The *msiK* gene, encoding the ATP-hydrolysing component of *N,N'*-diacetylchitobiose ABC transporters, is essential for induction of chitinase production in *Streptomyces coelicolor* A3(2)

Akihiro Saito,¹ Takeshi Fujii,² Tomonori Shinya,³ Naoto Shibuya,³ Akikazu Ando¹ and Kiyotaka Miyashita²

Correspondence

Akihiro Saito

takosaito@faculty.chiba-u.jp

¹Graduate School of Advanced Integration Science, Chiba University, Matsudo 648, Matsudo City, Chiba 271-8510, Japan

²National Institute of Agro-Environmental Sciences, Kannondai 3-1-1, Tsukuba, Ibaraki 305-8604, Japan

³Department of Life Sciences, School of Agriculture, Meiji University, Kawasaki, Kanagawa 214-8571, Japan

The *dasABC* genes encode an ATP-binding cassette (ABC) transporter, which is one of the uptake systems for *N,N'*-diacetylchitobiose [(GlcNAc)₂] in *Streptomyces coelicolor* A3(2), although the gene encoding the ABC subunit that provides ATP hydrolysis for DasABC has not been identified. In this study, we disrupted the sequence that is highly homologous to the *msiK* gene, the product of which is an ABC subunit assisting several ABC permeases in other *Streptomyces* species. Disruption of *msiK* severely affected the ability of *S. coelicolor* A3(2) to utilize maltose, cellobiose, starch, cellulose, chitin and chitosan, but not glucose. The *msiK* null mutant lacked (GlcNAc)₂-uptake activity, but GlcNAc transport activity was unaffected. The data indicated that *msiK* is essential for (GlcNAc)₂ uptake, which in *S. coelicolor* A3(2) is governed by ABC transporters including the DasABC–MsiK system, in contrast to *Escherichia coli* and *Serratia marcescens*, in which (GlcNAc)₂ uptake is mediated by the phosphotransferase system. Interestingly, the induction of chitinase production by (GlcNAc)₂ or chitin was absent in the *msiK* null mutant, unlike in the parent strain M145. The defect in chitinase gene induction was rescued by expressing the His-tagged MsiK protein under the control of the putative native promoter on a multicopy plasmid. The data suggest that uptake of (GlcNAc)₂ is necessary for induction of chitinase production. The *msiK* gene was constitutively transcribed, whereas the transcription of *dasA* [(GlcNAc)₂-binding protein gene], *malE* (putative maltose-binding protein gene), *cebE1* (putative cellobiose-binding protein gene) and *bxlE1* (putative xylobiose-binding protein gene) was induced by their corresponding sugar ligands. This is believed to be the first report to indicate that (GlcNAc)₂ uptake mediated by ABC transporters is essential for chitinase production in streptomycetes, which are known to be the main degraders of chitin in soil.

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INTRODUCTION

Streptomycetes are known as saprophytic soil bacteria and are the main decomposers of chitin, which is a polymer of *N*-acetylglucosamine (GlcNAc) linked by β -1,4 bonds. To metabolize chitin, streptomycetes produce a variety of chitinases (EC 3.2.1.14) in the presence of the substrate (for a review, see Saito *et al.*, 1999); the enzymes hydrolyse chitin into (GlcNAc)₂ as the predominant final product

(Blaak *et al.*, 1993; Ohno *et al.*, 1996). We previously reported that the *dasABC* gene cluster encodes subunits of an ATP-binding cassette (ABC) transporter for the uptake of (GlcNAc)₂ in *Streptomyces coelicolor* A3(2) (Saito *et al.*, 2007). The genes encode the (GlcNAc)₂-binding protein DasA and two putative integral membrane proteins, DasB and DasC, respectively. The *dasA* null mutant ASC2 shows a lower but significant rate (25 % of that of the parental strain M145) of decline of the (GlcNAc)₂ concentration in the culture supernatant, suggesting the presence of (GlcNAc)₂-uptake systems other than DasABC (Saito *et al.*, 2007), although the types of the remaining

Abbreviations: ABC, ATP-binding cassette; GlcNAc, *N*-acetylglucosamine; (GlcNAc)₂, *N,N'*-diacetylchitobiose; PTS, phosphotransferase system; SBP, sugar-binding protein.

Table 1. Plasmid vectors used in this study

Name	Description	Reference
pGEM-T Easy	Cloning vector for PCR products in <i>E. coli</i>	Promega
pBlueScript SK +	General cloning vector for <i>E. coli</i>	Alting-Mees & Short (1989)
pIJ2925	pUC18-derived vector with <i>Bgl</i> II sites flanking modified multiple cloning sites	Janssen & Bibb (1993)
pAS100	Derivative of the temperature-sensitive plasmid pGM160, from which the <i>Hind</i> III fragment including the <i>accC4</i> gene has been removed	Muth <i>et al.</i> (1989); Xiao <i>et al.</i> (2002)
pWHM3	<i>Streptomyces</i> – <i>E. coli</i> shuttle vector	Vara <i>et al.</i> (1989)

transporters are unknown. Interestingly, the *dasA* mutant ASC2 showed a longer duration of chitinase production in the presence of (GlcNAc)₂ (Saito *et al.*, 2007). Another *dasA* mutant, SAF3, which was independently generated, exhibits a chitin-degradation activity apparently stronger than that of the parental strain M145 (Colson *et al.*, 2008). The data imply that (GlcNAc)₂ uptake is important in controlling chitinase production in *S. coelicolor* A3(2).

Various genes encoding other oligosaccharide-uptake systems have been identified in streptomycetes: *cebEFG* for cellobiose and cellotriose in *Streptomyces reticuli* (Schlösser *et al.*, 1999); *malEFG* for maltose in *S. coelicolor* (van Wezel *et al.*, 1997a); *ngcEFG* for GlcNAc and (GlcNAc)₂ in *Streptomyces olivaceoviridis* (Xiao *et al.*, 2002); and *bxlEFG* for xylobiose in *Streptomyces thermoviolaceus* (Tsujiro *et al.*, 2004). Interestingly, all of these gene systems encode subunits of ABC transporters, with the *E* and *FG* genes of these uptake systems encoding sugar-binding proteins (SBPs) (CebE, MalE, NgcE and BxlE) and two putative integral membrane proteins (CebFG, MalFG, NgcFG and BxlFG), respectively. It is noteworthy that in streptomycetes, gene clusters that encode ABC transporters for oligosaccharides lack genes for ABC proteins, which provide the necessary energy for the corresponding transporters by hydrolysing ATP to ADP (Bertram *et al.*, 2004). The MsiK protein is known to be the ABC protein that assists the cellobiose-, xylobiose- and maltose-uptake systems in *Streptomyces lividans* (Hurtubise *et al.*, 1995; Schlösser *et al.*, 1997), and the trehalose-uptake system in *S. reticuli* (Schlösser, 2000). It is therefore assumed that the *msiK* gene is globally involved in oligosaccharide-uptake systems in *Streptomyces* species. Hurtubise *et al.* (1995) have indicated that the uptake of cellobiose and xylobiose assisted by the *msiK* product is essential for induction of cellulase and xylanase production, respectively, in *S. lividans*.

S. coelicolor A3(2) possesses the ORF SCO4240, which encodes an MsiK homologue sharing 93 and 91 % amino acid identities with those of *S. reticuli* and *S. lividans*, respectively. In this report, by constructing and analysing an *msiK* null mutant, we confirmed that the product of the *msiK* gene is required for (GlcNAc)₂ uptake in *S. coelicolor* A3(2). Unexpectedly, it was found that (GlcNAc)₂-uptake systems other than DasABC are also ABC transporters, in contrast to Gram-negative *Escherichia coli* and *Serratia*

marsescens, in which uptake of the disaccharides is mediated by the phosphotransferase system (PTS) (Keyhani *et al.*, 2000; Uchiyama *et al.*, 2003). The *msiK* mutant also allowed us to demonstrate that (GlcNAc)₂ uptake is necessary for the induction of chitinase production.

METHODS

Bacterial strains, plasmids and media. *S. coelicolor* A3(2) strain M145 was used (Kieser *et al.*, 2000). *E. coli* JM109 (Yanisch-Perron *et al.*, 1985) was used as the host for gene manipulation. *E. coli* ET12567 (*dam dcm hsdS*) (MacNeil *et al.*, 1992) was used to prepare plasmids for *S. coelicolor* A3(2) transformation, to avoid the methylation-specific restriction system of the bacterium. Plasmid vectors used are listed in Table 1. Luria–Bertani (LB) medium (Sambrook & Russell, 2001) was used for culture of *S. coelicolor* A3(2); *E. coli* transformants were grown in LB medium supplemented with 50 µg ampicillin ml^{−1} or 100 µg hygromycin B ml^{−1}. A minimal medium (10 mM K₂HPO₄, 10 mM KH₂PO₄, 1 mM CaCl₂, 0.5 mM MgCl₂, supplemented with 0.1 %, v/v, trace element solution) (Schlochtermeyer *et al.*, 1992) was used to investigate the responses of *S. coelicolor* A3(2) cells to various carbon sources. Soya flour–mannitol (SFM) agar medium (van Wezel *et al.*, 1997b) was used to prepare spores of *S. coelicolor* A3(2) strains.

Conditions for culture of *S. coelicolor* A3(2). To investigate cell response to various sugars, we cultured *S. coelicolor* A3(2) M145, its *msiK* mutant and transformants according to a method described previously (Saito *et al.*, 2000), with some modifications. Spores of *S. coelicolor* A3(2) strains that formed on SFM agar medium were inoculated into 30 ml LB medium in a 100 ml flask with a spring (Kieser *et al.*, 2000) and grown for 19 h at 30 °C on a rotary shaker at 150 r.p.m. Mycelia were harvested by centrifugation (3000 r.p.m., 3 min), washed with minimal medium without carbon sources, suspended in 60 ml minimal medium, and divided into several aliquots. Each aliquot was supplemented with a different carbon source: 250 µM of glucose, maltose, cellobiose, xylobiose, GlcNAc or (GlcNAc)₂, and 0.1 % (w/v) powdered chitin. For *S. coelicolor* transformants carrying pWHM3 or its derivative, 25 µg thiostrepton ml^{−1} was added to each culture. After sugar supplementation, cultures were again grown at 30 °C on a rotary shaker at 150 r.p.m. The culture fluids were sampled periodically, centrifuged to separate the supernatant and mycelia, and stored at −80 °C. The sugar concentration and chitinase activity of the supernatants were measured, whereas the mycelia were used for total RNA preparation.

Gene manipulation. Plasmid preparation and restriction enzyme digestion were done as described by Sambrook & Russell (2001). DNA fragments were ligated by using a DNA ligation kit (Takara Bio) according to the manufacturer's instructions.

Disruption of *msiK*. Regions (~1 kb) upstream and downstream of the *msiK* (SCO4240) gene were amplified by PCR using specific primers that we designed (Table 2). The products were cloned into pGEM-T Easy (Promega) and the sequences of the cloned fragments were confirmed to be identical to those registered in the genome database (http://www.sanger.ac.uk/Projects/S_coelicolor/). The fragment corresponding to the *msiK* downstream region was isolated with *EcoRI* and *HindIII*, and cloned into the corresponding sites of pBlueScript SK+ (Table 1) to obtain the plasmid pDMK03. The *HindIII*–*XhoI* fragment of the *msiK* upstream region was then inserted into pDMK03 to obtain pDMK04. The *HindIII* fragment of the *hyg* cassette (Blondelet-Rouault *et al.*, 1997) was integrated into the corresponding site on pDMK04. A plasmid clone in which the *hyg* gene was oriented opposite to the residual *msiK* gene (Fig. 1) was selected and named pDMK05. pDMK05 was digested with *XhoI* and *PstI*, and the fragment containing the upstream and downstream fragments of *msiK* and the *hyg* gene cassette was inserted into the *Sall*/*PstI*-digested pIJ2925 (Janssen & Bibb, 1993) to obtain pDMK06. The *BglII* fragment of pDMK06, which includes the entire *Sall*/*XhoI*–*PstI* fragment, was inserted into the *BamHI* site of the temperature-sensitive vector pAS100 (Table 1) to obtain the *msiK*-disruption plasmid pDMK07. *S. coelicolor* A3(2) M145 was transformed with pDMK07, which was prepared from *E. coli* ET12567 according to the method described by Kieser *et al.* (2000). After obtaining thiostrepton-resistant transformants at 30 °C, we selected strains that grew at 39 °C on SFM agar medium supplemented with 50 µg hygromycin B ml⁻¹. After streaking the obtained colonies on SFM agar medium containing hygromycin and culturing at 30 °C, we obtained strains that were resistant to hygromycin B but sensitive to

thiostrepton. Disruption of *msiK* was verified by Southern blot analysis, using the labelled *msiK* and *hyg* genes as probes.

Determination of sugar concentrations. GlcNAc and (GlcNAc)₂ concentrations in the culture supernatant were measured according to a previously described method (Saito *et al.*, 2007) by using high-performance anion-exchange chromatography (DX-500, Dionex) with pulsed amperometric detection (HPAEC-PAD; Dionex) and a CarboPac PA1 column (Dionex). GlcNAc and (GlcNAc)₂ were separated under isocratic conditions (18 mM sodium hydroxide) at a flow rate of 1.0 ml min⁻¹ and identified by their respective retention times.

Production of His-tagged MsiK protein in the *msiK* mutant. *msiK* and its flanking region (including the putative promoter sequence) were amplified by using the primers *msiKCf* and *msiKCr* (Table 2) to produce C-terminally His-tagged MsiK protein under the control of the putative native promoter. The amplified DNA fragment (1295 bp) was cloned into pGEM-T Easy (Table 1), and its sequence was confirmed to be identical to that registered in the genome database (http://www.sanger.ac.uk/Projects/S_coelicolor/). The *EcoRI*–*HindIII* fragment was integrated into pWHM3 (Table 1) to obtain the resulting plasmid pCMK02. The *msiK* null mutant ASC3 was transformed with pWHM3 or pCMK02, which was prepared from *E. coli* ET12567 by the method of Kieser *et al.* (2000).

RT-PCR. DNA-free total RNA was prepared from mycelia by our method (Saito *et al.*, 2007) and by using an SV Total RNA Isolation System (Promega). To characterize transcripts, RT-PCR analysis was

Table 2. Primers used in this study

Sequences corresponding to native sequences are shown in upper case, whereas introduced nucleotides are indicated in lower case.

Primer	Sequence (5'–3')	Description	Product size (bp)
<i>msiK</i> Uf	<u>ctcgag</u> AGGAAAGCAACGAGGACGAC	Used to amplify the upstream region of <i>msiK</i> for gene disruption. <i>XhoI</i> site is attached (underlined)	835
<i>msiK</i> Ur	<u>aagctt</u> AACAGTGGCCATGGTGAAAG	Used to amplify the upstream region of <i>msiK</i> for gene disruption. <i>HindIII</i> site is attached (underlined)	
<i>msiK</i> Df	<u>aagctt</u> CGCCTCACCGACTGAGAAAC	Used to amplify the downstream region of <i>msiK</i> for gene disruption. <i>HindIII</i> site is attached (underlined)	967
<i>msiK</i> Dr	<u>gaattc</u> AGCCACTTCTGGAGCACCTC	Used to amplify the downstream region of <i>msiK</i> for gene disruption. <i>EcoRI</i> site is attached (underlined)	
<i>msiK</i> Cf	AAAATTTTCGGCGGAATTCAC	Used to amplify <i>msiK</i> and its flanking region for production of His-tagged MsiK protein. <i>EcoRI</i> site is underlined	1295
<i>msiK</i> Cr	<u>aagctt</u> ctagtgggtgggtgggtgggtg GTCGGTGAGGCGCTCGCCCGTG	Used to amplify <i>msiK</i> and its flanking region for the production of the His-tagged MsiK protein. <i>HindIII</i> site (underlined) and codons for six histidines (italicized) are attached	
<i>msiK</i> f	ATGGCCACTGTTACGTTTCGACAAG	Used to detect <i>msiK</i> transcripts	483
<i>msiK</i> RTr	CTCGTCCATGAGGAACACCTG		
E4RTf	GCGTGAAGCGCAAGCTTATAG	Used to detect <i>dasA</i> transcripts (Saito <i>et al.</i> , 2007)	545
E4RTr	CTTGAGGTGCTGTAGAACTC		
malERTf	CATCACTTACTGGGACACCTC	Used to detect <i>malE</i> transcripts	375
malERTr	TTCTCGAACAGTTCCTTGTTG		
cebERTf	GAGCGGAACGAGAACTACTAC	Used to detect <i>cebE1</i> transcripts	449
cebERTr	TAGAACTTCTCGTCCTCACTG		
bxlERTf	AAGATCGAACAGCAGATCGTC	Used to detect <i>bxlE1</i> transcripts	479
bxlERTr	TAGAGGTACTGGAACCACATC

Fig. 1. (a) The *msiK* gene map on the *S. coelicolor* A3(2) M145 genome (top) compared with those of the *msiK* mutant ASC3 (middle) and the plasmid pCMK02 (bottom). An open square indicates the codons for six histidine residues that are attached to the C terminus of the MsiK protein. A scale bar (1 kb) is shown. The map of M145 was drawn from the data available on the *S. coelicolor* annotation server (<http://strepdb.streptomyces.org.uk>). (b) Southern blot analysis of the total DNA of *S. coelicolor* A3(2) M145 and its *msiK* null mutant ASC3. Total DNAs were digested with *Bam*HI or *Kpn*I. The *msiK* (left) and *hyg* (right) genes were used as probes. Approximate sizes of the main detected bands are indicated. M, M145; A, ASC3.

done by using AccuPower RT-PCR Premix (Bioneer), as reported previously (Saito *et al.*, 2007). Sets of primers specific for each transcript were designed to give PCR products ranging from 375 to 545 bp (Table 2). For PCR, the number of cycles was set to 20 to avoid saturation of PCR product formation. RT-PCR experiments without prior reverse transcription were done to ensure that no residual DNA was present in the RNA samples.

Chitinase assay. Chitinase activity was measured by using the fluorescent substrate 4-methylumbelliferyl-*N,N'*-diacetylchitobioside (Sigma) according to a previously described method (Miyashita *et al.*, 1991). One unit of chitinase activity was defined as the amount of enzyme that liberated 1 μ mol of 4-methylumbelliferone from the substrate in 1 min at 37 °C.

RESULTS AND DISCUSSION

Effects of *msiK* mutation on polysaccharide utilization

To investigate the effect of the mutation of the ABC protein-encoding gene *msiK*, which is involved in the uptake of cellobiose and maltose in *S. lividans* (Schlösser *et al.*, 1997), on chitin utilization in *S. coelicolor* A3(2) M145, *msiK* (SCO4240) was disrupted by homologous recombination in the bacterium. The profile of the

obtained strain ASC3, which was analysed by Southern blot hybridization using the *msiK* and *hyg* probes, clearly demonstrated the *msiK* disruption and the *hyg* insertion on the genome (Fig. 1b). The growth of the obtained *msiK* null mutant ASC3 was tested on minimal agar medium containing starch, cellulose, chitin and chitosan, and related mono- and disaccharides, and we judged the ability of the mutant to utilize those sugars by examining growth on each supplemented medium (Table 3). ASC3 did not seem to utilize any of the polysaccharides or maltose, whereas its growth on glucose-containing medium was comparable with that of the parental organism, M145. ASC3 seemed to utilize cellobiose but apparently less effectively than did M145. These data indicate that the protein product of *msiK* is essential for the ability to utilize polysaccharides and maltose, and that *msiK* is also involved in cellobiose utilization. The poor growth of the *msiK* mutant on a medium containing maltose or cellobiose suggests that *msiK* is involved in the uptake of those disaccharides in *S. coelicolor* A3(2), as demonstrated in *S. lividans* (Schlösser *et al.*, 1997). The defect of chitosan utilization in the *msiK* mutant may imply that *msiK* is involved in the uptake of chitosan-degradation products, such as chitobiose.

Table 3. Utilization of sugars by *S. coelicolor* A3(2) M145 and its *msiK* null mutant ASC3

Utilization was estimated by growth on minimal agar medium containing 0.5% (w/v) of the respective sugar. ++, good growth; +, intermediate growth; –, poor growth, comparable with that on the medium without sugar addition.

Strain	Sugar						
	Glucose	Maltose	Starch	Cellobiose	Cellulose	Chitin	Chitosan
M145	++	++	++	++	+	+	+
ASC3	++	–	–	+	–	–	–

***msiK* is essential for the uptake of (GlcNAc)₂**

To investigate the involvement of the *msiK* gene product in (GlcNAc)₂ uptake, mycelia of *S. coelicolor* A3(2) M145 and its *msiK* null mutant ASC3 were grown in LB medium, washed with minimal medium, and incubated in minimal medium containing 250 µM GlcNAc or (GlcNAc)₂. The culture was sampled periodically, and the GlcNAc and (GlcNAc)₂ concentrations in the culture supernatant were measured. The patterns of declining GlcNAc concentration in the culture supernatants of M145 and ASC3 were comparable to each other (Fig. 2a), suggesting that *msiK* is not involved in GlcNAc uptake. The data supported the finding of Nothaft *et al.* (2003) that *S. coelicolor* A3(2) exclusively uses the PTS for GlcNAc. In contrast, mutation of *msiK* dramatically altered the pattern of utilization of (GlcNAc)₂ in the culture supernatant (Fig. 2b). In M145, (GlcNAc)₂ was depleted within 4 h, as reported previously (Saito *et al.*, 2007). However, in the *msiK* mutant ASC3, the (GlcNAc)₂ concentration remained higher than 200 µM even 8 h after addition of the disaccharide. These data clearly demonstrate that the *msiK* gene product is required for (GlcNAc)₂ uptake in *S. coelicolor* A3(2). The slight decrease in the (GlcNAc)₂ concentration in the ASC3 culture supernatant may indicate the presence of a low level of *N*-acetylglucosaminidase (chitinase) activity. The poor GlcNAc uptake activity of the *msiK* mutant might reflect inhibition of the PTS for GlcNAc uptake (Nothaft *et al.*, 2003) due to the presence of (GlcNAc)₂ (Fig. 2b).

We recently identified the *dasABC* gene cluster, which encodes the components of an ABC transporter specific for (GlcNAc)₂ in *S. coelicolor* A3(2) (Saito *et al.*, 2007). The *dasA* null mutant ASC2 retains the ability to take up (GlcNAc)₂, although the level of activity is markedly reduced (25 % of that of the parental strain M145), thereby implying the presence of other (GlcNAc)₂-uptake systems

(Saito *et al.*, 2007). In contrast, the disruption of *msiK* leads to a virtually complete absence of (GlcNAc)₂ uptake (Fig. 2b). The data thus suggest that the (GlcNAc)₂-uptake systems other than DasABC must also be ABC transporters dependent on MsiK. (GlcNAc)₂ uptake therefore appears to be governed by ABC transporters in *S. coelicolor* A3(2), in contrast to *E. coli* (Keyhani *et al.*, 2000) and the Gram-negative chitin degrader *S. marcescens* (Uchiyama *et al.*, 2003), in which the uptake of (GlcNAc)₂ is mediated by a PTS. The gene cluster (SCO6005, SCO6006 and SCO6007) might be a good candidate for a (GlcNAc)₂ ABC uptake system (Bertram *et al.*, 2004) other than DasABC, although the putative products of the three ORFs share relatively low amino acid identity (31–48 %) with NgcE, F and G, which are components of the ABC transporter Ngc specific for GlcNAc and (GlcNAc)₂ in *S. olivaceoviridis* (Saito & Schrempf, 2004; Xiao *et al.*, 2002).

***msiK* is required for the induction of chitinase production**

In *S. coelicolor* A3(2), production of chitinase is induced by the chitin-degradation product (GlcNAc)₂ (Saito *et al.*, 2000). The defect of chitin utilization in the *msiK* null mutant (Table 3, Fig. 3a) implied that *msiK* might be required for the induction of chitinase production as well as for (GlcNAc)₂ uptake. To investigate the effect of *msiK* mutation on this induction, M145 and the *msiK* null mutant ASC3 were grown in LB medium and then incubated in minimal medium supplemented with 250 µM GlcNAc or (GlcNAc)₂. Chitinase production in M145 was induced by (GlcNAc)₂ but not by GlcNAc (Fig. 3b). In contrast, chitinase activity was not detected in the culture supernatant of ASC3 in the presence of either GlcNAc or (GlcNAc)₂ (Fig. 3b). Chitinase activity was detectable in the culture supernatant of M145 6 days after

Fig. 2. Dynamics of GlcNAc and (GlcNAc)₂ concentrations in the culture supernatants of *S. coelicolor* A3(2) M145 (circles) and its *msiK* null mutant ASC3 (triangles). Mycelia grown in LB medium were suspended at time zero in minimal medium (MM) supplemented with 250 µM GlcNAc (a) or (GlcNAc)₂ (b). The suspension was further incubated at 30 °C with shaking at 150 r.p.m. Supernatants were sampled periodically, and the GlcNAc (open symbols) and (GlcNAc)₂ (closed symbols) concentrations of the samples were measured. Mean values of two independent experiments are plotted. Error bars, SD.

Fig. 3. Chitinase activity in the *S. coelicolor* A3(2) strains. (a) Growth of M145 and its *msiK* null mutant ASC3 on minimal agar medium containing 0.2% (w/v) colloidal chitin. (b) Chitinase production in M145 (circles) and its *msiK* null mutant ASC3 (triangles) in the presence of 250 μ M GlcNAc (closed symbols) or (GlcNAc)₂ (open symbols). (c) Chitinase production in M145 (circles) and ASC3 (triangles) in the presence of 0.1% (w/v) powdered chitin. (d) Chitinase production in M145(pWHM3) (circles), ASC3(pWHM3) (triangles) and ASC3(pCMK02) (squares) in the presence of 250 μ M (GlcNAc)₂. See Fig. 2 legend for culture conditions. Chitinase activity was expressed as milliunits (mU) per millilitre of culture supernatant. In panels (b), (c) and (d), mean values of two independent experiments are plotted. Error bars, SD.

the addition of 0.1% (w/v) powdered chitin, whereas chitinase activity was not induced in the *msiK* null mutant ASC3 even after 13 days (Fig. 3c). The defect in chitinase production was rescued by introducing the multicopy plasmid pCMK02, which carries the gene for His-tagged MsiK protein with its putative native promoter (Figs 1 and 3d). These data demonstrate that the product of *msiK* is essential for the induction of chitinase production by (GlcNAc)₂ or chitin in *S. coelicolor* A3(2). Intracellular (GlcNAc)₂ would act as an inducer.

A cellulase/xylanase-negative mutant of *S. lividans*, which is defective in cellobiose and xylobiose uptake, carries a mutation in *msiK* (Hurtubise *et al.*, 1995). Our preliminary experiments suggested that *msiK* also is required for the induction of amylase production by maltose in *S. coelicolor* A3(2) (A. Saito and others, unpublished data). Therefore, we assume that uptake of cellobiose and xylobiose as well as maltose and (GlcNAc)₂ is necessary for the induction of the corresponding polysaccharide-hydrolysing enzymes. Just how disaccharide molecules that are taken into cells trigger the production of the corresponding glycosylhy-

drolases remains an open question. MalR, CebR and BxlR, which respectively regulate the transcription of the *malEFG*, *cebEFG* and *bxlEFG* operons (Schlösser *et al.*, 1999; Tsujibo *et al.*, 2004; van Wezel *et al.*, 1997b), and/or a pleiotropic regulator RegI (Nguyen *et al.*, 1997), which shares 95% amino acid identity with MalR of *S. coelicolor* A3(2), would also indirectly control amylase, cellulose and xylanase production.

msiK is constitutively transcribed

To elucidate the conditions for *msiK* expression in *S. coelicolor* A3(2), the *msiK* transcript was investigated by RT-PCR after growth in the presence of 250 μ M glucose, maltose, cellobiose, xylobiose, GlcNAc or (GlcNAc)₂. For comparison, we also analysed the transcription of genes for the following oligosaccharide-binding proteins of ABC transporters of OSP (oligosaccharide and polyols) family members: *dasA* [SCO5232, (GlcNAc)₂-binding protein gene; Saito *et al.*, 2007]; *malE* (SCO2231, putative maltose/maltotriose-binding protein gene; van Wezel

Fig. 4. RT-PCR analyses for detection of transcriptional products of *msiK*, *dasA* (Saito *et al.*, 2007), *malE* (van Wezel *et al.*, 1997a), *cebE1* (SCO2795, similar to the cellobiose/cellotriose-binding protein gene *cebE* in *S. reticuli*; Schlösser *et al.*, 1999) and *bxlE1* (SCO7028, homologue of the xylobiose-binding protein gene *bxlE* of *S. thermoviolaceus*; Tsujibo *et al.*, 2004). See Fig. 2 legend for the culture conditions of *S. coelicolor* A3(2) M145, except that 250 μ M of glucose, maltose, cellobiose, xylobiose, GlcNAc or (GlcNAc)₂ was added to the suspension at time zero. After 2 h of incubation, total RNA was prepared from the mycelia and subjected to RT-PCR analysis. The positions of PCR products and their expected sizes are indicated by arrows (sizes are approximate). Size markers (Φ X174/*Hinf*I) are also shown. As for *msiK*, the result with 15 cycles of PCR and that without RT reaction are indicated. The rRNA bands within RNA (0.15 μ g) on the ethidium bromide-stained gel are shown as a control. BP, binding protein; RT, reverse transcription.

et al., 1997a); *cebE1* (SCO2795, similar to the cellobiose/cellotriose-binding protein gene *cebE* in *S. reticuli*; Bertram *et al.*, 2004; Schlösser *et al.*, 1999); and *bxlE1* (SCO7028, similar to the xylobiose-binding protein gene *bxlE* of *S. thermoviolaceus*; Bertram *et al.*, 2004; Tsujibo *et al.*, 2004). The expression of *msiK* transcripts seemed to be constitutive under the various test conditions. In contrast, the transcription of *dasA*, *malE*, *cebE1* and *bxlE1* was induced in the presence of the corresponding (putative) ligand, although there did appear to be some background expression in each case (Fig. 4). The data extend the finding by Schlösser *et al.* (1999) that in *S. reticuli*, the regulation of MsiK production differs from that of the cellobiose/cellotriose-binding protein CebE. It is assumed that the MsiK protein interacts with the other components (such as DasBC and MalFG) of the ABC transporters, whose genes form clusters with the genes of the corresponding SBPs (i.e. *dasA* and *malE*, respectively) (Bertram *et al.*, 2004). This uptake machinery, which is unusual among bacteria, may be energetically efficient for streptomycetes; these organisms utilize a variety of oligosaccharides after degrading their cognate polysaccharides, which are present in soil.

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