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The *Streptomyces* ATP-Binding Component MsiK Assists in Cellobiose and Maltose Transport

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***Streptomyces reticuli* harbors an *msiK* gene which encodes a protein with an amino acid identity of 90% to a corresponding protein previously identified in *Streptomyces lividans*. Immunological studies revealed that *S. lividans* and *S. reticuli* synthesize their highest levels of MsiK during growth with cellobiose, but not with glucose. Moreover, moderate amounts of MsiK are produced by both species in the course of growth with maltose, melibiose, and xylose and by *S. lividans* in the presence of xylobiose and raffinose. In contrast, a recently identified cellobiose-binding protein and its distantly related homolog were only found if *S. reticuli* or *S. lividans*, respectively, was cultivated with cellobiose. Uptake of cellobiose and maltose was tested and ascertained for *S. reticuli* and *S. lividans*, but not for an *msiK S. lividans* mutant. However, transformants of this mutant carrying the *S. reticuli* or *S. lividans msiK* gene on a multicopy plasmid had regained the ability to transport both sugars. The data show that MsiK assists two ABC transport systems.**

Streptomycetes degrade a broad range of macromolecules, such as proteins, lipids, nucleic acids, chitin, cellulose, and starch (11). *Streptomyces reticuli* synthesizes an inducible cellulase (Avicelase, Cel1) with which crystalline cellulose is efficiently hydrolyzed to cellobiose (17, 18, 21–23). Our recent investigations revealed that *S. reticuli* expresses an inducible ATP-dependent uptake system specific for cellobiose and cellotriose. A corresponding binding protein was identified and purified in its palmitylated form (19).

A gene encoding the ATP-binding component MsiK (multiple sugar import protein), a characteristic element of ABC transporters, was cloned from *Streptomyces lividans* and sequenced. The *S. lividans* mutant 10-164 carrying a point mutation in a chromosomally located *msiK* gene no longer transports cellobiose and xylobiose; the uptake of xylose is reduced (8).

In this report, we analyze the *msiK* gene of *S. reticuli* and the synthesis of its gene product under various physiological conditions. Moreover, we show that MsiK interacts with two transport systems.

Characteristics of the *S. reticuli msiK* gene. Previously we purified the *S. reticuli* cellobiose- and cellotriose-binding protein (CBP) of an ATP-dependent uptake system. By reverse genetics, we cloned the *cpbE* gene (to be published). Sequences flanking this gene did not encode the ATP-binding component of the predicted ABC transport system for cellobiose and cellotriose uptake. Therefore, we used the C-terminal part of the recently described *S. lividans msiK* gene (8) as a probe to identify a homologous *S. reticuli* gene. Under stringent conditions, only one *Sma*I fragment and one *Bam*HI-*Kpn*I fragment of total DNA hybridized with the probe. Having established a subgenomic library in pUC18, the hybridizing 2.4-kb *Kpn*I-*Bam*HI DNA fragment was cloned and sequenced by a nonradioactive sequencing method (3). Further analyses revealed that the deduced *S. reticuli* MsiK protein has amino acids 90% identical to those of the deduced *S. lividans* MsiK protein (Fig. 1). The region situated downstream of the *S. reticuli msiK* gene does not encode a component of an ABC transport system (Fig. 1). Additional hybridization studies with total *S. reticuli* DNA (cleaved with various enzymes) proved that the *msiK* gene is not located in close vicinity to *cpbE* (data not shown).

Physiological studies. Like some other streptomycetes, *S. reticuli* is very difficult to transform, and thus we did not succeed in constructing a mutant which contains a disrupted *msiK* gene. For further studies, we therefore transformed the *S. lividans* mutant 10-164, which carries a point mutation in its *msiK* gene (8), by using the plasmid pMS1; pMS1 consists of the bifunctional vector pUWL201 and the *S. reticuli msiK* gene (Fig. 2).

The wild-type strains of *S. reticuli* and *S. lividans* grew at 30°C in minimal medium with glucose, cellobiose, maltose, raffinose, melibiose, xylobiose, and xylose. In contrast, *S. lividans* 10-164 (8) utilized only glucose well; with any other of the sugars tested, it grew poorly.

Transport of cellobiose and maltose. The strains of *S. reticuli* and *S. lividans* transported maltose and cellobiose with high affinity. Uptake of maltose and cellobiose was measured as follows. Spores (10^7 to 10^8/ml) were inoculated in minimal medium supplemented with a carbon source and shaken for 16 to 48 h. Mycelia were harvested by centrifugation (5 min at

TABLE 1. Competition experiments with *S. reticuli*[a]

Compound added	% of uptake of[b]:	
	Maltose	Cellobiose
None	100	100
Maltose	1	95
Cellobiose	89	2
Arbutin	95	33
Glucose	86	90
Lactose	84	80

[a] Similar results were obtained for *S. lividans*, *S. lividans* 10-164(pMS1), and *S. lividans* 10-164(pIAF48).

[b] For competition studies, a 100-fold excess of the unlabelled sugar (1 mM) was mixed with the mycelial suspension containing either [U-^{14}C]cellobiose (10 μM) or [U-^{14}C]maltose (10 μM). The transport rate with 10 μM radiolabelled sugar served as the control and was set as 100%.

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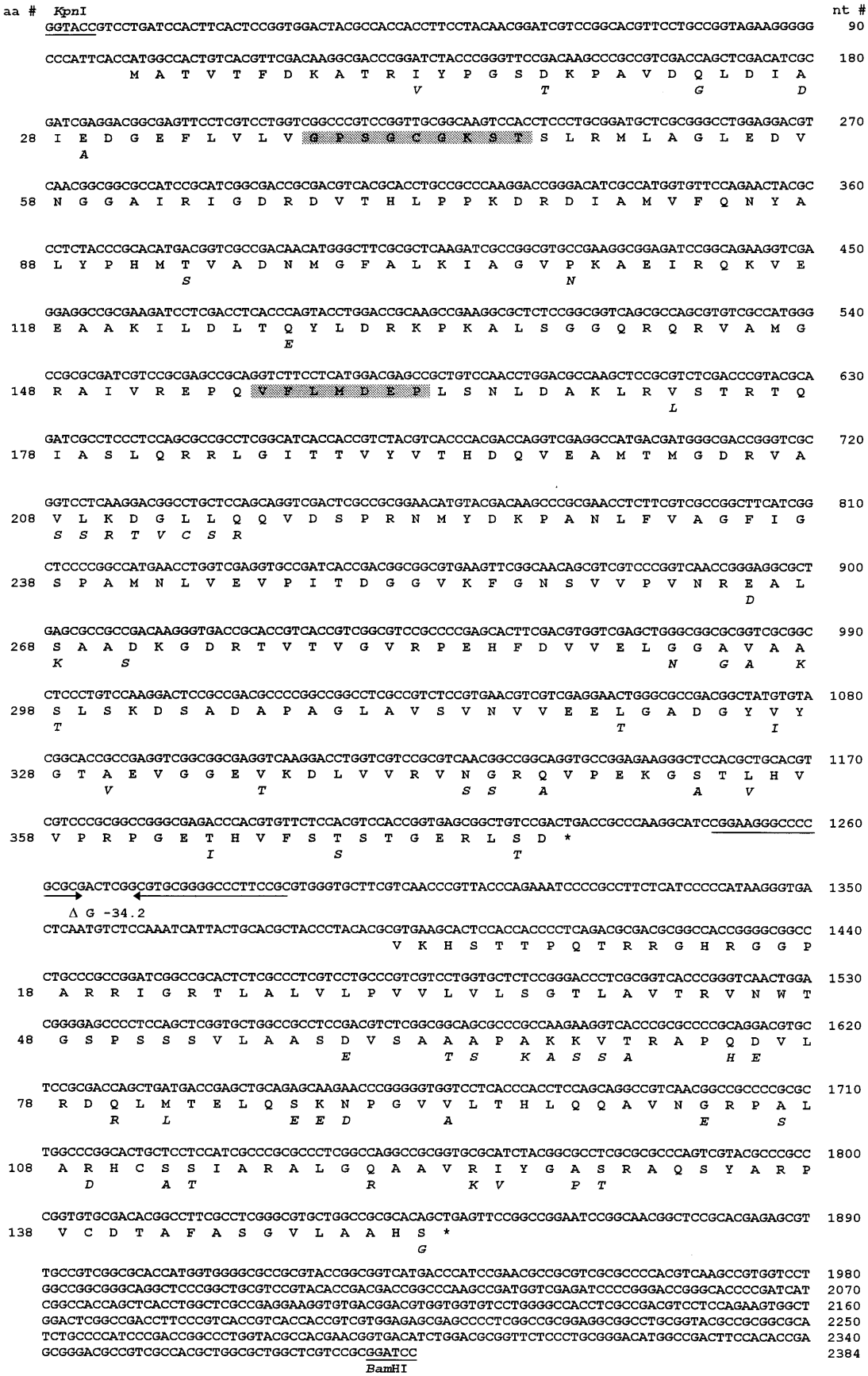

FIG. 1. Nucleotide sequence of the *S. reticuli msiK* gene and the deduced amino acid sequence. The sequence was established by a nonradioactive sequencing method (3). The deduced Walker A and B motifs are given in shaded boxes. The putative terminator is marked with arrows. Italics in the bottom line indicate the amino acids deduced from the *S. lividans* MsiK protein and the adjacent open reading frame, which differ from those of *S. reticuli*. The start codon of the adjacent open reading frame was not determined, however; considering the streptomycetes' codon usage, the indicated GTG has to be favored.

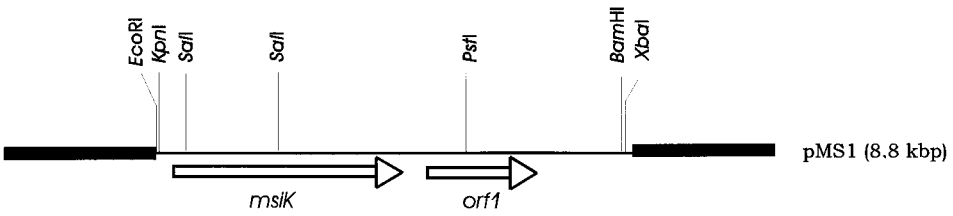

FIG. 2. Map of the plasmid pMS1. The bifunctional vector consists of pUC18 and a pIJ350 plasmid containing the thiostrepton resistance gene (7). The cloned region and the vector part are shown by the thin line and bold lines, respectively.

5,000 × *g*), washed twice in 50 mM phosphate buffer (pH 7.0), and suspended in the same buffer (0.5 to 1 mg [dry weight]/ml). This suspension was kept under aerobic conditions by shaking at 130 rpm in a water bath at 30°C. Samples of 1 ml each were taken at different time intervals, rapidly filtered through nitrocellulose filters (NC45), and washed twice with 5 ml of prechilled 0.1 M LiCl. The filters were dried and dissolved in Lsc cocktail Hydroluma (J. T. Baker, Deventer, The Netherlands), and radioactivity was determined. The initial uptake rates were derived from uptake values obtained after exposure of the mycelial suspension to a labelled sugar for 2 min.

[U-^{14}C]cellobiose (0.26 Gbq/mmol) was prepared from commercial ^{14}C-labelled cellulose (0.74 MBq/mg) by treatment with cellulase purified from an enriched commercial enzyme mixture (Sigma) of *Trichoderma reesei* enzymes. A total of 0.5 to 20 μM [U-^{14}C]cellobiose or [U-^{14}C]maltose (21.9 Gbq/mmol [Amersham]) was used for determination of the K_m and V_{max} values from Lineweaver-Burk plots. The uptake of both sugars was scarcely affected by glucose and lactose. The transport of cellobiose, but not that of maltose, was inhibited by an excess of cellobiose and arbutin, the structural analog of cellobiose (Table 1). For *S. lividans* 10-164, no uptake of these two sugars was recorded. However, in transformants of this strain carrying either plasmid pIAF48 or pMS1, the uptake of cellobiose and maltose was restored (Table 2).

Synthesis of MsiK and CBP. Anti-*S. lividans* MsiK antibodies cross-reacted with a protein with a size of about 41 kDa from *S. lividans* and *S. reticuli* mycelia which had been cultivated with different sugars (Fig. 3). Within *S. reticuli*, the largest amounts of MsiK were identified in the presence of cellobiose; smaller amounts were found upon cultivation with maltose, melibiose, xylobiose, and xylose. During growth with glucose, hardly any MsiK could be detected. *S. lividans* synthesized the largest amounts of MsiK when cultivated with cellobiose, the second largest amounts when cultivated with xylobiose, moderate amounts during growth with maltose, raffinose, xylose, and melibiose, and extremely small amounts in the presence of glucose. In the mutant strain *S. lividans* 10-164, the synthesis of the defective MsiK was very high, independent of the absence or presence of any sugar tested, including glucose. These observations are in agreement with the earlier finding that *Escherichia coli* cells containing a defective *malK* or *malT* gene overproduced the mutated proteins. It had been suggested that there might be an endogenous inducer for the maltose regulon which accumulates in *malK* mutants (15).

Antibodies raised against the CBP of *S. reticuli* (19) also cross-reacted weakly with a protein of corresponding size (45 kDa) in the *S. lividans* wild type and in the *S. lividans* mutant 10-164. These results indicate that *S. lividans* has a homolog which is merely distantly related to the *S. reticuli* CBP. So far, this homologous protein has not been identified; consequently, its binding specificity has not yet been investigated. The synthesis of CBP was only induced if all strains had been cultivated with cellobiose.

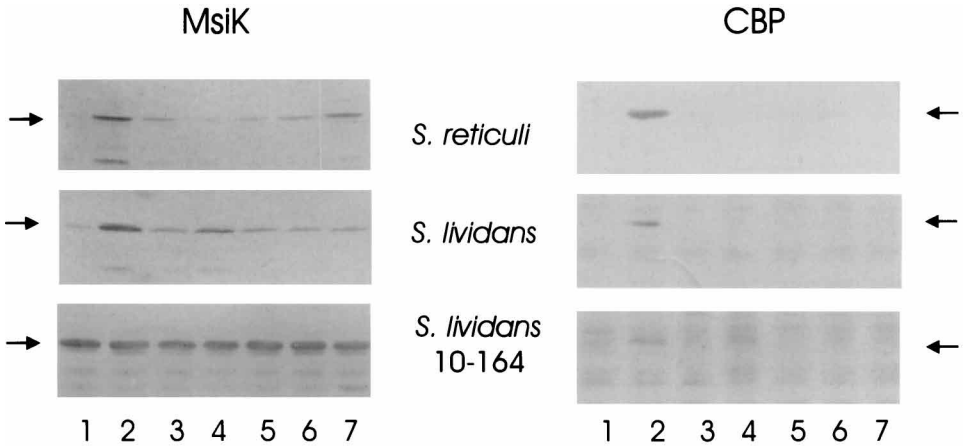

FIG. 3. Immunodetection of MsiK and CBPs. Strains were grown at 30°C in minimal medium at pH 7.4 [100 mM K_2HPO_4, 100 mM KH_2PO_4, 10 mM $(NH_4)_2SO_4$, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$] supplemented with trace element solution (7), to which 0.5% of the desired carbon source had been added. Mycelia from the indicated *Streptomyces* strain were disrupted (1 min in a Branson Sonifier 250, Microtip 6, 50% duty cycle), and the protein concentration was determined as described previously (13). Proteins (20 μg/lane) of the crude cell extract were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12). For immunodetection, proteins were transferred onto nylon filters (Fluorotrans; Pall), treated with 2% bovine serum albumin in 150 mM NaCl–50 mM Tris-HCl (pH 7.4) for 1 h, and then incubated for 3 h in the same buffer containing a 1:10,000 dilution of anti-MsiK antibodies or a 1:200,000 dilution of antibodies raised against a recently purified CBP (19). After three washes in 50 mM Tris-HCl (pH 7.4)–150 mM NaCl, the membranes were treated for 1 h with alkaline phosphatase-conjugated goat antibodies [immunoglobulin G $F(ab)_2$, from Sigma, Heidelberg, Germany] diluted 1:15,000. Color was developed as depicted by Blake et al. (4). Mycelia from the indicated strains were grown with glucose (lanes 1), cellobiose (lanes 2), maltose (lanes 3), xylobiose (lanes 4), raffinose (lanes 5), melibiose (lanes 6), and xylose (lanes 7).

TABLE 2. Kinetic parameters of sugar uptake in *Streptomyces* strains[a]

Strain	Uptake of:			
	Maltose		Cellobiose	
	K_m (μM)	V_{max}[b]	K_m (μM)	V_{max}[b]
S. lividans (wild type)	4.5	0.8	5.4	4.7
S. lividans 10-164		0		0
S. lividans 10-164(pIAF48)	3.4	1.1	6.3	2.3
S. lividans 10-164(pMS1)	5.2	1.4	5.8	4.3
S. reticuli (wild type)	1.5	1.3	2.1	5.1

[a] For determination of kinetic parameters, see the text.
[b] Nanomoles per minute per milligram of mycelia (dry weight).

Conclusions. Up to now, genes encoding ABC transporters for various sugars, peptides, or oligopeptides have been found to be encoded by corresponding operons (6). These include the operons for well-studied maltose uptake systems (Mal) in *E. coli* (9) and *Salmonella typhimurium* (2), the multiple sugar transport system (Msm) of *Streptococcus mutans* (16), and various ABC transporters for peptides (14) and oligopeptides (1) from gram-negative and gram-positive bacteria. In contrast, the genes encoding the ATP-binding component MsiK from *S. reticuli* and *S. lividans* are not situated within operons.

The studies presented allow the following conclusions. (i) *S. reticuli* and *S. lividans* have separate uptake systems for the disaccharides cellobiose and maltose. (ii) Each of the strains has a CBP and a separate protein binding maltose. (iii) The inducible ATP-binding component (MsiK or its homolog) assists both the cellobiose and the maltose ABC transport systems. (iv) The synthesis of the specific binding protein is inducible during growth on cellobiose, maltose, and some other sugars.

Previous findings indicated that HisP of the high-affinity ABC histidine transport system can also assist arginine transport in *S. typhimurium* (10). Up to now, the structural interactions of the membrane-integral permeases and the ATP-binding protein of an ABC transporter have been ill defined. Recently, however, it was demonstrated that the N-terminal part of MalK can be replaced by a corresponding part of HisP (20). Additional data revealed that MalK and UgpC (uptake system for *sn*-glycerol-3-phosphate) of *E. coli* belong to the same subfamily of ATP-binding proteins and are functionally exchangeable (5). The *Agrobacterium radiobacter lacK* gene (encoding the ATP-binding subunit LacK of the lactose ABC transporter) proved to be sufficient to restore growth of a *malK S. typhimurium* strain and complemented an *E. coli ugpC* mutant (24). Phylogenetic relationships suggest that the MsiK from *S. lividans* (8) and the one from *S. reticuli* (data not shown) have amino acids about 32% similar to those of MalK (24). The MalK counterpart of a *Streptomyces* strain has not yet been characterized. However, our data clearly indicate that the *Streptomyces* MsiK protein interacts with two different binding proteins. It will be interesting to test whether MsiK assists not only two but more ABC transport systems.

Nucleotide sequence accession number. The sequence of the *msiK* region from *S. reticuli* has been deposited in GenBank under accession number Y08921.

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