, our analysis did not provide an unambiguous hint as to a possible substrate, and *dasABC* was therefore classified as a less-well-definable system.

Putative galactose-Na^+ symporter of the SSS family. Galactose can be readily utilized by *S. coelicolor*. Genes for the utilization of galactose have been identified in *S. lividans*, and the homologues of *S. coelicolor* have been noted (1, 7). The products of the *galKE1T* operon in *S. lividans* mediate the funneling of galactose into glycolysis via phosphorylation (GalK), epimerization (GalE1) and transfer of an uridylyl group, mediated by GalT. The promoter region of the *galKEIT* operon has been analyzed with respect to its role in glucose repression (23). The galactose transporter-encoding gene, however, has so far not been elucidated. This could be *galP*, an *orf* that is divergently orientated upstream of *galT*. GalP shares 28% similarity with the Na^+-galactose symporter SglS of *Vibrio parahaemolyticus* (38, 55) (Fig. 5A). The postulated galactose permease belongs to the SSS family and appears to be the only member of this family within the *S. coelicolor* genome that serves as a carbohydrate permease (36). Possible regulatory elements are a 10-bp palindrome 14 bp upstream of *galP* as well as two 12-bp direct repeats separated by 33 bp and located 85 bp in front of *galP*.

Glucose permease of the MFS. Glucose is a preferred carbon source of *S. coelicolor*, and many reports have been published that deal with the mechanism of glucose repression (2, 19, 22). However, a glucose transporter has not yet been identified. When the genome was screened with the non-PTS glucose permeases GlcP of *Synechocystis* sp. strain PCC6803 and Glf of *Zymomonas mobilis*, a homologous protein with 51 and 33% identity, respectively, was detected and designated GlcP (61, 65). Interestingly, the corresponding gene occurs in two copies on the chromosome, *glcP1* (SC7A1.22) and *glcP2* (SC9A4.15), which exhibit 99% DNA sequence identity to each other and encode identical gene products (Fig. 5B and C). DNA sequence similarity is extended to 39 bp (except two nucleotides) in the upstream area and only 4 bp in the downstream region. A common dyad repeat is located 18 bp upstream of both *glcP* genes. Furthermore, a 10-bp palindromic region is present 70 bp upstream of *glcP1*, and another dyad sequence is exclusively found 41 bp in front of *glcP2*. The adjacent genes do not encode potential metabolic or regulatory genes that may be functionally associated.

BLAST searches with other sugar permeases of the MFS, such as the xylose permease XylE and arabinose permease AraE of *E. coli*, revealed that *S. coelicolor* has more than 50 members of this family. However, no further genes besides *glcP1* and *glcP2* were detected that encode an obvious permease for carbohydrates.

Glycerol permease of the MIP family. To complete the analysis on carbohydrate uptake systems, it should be noted that glycerol is efficiently metabolized by the genes of the *gylRCABX* region (14) (Fig. 5D). Deletion of *gylR* demonstrated that substrate induction and catabolite repression of the *gyl* operon are mediated through GylR. GylC shares 49% amino acid identity to GlpF of *B. subtilis* (5). GylA and GylB exhibit 52 and 32% identity to glycerol kinase GlpK and glycerol-3-phosphate dehydrogenase GlpD of *E. coli*, respectively (4, 26). GylX shares similarity with proteins of unknown function present in other actinomycetes and archaea. We observed that glycerol transport is inducible by this substrate and repressible by glucose (unpublished data). Furthermore, we demonstrated that the specific repressor GylR, a member of the IclR family, binds within the upstream region (400 bp) of *gylC* (unpublished data) (37). There are three potential palindromic binding sites of 8 to 10 bp for GylR.

Which carbohydrate genes are expressed and what is the true substrate? The substrate definition of the well-definable

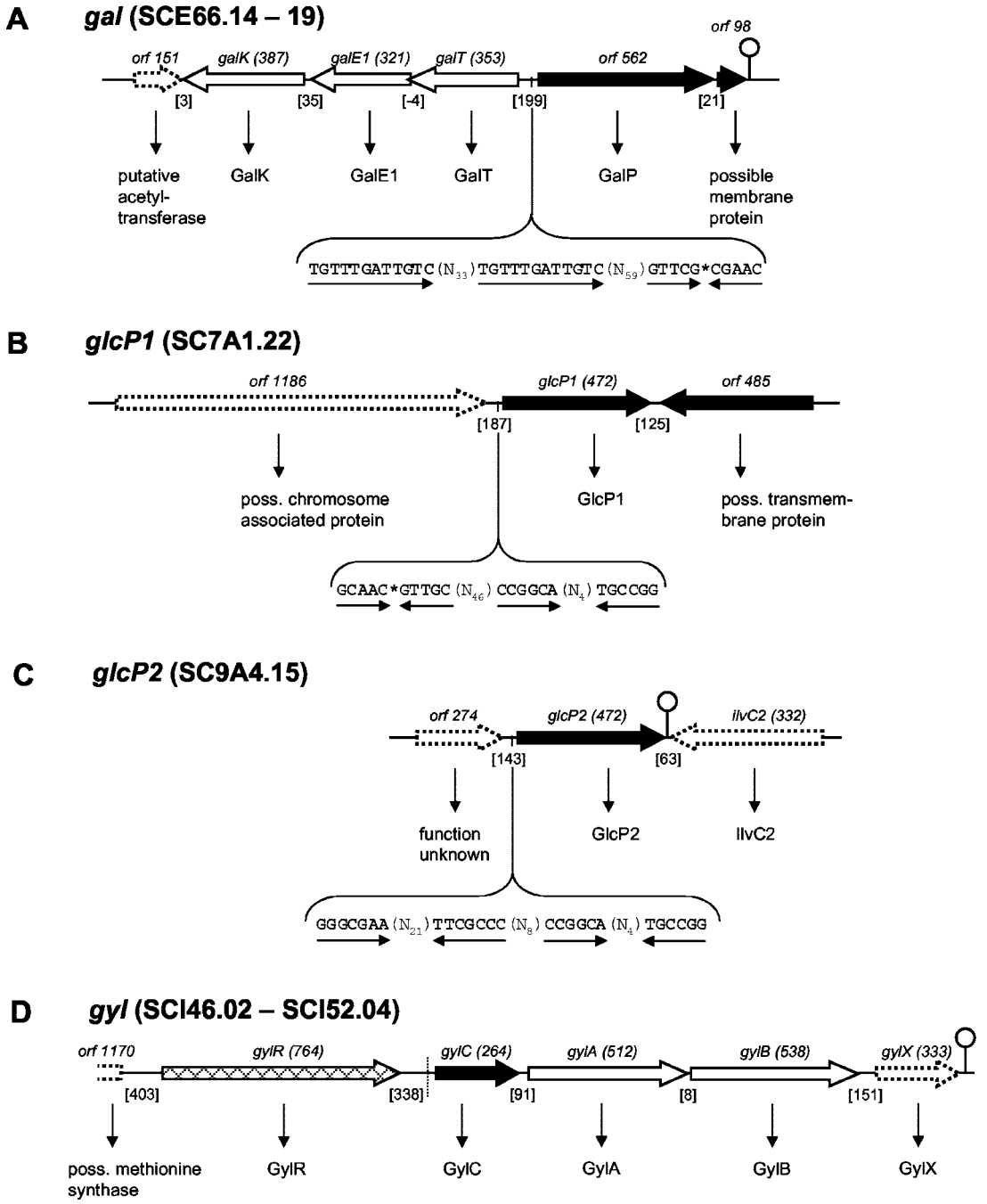

FIG. 5. The *gal*, *glcP1*, *glcP*, and *gyl* loci are shown. The conventions of presentation and designations are described in the legend to Fig. 2A.

carbohydrate transport systems that are compiled in Table 1 has been derived from available molecular genetic, biochemical, and in silico data. From transcriptional and growth analyses of *malEFG* and from the mutation of *malE* and the adjacent regulatory gene *malR*, it is obvious that this ABC permease is inducible by amylose and maltotriose (44). It transports not only maltotriose with the highest specificity but also maltose and other maltodextrins. The regulator MalR reacts best on maltopentaose and confers substrate induction and glucose repression (44, 58). From the published data on the glycerol-, galactose-, fructose-, and *N*-acetylglucosamine-utilizing systems, it is rather evident that GylC, GalP, FruA, and NagE2 are the respective permeases (14, 23, 29, 30, 47).

For the remaining systems of Table 1, we suggested their possible function solely on the basis of in silico data. To support our findings, we performed RT-PCR experiments to examine whether the particular system is expressed and whether it is induced by the suggested substrate. Therefore, we measured the mRNA levels of a representative gene of the respective operon (see Materials and Methods for details). As can be

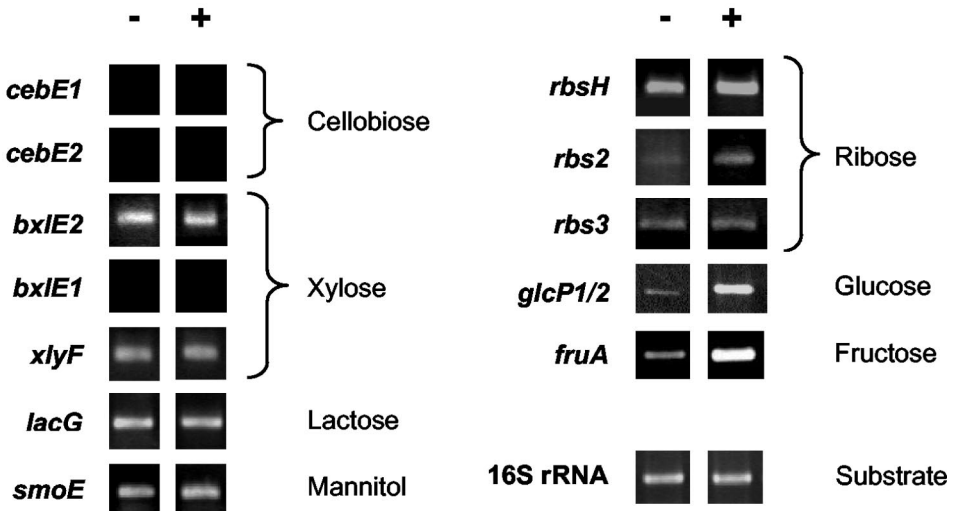

FIG. 6. Expression profiling of proposed carbohydrate uptake systems. For each system, RNA from *S. coelicolor* A3(2) grown without (left column, −) or in the presence of (right column, +) the indicated carbon source was used for RT-PCR. As an example of undoubted substrate induction, transcriptional analysis of *fruA* (encoding the specific fructose-PTS enzyme IIABC) is depicted (30).

seen from Fig. 6, *cebE1*, *cebE2*, and *bxlE1* were not expressed, which indicates that these are silent genes or that their expression occurs only under very specific growth conditions. mRNA of *bxlE2* was readily detectable. Since it may transport β-xylosides, we checked whether the presence of xylose could induce *bxlE2*. This was not the case. Due to cost restriction, we did not try induction by the more likely inducer xylobiose (18). Gene expression of *xylF*, *lacE*, and *smoE* was as well detectable by RT-PCR. However, the addition of xylose, lactose, or mannitol to the growth medium, respectively, did not reveal induction of gene expression. Thus, these systems are constitutively expressed in the complex rich medium we used. In correlation with the mRNA *lacE* data, we measured that lactose uptake is also constitutive (our unpublished data). Of the three potential ribose permease systems, we found that they are all expressed. Here, the amounts of *rbsE2* mRNA were elevated when ribose was present in the growth medium, which strengthens the suggestion that the *rbs2* gene locus encodes a ribose-specific permease. Expression of *glcP* was clearly glucose dependent and therefore confirmed our suggestion. It should be noted that we could not distinguish between *glcP1* and *glcP2* gene activity, due to the almost identical sequence conservation. RT-PCR of *fruA* (Fig. 6) and *crr* (IIA domain of the PTS permease for *N*-acetylglucosamine) further revealed the specificity of the suggested systems (29, 30, 33). Due to the uncertainty of a possible substrate and cost restriction (chitobiose), we refrained from analyzing the *agl* and *ngc* loci.

Chromosomal distribution. Different from the genomes of most other bacteria, the chromosome of *S. coelicolor* is linear. A central core appears to be flanked by two arms, which are subject to more-intensive genetic variation. These arms range from 0 to about 1.5 Mbp (left arm) and from about 6.4 Mbp to the end of the chromosome (right arm) (7). The genetic localization of the newly identified carbohydrate uptake systems shows a distribution both in the core and in the variable region (Fig. 7). It is remarkable that *ceb1*, *glcP1*, and *rbs1* are located in the core region, whereas the *ceb2*, *glcP2*, *rbs2*, and *rbs3* systems lie within the variable region. This supports the thesis that the variable region harbors nonessential and duplicate genes (7).

This report provides a comprehensive survey of the carbohydrate permeases present in *S. coelicolor*. We were able to collect a total of 53 potential systems and conclude the following. (i) The huge number of detected carbohydrate uptake systems reflects perfectly the lifestyle of streptomycetes. They live in the soil and feed primarily on complex long-chain carbohydrates from plants, insects, and fungi. These polysaccharides are broken down by exoenzymes and internalized as mono- and disaccharides. (ii) It seems that *S. coelicolor* may predominantly use permeases of the ABC type for the internalization of carbohydrates, a feature that has recently been

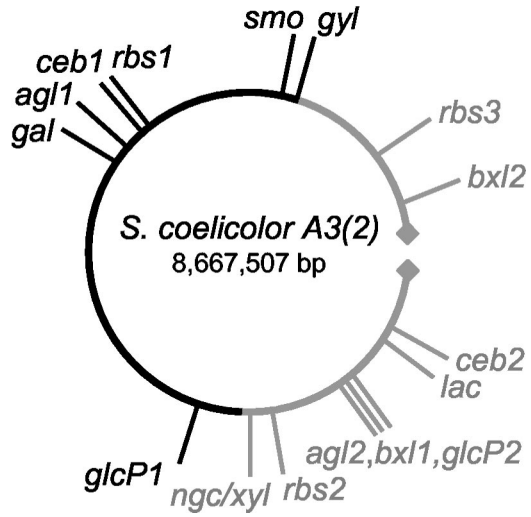

FIG. 7. Genetic map of *S. coelicolor*. A depiction of the positions of the gene clusters of newly identified transport systems (*bxl2*, *rbs3*, *smo*, *rbs1*, *ceb1*, *agl1*, *gal*, *glcP1*, *gyl*, *ngc*/*xyl*, *rbs2*, *agl2*, *bxl1*, *glcP2*, *lac*, and *ceb2*) is shown. The constant region of the chromosome is shown in black; the variable ends are in grey. The direction of the chromosome is counterclockwise.

found in the archaean *Sulfolobus solfataricus* as well (11). It can be inferred that α-glucosides, lactose, maltodextrins, maltose, ribose, β-xylosides, xylose, and sugar alcohols are likely to be transported by ABC systems, but only the maltose-maltodextrin system MalEFG has been experimentally proven (44, 57). Another 31 ABC systems are present that may contribute to the uptake of further carbohydrates. Genes encoding the required ATPase for ABC permease function were only within the permease gene cluster in four cases. This finding, which is unusual among bacteria, supports the results from previous studies with *S. reticuli* and *S. lividans*, where the ATPase MsiK assists several ABC permeases (18, 42). (iii) Fructose, *N*-acetylglucosamine, and probably two yet-unidentified sugars are transported by PTSs (29, 30, 33). The fructose and *N*-acetylglucosamine PTSs are the only uptake systems for the respective substrate in *S. coelicolor*. They are unequivocally characterized by mutation and extensive biochemical analysis (29, 30). (iv) *S. coelicolor* probably has just one porter of the SSS family (galactose), the MFS (glucose), and the MIP family (glycerol) at its disposal. It is noteworthy that a potential permease of the preferred carbon source glucose is encoded by two identical gene copies. Research on the glycerol and galactose operons strongly suggests that, although not biochemically characterized, the permease gene is carried within the respective operon (14). (v) Three systems are present in duplicated form. It appears that the two potential permeases for cellobiose and cellotriose, Ceb1 and Ceb2, are not expressed. The genes encoding the potential β-xyloside permease Bxl2 are expressed, whereas Bxl1 may be silent. *glcP* expression was clearly glucose inducible. Due to the high sequence identity, it was not possible to determine to which extent the *glcP1* or *glcP2* allele is transcribed. However, it should be noted that for *ceb*, *bxl*, and *glcP*, both loci putatively encode isofunctional transporters that may be expressed at different growth phases or under different growth conditions, as is true also for anabolic genes, such as that for glycogen synthesis (45). (vi) A gene encoding a regulator was found in most gene clusters. Just the regulators, ROK7B71, SmoR, and GylR, belong to the ROK, DeoR, and IclR families, respectively. All others are members of the LacI family of bacterial regulators. This group appears to be rather heterogeneous, which holds true also for the primary sequence of the α-helix–turn–α-helix motifs (28). Therefore, the respective cognate operators can display pronounced variation, thus diminishing undesired cross-recognition. Nevertheless, we have included in our analysis the presence of putative *cis* active elements that may be recognized by these regulatory proteins. A divergent orientation of repressor and metabolic genes indicates that the metabolic operon and the repressor itself might be simultaneously controlled by one or more copies of a regulator binding site in between. Such autogenous regulation has been found in divergeons for sucrose and arabinose utilization (21, 25). (vii) The presence of metabolic genes within a permease gene cluster occurred at a rate of 74%. It indicates that a number of catabolic enzymes are not coordinately regulated with the respective permease. This is reasonable when low- and high-affinity transport systems for the same substrate are expressed independently of an abundant or scarce supply of carbon source or when the catabolic enzyme is required for metabolism of different substrates. Glucose kinase, for instance, should be present whenever free internal glucose from glucose-containing saccharides is delivered and is thus independently regulated (3, 22).

This study documents that our molecular understanding of streptomycete nutrition concerning the intake of carbohydrates is still at the very beginning. We hope that the compilation presented here may serve as a guide to stimulate further investigation of *S. coelicolor* and other relevant *Streptomyces* species.

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