APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2007, p. 3000–3008 Vol. 73, No. 9
0099-2240/07/$08.00+0 doi:10.1128/AEM.02612-06

The *dasABC* Gene Cluster, Adjacent to *dasR*, Encodes a Novel ABC Transporter for the Uptake of *N*,*N*′-Diacetylchitobiose in *Streptomyces coelicolor* A3(2)[▽]

Akihiro Saito,[1]* Tomonori Shinya,[2] Katsushiro Miyamoto,[3] Tomofumi Yokoyama,[4] Hanae Kaku,[2] Eiichi Minami,[5] Naoto Shibuya,[2] Hiroshi Tsujibo,[3] Yoshiho Nagata,[1] Akikazu Ando,[4] Takeshi Fujii,[6] and Kiyotaka Miyashita[6]

Department of Bioresources Chemistry, Faculty of Horticulture, Chiba University, Matsudo 648, Matsudo City, Chiba 271-8510,[1] *Department of Life Sciences, School of Agriculture, Meiji University, Kawasaki, Kanagawa 214-8571,*[2] *Department of Microbiology, Osaka University of Pharmaceutical Sciences, Takatsuki, Osaka 569-1094,*[3] *Department of Biotechnology, Graduate School of Science and Technology, Chiba University, Matsudo 648, Matsudo City, Chiba 271-8510,*[4] *National Institute of Agrobiological Sciences, Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602,*[5] *and National Institute of Agro-Environmental Sciences, Kannondai 3-1-1, Tsukuba, Ibaraki 305-8604,*[6] *Japan*

Received 9 November 2006/Accepted 19 February 2007

***N*,*N*′-Diacetylchitobiose [$(GlcNAc)_2$] induces the transcription of chitinase (*chi*) genes in *Streptomyces coelicolor* A3(2). Physiological studies showed that $(GlcNAc)_2$ addition triggered *chi* expression and increased the rate of $(GlcNAc)_2$ concentration decline in culture supernatants of mycelia already cultivated with $(GlcNAc)_2$, suggesting that $(GlcNAc)_2$ induced the synthesis of its own uptake system. Four open reading frames (SCO0531, SCO0914, SCO2946, and SCO5232) encoding putative sugar-binding proteins of ABC transporters were found in the genome by probing the 12-bp repeat sequence required for regulation of *chi* transcription. SCO5232, named *dasA*, showed transcriptional induction by $(GlcNAc)_2$ and *N*,*N*′,*N*″-triacetylchitotriose [$(GlcNAc)_3$]. Surface plasmon resonance analysis showed that recombinant DasA protein exhibited the highest affinity for $(GlcNAc)_2$ (equilibrium dissociation constant [K_D] = 3.22×10^{-8}). In the *dasA*-null mutant, the rate of decline of the $(GlcNAc)_2$ concentration in the culture supernatant was about 25% of that in strain M145. The in vitro and in vivo data clearly demonstrated that *dasA* is involved in $(GlcNAc)_2$ uptake. Upstream and downstream of *dasA*, the transcriptional regulator gene (*dasR*) and two putative integral membrane protein genes (*dasBC*) are located in the opposite and same orientations, respectively. The expression of *dasR* and *dasB*, which seemed independent of *dasA* transcription, was also induced by $(GlcNAc)_2$ and $(GlcNAc)_3$.**

Streptomycetes are known as saprophytic soil bacteria and the main decomposers of chitin, which is a polymer of *N*-acetylglucosamine (GlcNAc) linked by β-1,4 bonds. Streptomycetes degrade chitin by producing a variety of chitinase (EC 3.2.1.14) (for a review, see reference 27) that hydrolyzes chitin into *N*,*N*′-diacetylchitobiose [$(GlcNAc)_2$] as the final product (3, 21). *Streptomyces coelicolor* A3(2) strain M145 possesses 13 chitinase (*chi*) genes in its genome, although 6 of them are putative ones (2, 6, 12, 29). The expression of five *chi* genes (*chiA*, *chiB*, *chiC*, *chiD*, and *chiF*) is induced by chitin in the bacterium (30), and each of the chitinases (ChiA, ChiC, ChiD, and ChiF) has its own enzymatic properties (12). It is assumed that these chitinases act synergistically with each other in chitin degradation, as has been reported in the chitinase system of a marine bacterium, *Alteromonas* sp. strain O-7 (22).

It is generally known that chitinase genes in streptomycetes are induced in the presence of the chitin substrate and repressed when both glucose (Glc) and chitin are present (for a review, see reference 27). The regulation mechanisms are, however, still unclear. The 12-bp direct repeat (DR) sequences overlapping the promoters are required for regulation of the *chi63* gene in *Streptomyces plicatus* and the *chiA* gene in *Streptomyces lividans* (8, 16, 20). The DR-like sequences are also present upstream of other chitinase genes, the expression of which is regulated as described above. It is therefore believed that the DR-like sequences are common *cis*-acting elements involved in the regulation of chitinase genes in streptomycetes. The transcription of *chi* genes in *S. coelicolor* A3(2) and *S. lividans* is induced by $(GlcNAc)_2$ as well as by chitin (17, 30). Because of the more immediate induction activity of $(GlcNAc)_2$, it has been suggested that $(GlcNAc)_2$ production, which occurs by hydrolysis of chitin, is necessary for the induction of *chi* gene expression (30).

Although chitinase systems have been studied in streptomycetes for decades, the nature of the systems of transport of chitin degradation products was unclear until two transporters for GlcNAc uptake were identified in *Streptomyces olivaceoviridis*. One is Ngc, an ABC (ATP-binding cassette) transport system for GlcNAc and probably for $(GlcNAc)_2$ (40). The corresponding solute-binding protein NgcE has high affinities for GlcNAc and $(GlcNAc)_2$. The other transporter protein is PtsC2 of the phosphotransferase system which is specific for GlcNAc (38).

During the course of our studies, we obtained data suggesting that $(GlcNAc)_2$ uptake activity was induced by the amino sugar itself. In light of this implication, we explored transporter

* Corresponding author. Mailing address: Department of Bioresources Chemistry, Faculty of Horticulture, Chiba University, Matsudo 648, Matsudo City, Chiba 271-8510, Japan. Phone and fax: (81-47) 308-8871. E-mail: takosaito@faculty.chiba-u.jp.

▽ Published ahead of print on 9 March 2007.

TABLE 1. Plasmid vectors and primers used in this study

Type of construct, purpose, and name	Description	Sequence[a]	Reference or source	Target	Product size (bp)
Plasmid vector					
pGEM-T Easy	Cloning vector for PCR products in *Escherichia coli*		Promega		
pET16b	Expression vector to overproduce recombinant proteins in *Escherichia coli*		Novagen		
pBlueScript SK+	General cloning vector for *Escherichia coli*		1		
pAS100	Derivative of the temp-sensitive plasmid pGM160 (19), of which the HindIII fragment including the *accC4* gene has been removed		40		
Primer					
For RT-PCR					
E1RTf		5′-GTTCCAGAAGGAGTTCGAGAC-3′		SCO0531	455
E1RTr		5′-GGACAGCACATACCAGTTCTG-3′			
E2RTf		5′-TGTGGCTGATGAAGGACAGTG-3′		SCO0914	442
E2RTr		5′-TTCTTGTCGAGCTTCTCCGTG-3′			
E3RTf		5′-AGGAAAGACTCCATGAAGCTC-3′		SCO2946	455
E3RTr		5′-TAGACCTTTCCGTCCACGGTG-3′			
E4RTf		5′-GCGTGAAGCGCAAGCTTATAG-3′		SCO5232 (*dasA*)	545
E4RTr		5′-CTTGAGGTCGTTGTAGAACTC-3′			
R4RTf		5′-GAAGCATCTGCTCGACATGAC-3′		SCO5231 (*dasR*)	438
R4RTr		5′-GTAGAGGGACGTGTACTTCAC-3′			
F4RTf		5′-ATGCGGTTCGTGCTGATGATC-3′		SCO5233 (*dasB*)	393
E4DWr	XhoI site is attached (underlined)	5′-ctcgagGAAGATCCAGATGACTTCGAG-3′			
E4DWf	HindIII site is attached (underlined)	5′-aagcttGCTGAGGGCGAGTTCAACAAG-3′		*dasAB*	549
E4F4RTr		5′-ATCATCAGCACGAACCGCATC-3′			
F4G4RTf		5′-CTCGAAGTCATCTGGATCTTC-3′		*dasBC*	615
F4G4RTr		5′-TCACGCACGATCATGTAGATC-3′			
chiCRTf		5′-ACATGCGCTTCAGACACAAAG-3′		*chic*	533
chiCRTr		5′-CAGGACGTCGTAGTTCTTGAC-3′			
RThrdBf		5′-AAGGAAGACGGCGAGCTTCTC-3′		*hrdB*	431
RThrdBr		5′-ATGAGGTCCAGGAAGAGCATG-3′			455
For overproduction of DasA					
cbiE4f	NdeI site is attached (underlined)	5′-catatgGGGGGCGACAGCGACGACGAC-3′			
cbiE4r	XhoI site is attached (underlined)	5′-ctcgagCTACTGCTGCTGCGCCAGGCGCTT-5′			
For *dasA* disruption					
E4UPf	EcoRV site is attached (underlined)	5′-gatatgGTAGAGGGACGTGTACTTCAC-3′			
E4UPr	HindIII site is underlined	5′-GGCTATAAGCTTGCGCTTCAC-3′			
E4DWf	HindIII site is attached (underlined)	5′-aagcttGCTGAGGGCGAGTTCAACAAG-3′			
E4DWr	XhoI site is attached (underlined)	5′-ctcgag*G*AAGATCCAGATGACTTCGAG-3′			

[a] In primers, sequences corresponding to the native ones are shown in uppercase, whereas nucleotides artificially attached are indicated in lowercase.

genes for $(GlcNAc)_2$ in the genome of *S. coelicolor* A3(2) by using the DR sequence as a probe. We successfully identified a novel ABC transporter gene for $(GlcNAc)_2$, the deduced products of which exhibit properties distinct from those of Ngc of *S. olicaceoviridis*. Interestingly, the gene cluster for the new transporter is located adjacent to the *dasR* gene, which is involved in the regulation of morphological differentiation in *Streptomyces griseus* (35) as well as in *S. coelicolor* A3(2) (23).

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. *Streptomyces coelicolor* A3(2) strain M145 was used (13). *Escherichia coli* JM109 (41) was routinely used as the host for gene manipulation. *E. coli* ET12567 (*dam dcm hsdS*) (15) was used to prepare plasmids for *S. coelicolor* A3(2) transformation to avoid the methylation-specific restriction system of the bacterium. Luria-Bertani (LB) medium (32) and LB medium supplemented with 50 μg/ml ampicillin or 100 μg/ml hygromycin B were used for the culture of *S. coelicolor* A3(2) and the *E. coli* transformants, respectively. A minimal medium (MM) (10 mM K_2HPO_4, 10 mM KH_2PO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$ supplemented with 0.1% [vol/vol] trace element solution) (34) was used to investigate the responses of *S. coelicolor* A3(2) cells to various carbon sources. Soya flour-mannitol (SFM) agar medium (37) was used to prepare spores of *S. coelicolor* A3(2) strains. The plasmid vectors used are listed in Table 1.

Culture conditions for *S. coelicolor* A3(2). To investigate the responses of the cells to sugars, *S. coelicolor* A3(2) M145 and its mutant were cultivated by following a method described previously (30), with some modifications. Spores of *S. coelicolor* A3(2) strains formed on SFM agar medium were inoculated into 30 ml LB medium in a 100-ml flask with a spring (13) and grown for 19 h at 30°C on a rotary shaker at 150 rpm. Mycelia were harvested by centrifugation (3,000 rpm; 3 min), washed with MM without carbon sources, suspended in 60 ml MM, and divided into several aliquots. Each aliquot was supplemented with various carbon sources, i.e., 1 mM or 250 μM of Glc, GlcNAc, $(GlcNAc)_2$, *N*,*N*′,*N*″-triacetylchitotriose $[(GlcNAc)_3]$, glucosamine (GlcN), or chitobiose $[(GlcN)_2]$ (Seikagaku Corporation, Tokyo, Japan), or 0.05% (wt/vol) colloidal chitin. The culture was further grown at 30°C on a rotary shaker at 150 rpm. Portions of the culture fluids were sampled periodically, centrifuged to separate the supernatants and the mycelia, and stored at −80°C. The supernatants were subjected to measurements of amino sugar [GlcNAc, $(GlcNAc)_2$, and $(GlcNAc)_3$] concentrations and chitinase activities, whereas the mycelia were used for total RNA preparation, immunoblot analysis, and protein assay. To investigate the growth rates of *S. coelicolor* A3(2) strains, spores were inoculated onto MM supplemented with 0.5% (wt/vol) chitin (Wako Pure Chemical Industries, Osaka, Japan) and cultivated at 30°C on a rotary shaker at 150 rpm. Periodically, 1-ml samples were taken from the culture, and growth was evaluated by measuring the amounts of protein in the mycelia and chitin, as reported previously (28).

Gene manipulation. Plasmid preparation and restriction enzyme digestion were done as described by Sambrook and Russell (32). Dephosphorylation and ligation of DNA fragments were done by using bacterial alkaline phosphatase (Toyobo, Osaka, Japan) and a DNA ligation kit (Takara Bio, Shiga, Japan), respectively, according to the manufacturers' instructions.

Total RNA preparation. DNA-free total RNAs were prepared by following the reported method (30), with some alterations. The mycelia of *S. coelicolor* A3(2) harvested from 5 ml of culture fluid were disrupted by using alumina type A-5 (Sigma) and a pellet mixer (Treff AG, Degersheim, Switzerland), following the addition of 75 μl of the lysis buffer supplied in an SV total RNA isolation system (Promega). DNA-free total RNA was subsequently prepared using the system and subjected to reverse transcription (RT)-PCR analysis as described below.

RT-PCR. To investigate transcripts, RT-PCR analysis was done using an AccuPower RT-PCR premix (Bioneer, Daejeon, Korea) as follows. Sets of primers specific for each transcript were designed to give PCR products ranging from 393 to 615 bp (Table 1). Each reaction mixture (20 μl) included 25 pmol of each primer and 0.5 μg total RNA. Annealing of RNA with a reverse primer and RT-PCR were performed by following the manufacturer's instructions. For PCR, the number of cycles was set to 20 to avoid saturation of PCR product formation. RT-PCR experiments without prior RT were done to ensure that no residual DNA was present in the RNA samples.

Overproduction and purification of recombinant DasA protein. An expression plasmid that encoded DasA protein without the signal sequence and with six histidine residues at the N terminus was constructed as follows. A set of primers (Table 1) was designed to amplify part of the *dasA* gene, which encodes that part of the DasA protein without the putative signal peptide (20 amino acids from the N terminus). The nucleotide sequences for the N-terminal cysteine residue of the presumed mature DasA protein were replaced with those for a methionine residue in the primer cbiE4f. Total DNA was prepared from *S. coelicolor* A3(2) M145 by following the method described previously (13), and the above-mentioned part of the *dasA* gene was amplified with the primer set by using the total DNA as a template. The PCR product was cloned in the plasmid vector pGEM-T Easy to obtain the plasmid pCBI402. The nucleotide sequence of the cloned fragment was ascertained, and the NdeI-XhoI fragment of pCBI402 was then ligated to the corresponding sites of pET16b to overproduce recombinant DasA protein that was N-terminally tagged with six histidine residues. The resulting plasmid, pCBI403, was introduced into *E. coli* BL21(DE3)pLysS (Novagen). The recombinant DasA protein was successfully overproduced in a soluble form and purified in accordance with Novagen's instructions. Before confirming the N-terminal amino acid sequence of the purified recombinant DasA protein, we removed the six N-terminal histidine residues using a factor Xa cleavage capture kit (Novagen) in accordance with the manufacturer's instructions. The N-terminal amino acid sequence was determined by Edman degradation using an LF3000 automated protein sequencer (Beckman Coulter).

Immunoblot analysis. *Streptomyces coelicolor* A3(2) mycelia were incubated for 3 or 6 h in MM supplemented with 1 mM of Glc, galactose (Gal), GlcNAc, $(GlcNAc)_2$, or $(GlcNAc)_3$ or 0.05% (wt/vol) colloidal chitin. They were then harvested by centrifugation (18,000 × *g*; 3 min; 4°C), suspended in phosphate-buffered saline (32), and disrupted by sonication (15 s × 8 pulses) on ice. The suspension was centrifuged at 10,000 × *g* for 5 min at 4°C, and the protein concentration of the supernatant was measured by Bradford's method (5). Proteins corresponding to 50 μg were separated with 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (14) and blotted onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore). For immunoblot analysis, *anti*-DasA antiserum was prepared by injecting the purified His-tagged DasA protein into a rabbit.

Surface plasmon resonance analyses. The binding specificities and affinities of the His-tagged DasA protein were determined by using a Biacore X system and carboxymethylated dextran (CM5) sensor chips (GE Healthcare), as reported elsewhere (36, 40). GlcNAc, $(GlcNAc)_2$, $(GlcNAc)_3$, *N*,*N*′,*N*″,*N*‴-tetraacetylchitotetraose [$(GlcNAc)_4$], *N*,*N*′,*N*″,*N*‴,*N*⁗-pentaacetylchitopentaose [$(GlcNAc)_5$], GlcN, $(GlcN)_2$, and chitotriose [$(GlcN)_3$] were subjected to the analysis at concentrations ranging from 10 nM to 1 mM.

Chitinase and *N*-acetylglucosaminidase assay. Chitinase activity was measured using the fluorescent substrate 4-methylumbelliferyl-*N*,*N*′-diacetylchitobioside [4MU-$(GlcNAc)_2$] (Sigma) by following a method described previously (18). The total protein amounts per mycelia in 1-ml volumes of culture fluid were measured as reported by Saito et al. (28). Specific chitinase activity was expressed as activity (units) per gram of protein in the mycelia, as used in a previous paper (28). *N*-Acetylglucosaminidase activity was measured at 37°C for 30 min at a pH of 7.0 by using *p*-nitrophenyl-*N*-acetylglucosaminide (pNP-GlcNAc) or 4-methylumbelliferyl-*N*-acetylglucosaminide (4MU-GlcNAc) (Sigma) as the substrate.

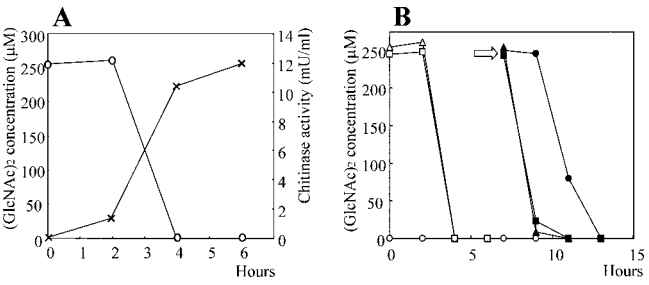

FIG. 1. Dynamics of $(GlcNAc)_2$ concentration in the culture supernatant of *Streptomyces coelicolor* A3(2) M145. (A) Mycelia grown in LB medium were suspended in MM supplemented with 250 μM $(GlcNAc)_2$ at time zero. The suspension was further incubated at 30°C with shaking at 150 rpm. Chitinase activity (crosses) and $(GlcNAc)_2$ concentration (circles) in the culture supernatant were measured. (B) Mycelia pregrown in LB medium were suspended in MM with (open triangles and open squares) or without (open circles) 250 μM $(GlcNAc)_2$ at time zero. Following incubation at 30°C with shaking at 150 rpm for 7 h, 250 μM $(GlcNAc)_2$ was directly added to the mycelial suspensions, which had already been preincubated with (closed triangles) or without (closed circles) $(GlcNAc)_2$. Mycelia that had been subjected to $(GlcNAc)_2$ once (open squares) were washed and resuspended in fresh MM containing 250 μM $(GlcNAc)_2$ at 7 h (closed squares). The mycelia were further cultivated at 30°C with shaking at 150 rpm, and the $(GlcNAc)_2$ concentrations in the culture supernatants were measured. The point of $(GlcNAc)_2$ addition at 7 h is indicated by the arrow. The experiment was performed twice with similar results, one of which is indicated.

Determination of GlcNAc, $(GlcNAc)_2$, and $(GlcNAc)_3$ concentrations. To measure the concentrations of amino sugars, culture supernatant obtained by centrifugation was assayed with DX-500 high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using a CarboPac PA1 column (Dionex, Sunnyvale, CA). GlcNAc, $(GlcNAc)_2$, and $(GlcNAc)_3$ were separated under isocratic conditions (18 mM sodium hydroxide) at a flow rate of 1.0 ml/min and identified by their respective retention times.

Disruption of the *dasA* gene. The upstream and downstream regions (circa 1 kb) of the *dasA* gene were amplified by PCR using the specific primers we had designed (Table 1). The products were cloned into pGEM-T Easy 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 *dasA* upstream region was digested by EcoRI-HindIII and cloned into the corresponding sites of pBlueScript SK+ to obtain the plasmid pDE401 (Table 1). The HindIII-XhoI fragment of the *dasA* downstream region was then inserted into pDE401 to obtain pDE402. The HindIII fragment of the *hyg* cassette (4) was integrated into the corresponding site on pDE402. The resulting plasmid, pDE403, was digested with XhoI and BamHI, and the fragment containing the upstream and downstream fragments of *dasA* and the *hyg* gene cassette was inserted into the SalI-BamHI sites on the temperature-sensitive plasmid pAS100 (Table 1) to get the *dasA* disruption plasmid pDE404. *Streptomyces coelicolor* A3(2) M145 was transformed with pDE404, which was prepared from *E. coli* ET12567, by following the method described previously (13). After obtaining thiostrepton-resistant transformants at 30°C, we selected strains that grew at 39°C on SFM agar medium supplemented with 50 μg/ml hygromycin B. After streaking the colonies so obtained onto SFM agar medium containing hygromycin at 30°C, we obtained strains that were resistant to hygromycin B but sensitive to thiostrepton. The disruption of *dasA* was checked by Southern blot analysis using the labeled *dasA* and *hyg* genes as probes.

RESULTS

Decline of $(GlcNAc)_2$ concentration is induced by $(GlcNAc)_2$. Chitinase production is known to be induced by $(GlcNAc)_2$ in *S. coelicolor* A3(2) (30). The level of induced chitinase activity peaked 4 h after the addition of 250 μM $(GlcNAc)_2$ (Fig. 1A). To investigate the dynamics of the inducer, we measured the $(GlcNAc)_2$ concentration in the culture supernatant. Although

the concentration remained at the initial level for the first 2 h, $(GlcNAc)_2$ was not detected in the culture supernatant 4 h after its addition (Fig. 1A). The results implied that the addition of $(GlcNAc)_2$ induced chitinase production, which was accompanied by a decline in the concentration of the added $(GlcNAc)_2$ in the supernatant. To characterize the phenomenon in more detail, another physiological experiment was performed. When mycelia of *S. coelicolor* A3(2) were preincubated at 30°C for 6 h in MM supplemented with 250 μM $(GlcNAc)_2$, the concentration of a second dose of 250 μM $(GlcNAc)_2$ added at 7 h declined rapidly and reached the detection limit (10 μM) in 2 h. However, when mycelia were preincubated in MM without $(GlcNAc)_2$, the concentration of the second dose of $(GlcNAc)_2$ remained at the initial level for 2 h and did not reach the detection limit until 4 h had elapsed (Fig. 1B). This induction of a more rapid decline in $(GlcNAc)_2$ concentration in the mycelia after the addition of a second dose was retained even after the mycelia had been washed with MM before the 6-h preincubation with fresh $(GlcNAc)_2$. The data clearly indicated that preincubation with an initial dose of $(GlcNAc)_2$ led to a more rapid decline in $(GlcNAc)_2$ concentration in the culture supernatant of *S. coelicolor* A3(2) when a second dose was added. GlcNAc was never detected in the culture supernatant of the bacterium under these conditions (data not shown). Using 4MU-GlcNAc and pNP-GlcNAc as substrates, *N*-acetylglucosaminidase activity was not detected in the culture supernatant or in the intact mycelia (data not shown). These results thus suggest that $(GlcNAc)_2$ uptake was induced in the mycelia by priming with $(GlcNAc)_2$.

Four open reading frames were found as candidates for $(GlcNAc)_2$ transporter genes. In streptomycetes, the transcription of chitinase genes is induced by chitin but repressed in the presence of Glc and chitin together (3, 9, 16, 25, 28), and a pair of 12-bp DR sequences present at the promoter is involved in the regulation of chitinase genes (8, 16, 20). The transcription of five chitinase genes in *S. coelicolor* is also induced by chitin, and it has been suggested that $(GlcNAc)_2$ production derived from chitin hydrolysis is involved in this induction (30). DR-like sequences similar to the pair of DR sequences are also present at the promoter regions of these five chitinase genes (30). On the basis of the hypothesis that DR-like sequences might also be present at the promoter regions of genes for $(GlcNAc)_2$ uptake, putative sugar transporter genes that possessed DR-like sequences in their upstream regions were searched for in the genome of *S. coelicolor* A3(2), using the DR sequence of the *chiC* gene (5′-TGGTCCAGACCT-3′) as a probe. Four open reading frames (ORFs) (SCO0531, SCO0914, SCO2946, and SCO5232) encoding putative sugar-binding proteins of ABC transporters were found. One unit of a DR-like sequence was present upstream of SCO0531 and of SCO0914, whereas two and three units were located at the possible promoter regions of SCO2946 and SCO5232, respectively (Fig. 2A). The amino acid sequences of the deduced products of the four ORFs exhibited sequence similarities to the solute-binding proteins of the CUT-1 (*c*arbohydrate *u*ptake *t*ransporter-1) family (alternatively, the OSP [*o*ligosaccharides and *p*olyols] family) of ABC transporters (7, 26). However, the specific ligands for the sugar-binding proteins encoded by the ORFs could not be predicted from the amino acid sequences. Among the four ORFs, SCO5232 was named *dasA*, as its product

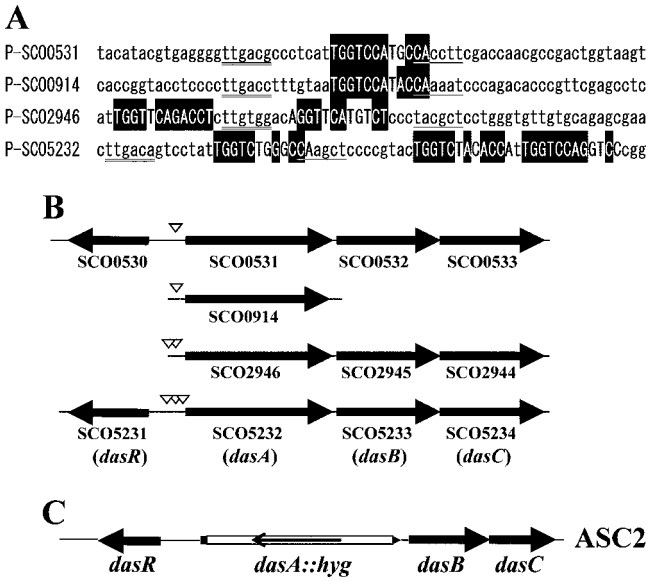

FIG. 2. (A) Structures of upstream regions of four ORFs (SCO0531, SCO0914, SCO2946, and SCO5232) encoding putative sugar-binding proteins. Sequences similar to the DR sequence, which is involved in the regulation of chitinase genes, are shown in white on a black background. Putative −35 and −10 sequences are double- and single-underlined, respectively. (B) Gene organization around SCO0531, SCO0914, SCO2946, and SCO5232. Positions of DR-like sequences are indicated by open arrowheads. (C) Physical map of the *das* locus in the *dasA* mutant ASC2. A hygromycin resistance gene (*hyg*) cassette is shown by a white box with an arrow indicating the *hyg* orientation.

showed 34% similarity to the amino acid sequence of DasA of *S. griseus*, a protein that is involved in the regulation of morphological differentiation (35). Two ORFs encoding putative permeases were located in the downstream regions of SCO0531, SCO2946, and SCO5232 (*dasA*) (Fig. 2B). Each of the six putative permeases contains a region corresponding to the consensus sequence $EAAX_3GX_9IXLP$ (Table 2) which is characteristic of membrane proteins from binding protein-dependent ABC transporters (33). An ORF for a putative transcriptional regulator and an ORF for the *gntR* family transcriptional regulator gene *dasR* (24) were divergently located upstream of SCO0531 and SCO5232 (*dasA*), respectively (Fig. 2B).

Expression of the *dasA* gene is induced by $(GlcNAc)_2$ and $(GlcNAc)_3$. The transcripts of the four ORFs (see above) that encoded putative sugar-binding proteins of ABC transporters were investigated in the presence of 1 mM Glc, GlcNAc, $(GlcNAc)_2$, $(GlcNAc)_3$, GlcN, or $(GlcN)_2$. Total RNAs were prepared from the mycelia, which were incubated with each of the sugars for 2 or 4 h. The transcript of SCO5232 (*dasA*) was strongly induced in the presence of $(GlcNAc)_2$ and $(GlcNAc)_3$ (Fig. 3). It is noteworthy that upregulation of SCO5232 (*dasA*) transcription was also observed in mycelia that were exposed to $(GlcN)_2$. The transcript level of SCO0531 was higher in mycelia exposed to $(GlcNAc)_3$ for 2 h than in other conditions (data not shown). SCO0914 transcription was not detected, while SCO2946 transcription seemed constitutive but very low (data not shown).

To investigate the expression of SCO5232 (*dasA*) at the level

TABLE 2. Presence of the signature sequence for membrane proteins of binding protein-dependent ABC transporters

ORF (product)	Size of the deduced product (no. of amino acid residues)	Amino acid sequence[a]	Position of the signature sequence
SCO0532	302	**ESA**RMD**G**AGAARVERS**V**T**FP**	197–216
SCO0533	285	**EAA**QVD**G**CTRAGAFRR**V**A**FP**	178–197
SCO2945	316	**EAA**ALD**G**ASQWRIWRS**V**LA**P**	211–230
SCO2944	281	**EAA**YID**G**ASRTRFLWQ**I**L**FP**	175–194
SCO5233 (DasB)	328	**EAA**ALD**G**AGMWRSFTS**V**T**LP**	223–252
SCO5234 (DasC)	276	**ESA**MVD**G**CTRAQAFRR**V**I**LP**	170–189

[a] Amino acids corresponding to the signature sequence ($EAAX_3GX_9IXLP$) for membrane proteins of binding-protein-dependent ABC transporters (33) are shown in bold, and those identical to the signature are underlined.

of protein production, an immunoblot analysis was performed using antibodies against recombinant DasA protein overproduced in *E. coli* (Fig. 4A). Although the protein was not detected in mycelia that were prepared just after replacement of the LB medium with MM (0 h), DasA production was observed at 3 and 6 h after the addition of 1 mM Glc, Gal, GlcNAc, $(GlcNAc)_2$, $(GlcNAc)_3$, or colloidal chitin (Fig. 4B). The level of production was obviously higher in the presence of $(GlcNAc)_2$, $(GlcNAc)_3$, or colloidal chitin. It was thus demonstrated that expression of the *dasA* gene was strongly induced by $(GlcNAc)_2$, $(GlcNAc)_3$, and colloidal chitin.

DasA binds specifically to $(GlcNAc)_2$. To clarify the biological function of DasA in vitro, recombinant DasA protein without the original signal peptide and with six histidine residues N-terminally added (six-His tag) was overproduced in *E. coli* in a soluble form and purified (Fig. 4A). The N-terminal amino acid sequence of the recombinant DasA protein, which was cleaved with factor Xa to remove the six-His tag, was determined to be HMGGD—identical to that encoded on the designed recombinant gene. Using the six-His-tagged DasA protein, the binding specificities and affinities of the protein were investigated by surface plasmon resonance. DasA was fixed on the surface of a sensor chip, and each sugar was tested to see whether it would interact with the protein. GlcN, $(GlcN)_2$, and $(GlcN)_3$, as well as GlcNAc, $(GlcNAc)_2$, $(GlcNAc)_3$, $(GlcNAc)_4$, and $(GlcNAc)_5$, were selected as candidate ligands. DasA was shown to interact with GlcNAc, $(GlcNAc)_2$, $(GlcNAc)_3$, $(GlcNAc)_4$, and $(GlcNAc)_5$, whereas it did not exhibit binding to GlcN, $(GlcN)_2$, or $(GlcN)_3$ even at 100 μM (data not shown). Of the five sugars interacting with DasA, $(GlcNAc)_2$ exhibited the highest affinity (equilibrium dissociation constant [K_D] = 3.22×10^{-8}) (Table 3). The K_D values obtained for GlcNAc, $(GlcNAc)_3$, $(GlcNAc)_4$, and $(GlcNAc)_5$ were greater than the value for $(GlcNAc)_2$. The value for $(GlcNAc)_2$ was comparable to those reported for other sugar-binding proteins of ABC transporters: the GlcNAc-binding protein NgcE for GlcNAc ($K_D = 8.28 \times 10^{-9}$) (40) and the xylobiose-binding protein BxlE for xylobiose ($K_D = 8.75 \times 10^{-9}$) (36). It was thus concluded that DasA protein binds specifically to $(GlcNAc)_2$.

The *dasA* gene is involved in $(GlcNAc)_2$ uptake. To assess its in vivo function, the *dasA* gene on the genome was disrupted in *S. coelicolor* A3(2) strain M145 (Fig. 2C). Following cultivation in LB medium, the mycelia of strain M145 or the *dasA*-null

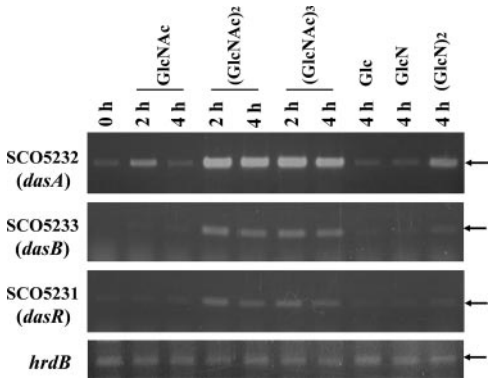

FIG. 3. RT-PCR analyses for detection of transcriptional products of SCO5232 (*dasA*), SCO5233 (*dasB*), and SCO5231 (*dasR*). Refer to the legend of Fig. 1A for the culture conditions of *Streptomyces coelicolor* A3(2) M145, except that 1 mM of GlcNAc, $(GlcNAc)_2$, $(GlcNAc)_3$, Glc, GlcN, or $(GlcN)_2$ was added to the suspension at time zero. After 2 and 4 h of incubation, total RNA was prepared from the mycelia and subjected to RT-PCR analysis. Transcripts of the *hrdB* gene are shown as controls. Positions of PCR products corresponding to the expected sizes are indicated by arrows.

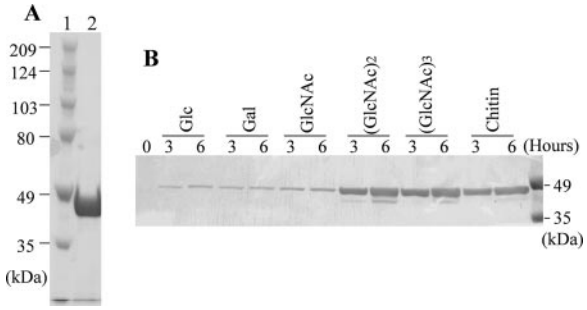

FIG. 4. (A) Purification of recombinant DasA protein. Six-His-tagged DasA protein was overproduced as a soluble form in the cytoplasmic fraction of *Escherichia coli* and purified using nickel affinity chromatography. The purified protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Size markers are given. Lanes: 1, molecular size standards; 2, DasA. (B) Production of DasA protein in *Streptomyces coelicolor* A3(2) M145 in the presence of a series of sugars. Refer to the legend of Fig. 1A for the culture conditions of *Streptomyces coelicolor* A3(2) M145, except that 1 mM of Glc, Gal, GlcNAc, $(GlcNAc)_2$, or $(GlcNAc)_3$ or 0.05% (wt/vol) colloidal chitin was added at time zero. Mycelia were harvested at 3 and 6 h and disrupted by sonication. Soluble proteins (50 μg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then DasA protein was detected using *anti*-DasA antiserum. Size markers are indicated in kDa.

TABLE 3. Kinetic parameter for binding of various saccharides to DasA

Saccharide	Kinetic parameter		
	k_a (M^{-1} s^1)[a]	k_d (s^{-1})[b]	K_D (M)
(GlcNAc)	1.77×10^2	4.49×10^{-3}	2.54×10^{-5}
$(GlcNAc)_2$	9.36×10^5	3.01×10^{-2}	3.22×10^{-8}
$(GlcNAc)_3$	1.16×10^5	1.16×10^{-1}	9.99×10^{-7}
$(GlcNAc)_4$	2.29×10^3	2.09×10^{-2}	9.13×10^{-6}
$(GlcNAc)_5$	2.04×10^3	1.79×10^{-2}	8.76×10^{-6}

[a] Rate constant for association.
[b] Rate constant for dissociation.

mutant ASC2 were washed, suspended in MM supplemented with 250 μM GlcNAc, $(GlcNAc)_2$, or $(GlcNAc)_3$, and further cultivated at 30°C. The culture was sampled periodically, and the GlcNAc, $(GlcNAc)_2$, and/or $(GlcNAc)_3$ concentrations in the culture supernatant were measured. When GlcNAc was added to the culture, the patterns of decline of the GlcNAc concentration in the M145 and ASC2 culture supernatants were comparable to each other (Fig. 5A). The data indicated that the *dasA* disruption did not affect GlcNAc transport. In contrast, when $(GlcNAc)_2$ was added, the sugar decline pattern in ASC2 differed from that in M145 (Fig. 5B). In strain M145, $(GlcNAc)_2$ had declined markedly, to 37 μM, at 4 h and was no longer detected after 6 h, whereas in ASC2 the $(GlcNAc)_2$ concentrations at 6 and 8 h were 119 μM and 54 μM, respectively. Hydrolysis of $(GlcNAc)_2$ into GlcNAc in the culture supernatant was minimal in both strains. The rates of decline of the $(GlcNAc)_2$ concentration, which were calculated from the data obtained at 2 and 4 h, were 83 μM/h in M145 and 17 μM/h in ASC2. The data clearly indicated that the *dasA* gene is involved in $(GlcNAc)_2$ uptake in M145. When $(GlcNAc)_3$ was added at 0 h, it was apparent that $(GlcNAc)_3$ immediately began to be hydrolyzed into GlcNAc and $(GlcNAc)_2$, and the mono- and disaccharides accumulated until the 4-h point in both strains (Fig. 5C). The accumulated sugars were not detected at 6 h in M145, whereas they remained in the culture supernatant of ASC2. The data suggested that $(GlcNAc)_3$ was not directly transported but was instead taken up following hydrolysis to GlcNAc and $(GlcNAc)_2$. We thus concluded that *dasA* is involved in $(GlcNAc)_2$ uptake, but not in GlcNAc transport, and is unlikely to be involved in $(GlcNAc)_3$ transport. The growth rate of ASC2 in MM containing chitin was comparable to that of M145 (data not shown), possibly because of the remaining $(GlcNAc)_2$ uptake system in ASC2.

The *dasABC* gene cluster is apparently composed of two transcriptional units. Upstream of the *dasA* gene, the transcriptional regulator gene *dasR* (24) is divergently located, whereas two ORFs (SCO5233 and SCO5234) encoding putative integral membrane proteins are present downstream of *dasA* (see Fig. 2B). On the basis of both the amino acid sequences and the gene organization, the two putative proteins encoded by SCO5233 and SCO5234 presumably act with DasA as subunits of an ABC transporter and were named *dasB* and *dasC*, respectively, as in *S. griseus* (35). First, we investigated the transcriptional unit, as the *dasABC* genes seemed to be organized like an operon. Five primer sets were designed to detect the transcripts of *dasA*, *dasAB*, *dasB*, *dasBC*, and *dasR* (Table 1; Fig. 6A). Total RNA was prepared from mycelia submerged for 2 h in MM containing 250 μM $(GlcNAc)_2$. The transcripts of *dasA*, *dasB*, *dasBC*, and *dasR* were reverse transcribed and amplified by PCR, but no part of the polysystronic transcripts of *dasAB* was detected (Fig. 6B). The data suggested that the *dasA* gene was monosystronically transcribed, whereas *dasB* and *dasC* were transcribed together as one unit.

Transcription of *dasR* and *dasB* is also induced by $(GlcNAc)_2$ and $(GlcNAc)_3$. Physiological conditions for the transcription of *dasR* and *dasB* were investigated by preparing total RNAs from mycelia exposed to a series of sugars. As we had already observed in *dasA*, transcription of *dasR* and *dasB* was induced in the presence of $(GlcNAc)_2$ and $(GlcNAc)_3$, although the *dasR* and *dasB* transcripts, unlike *dasA*, did not increase in the presence of $(GlcN)_2$ (see Fig. 3). To gain an understanding of the mechanisms of transcriptional regulation of the three genes, we compared the induction patterns of *dasA*, *dasB*, and *dasR* with that of *chiC*, which is one of the chitinase genes. The transcription of *chiC* peaked 2 h after the addition of 250 μM $(GlcNAc)_2$, had declined dramatically by 4 h, and was not apparent at 6 h (Fig. 6C), as reported previously (30). The transcriptional patterns of *dasR* and *dasB* were similar to the pattern of *chiC*, whereas *dasA* showed a different expression pattern (Fig. 6C). Induction of *dasA* transcription was observed 1 h earlier than that of *chiC*, and the transcription level remained high for longer (a total of at least 3 h). The results

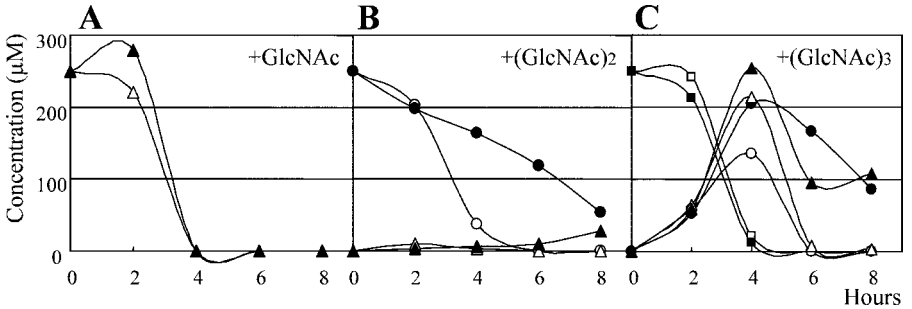

FIG. 5. Dynamics of GlcNAc, $(GlcNAc)_2$, and $(GlcNAc)_3$ concentrations in the culture supernatants of *Streptomyces coelicolor* A3(2) M145 (open symbols) and its *dasA* mutant ASC2 (closed symbols). Refer to the legend of Fig. 1A for the culture conditions of *Streptomyces coelicolor* A3(2) strains, except that 250 μM GlcNAc (A), $(GlcNAc)_2$ (B), or $(GlcNAc)_3$ (C) was added at time zero. The supernatants were periodically sampled and subjected to measurement of the concentrations of GlcNAc (triangles), $(GlcNAc)_2$ (circles), and $(GlcNAc)_3$ (squares). The experiment was performed twice with similar results, one of which is shown.

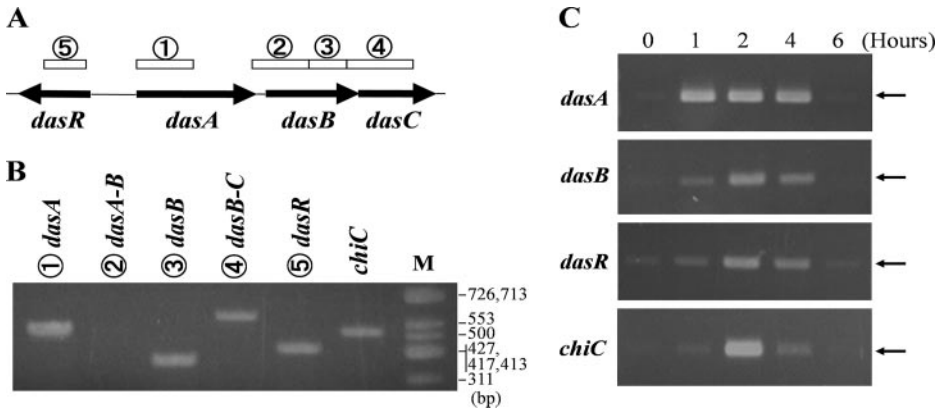

FIG. 6. Transcriptional analyses of the *dasRABC* cluster. (A) Positions of DNA fragments targeted to be amplified by the designed primers. Five primer sets were designed to detect transcripts with RT-PCR: 1, *dasA*; 2, *dasAB*; 3, *dasB*; 4, *dasBC*; and 5, *dasR* (see also Table 1). (B) RT-PCR for determination of transcriptional linkages of *dasABC*. Refer to the legend of Fig. 1A for the culture conditions of *Streptomyces coelicolor* A3(2) M145. Total RNAs were prepared from mycelia incubated for 2 h in the presence of 250 μM $(GlcNAc)_2$. PCR products were separated in an agarose gel. Size markers (ϕX174/HinfI) are shown in kilobases and kilobase pairs. (C) Patterns of transcriptional induction of the *dasA*, *dasB*, *dasR*, and *chiC* genes. Following pregrowth in LB medium, mycelia were incubated in MM with 250 μM $(GlcNAc)_2$ at 30°C with shaking at 150 rpm (see Materials and Methods for detailed conditions). Total RNAs were prepared from mycelia incubated for 1, 2, 4, and 6 h and subjected to RT-PCR analysis. The PCR products were separated in agarose gels. Positions of PCR products corresponding to the expected sizes are indicated by arrows.

implied that the mechanisms of induction of transcription of *dasA* might differ from those of *dasB*, *dasR*, and *chiC*.

***The dasA*-null mutant shows higher chitinase activity.** As $(GlcNAc)_2$ induces chitinase production in *S. coelicolor* A3(2) M145 (30), we hypothesized that disruption of *dasA*, which is involved in the uptake of $(GlcNAc)_2$, would affect chitinase production. In the *dasA*-null mutant ASC2, chitinase production was induced by the addition of 250 μM $(GlcNAc)_2$, and the pattern was similar to that in M145 until 2 h after addition (Fig. 7A). However, chitinase activity in ASC2 was still increasing even after 8 h, whereas the activity reached its highest level at 4 h in M145. The data indicated that the duration of chitinase production in ASC2 was longer than in M145. To confirm the longer duration of chitinase production at the level of transcription, *chiC* expression was investigated by RT-PCR. *chiC* transcription continued for longer than 4 h in ASC2, whereas in M145 it declined after peaking at 2 h (Fig. 7B). The data demonstrated that *dasA* disruption conferred longer induction of *chiC* transcription and higher chitinase activity as a result. The reduced rate of uptake of $(GlcNAc)_2$ in ASC2 and the resulting longer presence of residual $(GlcNAc)_2$ in the culture supernatant (Fig. 5B) would account for the longer duration of chitinase production and *chiC* transcription.

DISCUSSION

Although chitin degradation systems have been investigated in streptomycetes for decades, systems for the uptake of the products of degradation of chitin had not been identified until the *ngcEFG* gene cluster was identified as encoding the ABC transporter Ngc, which is involved in the uptake of GlcNAc, and probably $(GlcNAc)_2$, in *S. olivaceoviridis* (40). The sugar-binding protein NgcE of the Ngc system exhibits the highest affinity for GlcNAc (K_D = 8.3 nM), followed by $(GlcNAc)_2$ (K_D = 29 nM), and has lower affinities for higher oligomers (40). In contrast, the DasA protein binds specifically to $(GlcNAc)_2$ in vitro (Table 3). It is generally known that solute-binding proteins confer specificity on cognate ABC transporters, capturing extracellular ligands with high affinity and delivering them to the membrane assembly for transport (39). The distinctive in vitro properties of DasA and NgcE are observed

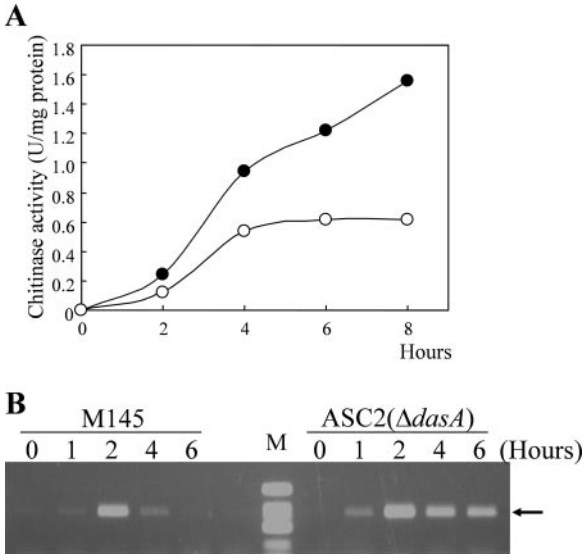

FIG. 7. Chitinase activity (A) and *chiC* transcription (B) in *Streptomyces coelicolor* A3(2) M145 (open circles) and its *dasA* mutant ASC2 (closed circles). Refer to the legend to Fig. 1A for the culture conditions of *Streptomyces coelicolor* A3(2) strains. At time zero, 250 μM $(GlcNAc)_2$ was added. Chitinase activity in the culture supernatant was expressed as mU per mg protein in mycelia. The *chiC* transcripts were detected by RT-PCR using specific primers (Table 1). Positions of PCR products corresponding to the expected size are indicated by the arrow. Size markers (M, ϕX174/HinfI) are also shown. The experiment was repeated three times to ensure reproducibility, and a typical result is indicated.

in vivo as well. The *ngcE* mutant shows reduced ^{14}C-labeled GlcNAc uptake activity, which is competitively inhibited by $(GlcNAc)_2$ (31, 40). On the other hand, *dasA* disruption does not affect the pattern of decline of the GlcNAc concentration in the culture supernatant, but it causes a marked reduction in the rate of decline of the $(GlcNAc)_2$ concentration (Fig. 5A and B). DasA and NgcE share only 20% amino acid identity. The tyrosine 201 and tryptophan 280 residues in NgcE, which contribute to the GlcNAc-binding avidity of the protein (31), are not conserved in the DasA protein. The distinctive binding characteristics and the low level of amino acid conservation imply the individual nature of the structures of the sugar-binding sites in DasA and NgcE.

The decline in $(GlcNAc)_2$ uptake activity was markedly reduced in the *dasA* mutant but still occurred (Fig. 5B). Putative ORFs (SCO6005, SCO6006, and SCO6007) corresponding to the *ngcEFG* genes of *S. olivaceoviridis* are present in the *S. coelicolor* A3(2) genome. NgcE has 34% amino acid identity with the deduced product of SCO6005. The *ngcEFG* homologs may act as other transporters of $(GlcNAc)_2$ uptake in the bacterium, as has been proposed previously (23). As is shown in Fig. 5A, *dasA* is not involved in the uptake of GlcNAc, whereas the uptake of GlcNAc generated by the breakdown of $(GlcNAc)_3$ was not completed in ASC2 (Fig. 5C). $(GlcNAc)_2$ might have inhibited the NgcE-mediated GlcNAc uptake, which in *S. olivaceoviridis* is involved in the uptake of GlcNAc as well as $(GlcNAc)_2$ (31, 40).

The transcript of the *dasA* gene is likely to be monocistronic, whereas the *dasB* and *dasC* genes are apparently transcribed together (Fig. 6B), as has already been observed in the *dasABC* cluster in *S. griseus* (35). The distinctive patterns of transcriptional induction (Fig. 6C) support the conclusion that the *dasA* and *dasB* genes are transcribed individually. A putative promoter sequence (-35 and -10) and an inverted repeated sequence, possibly functioning as a rho-independent transcriptional terminator, are present upstream and downstream, respectively, of *dasA* in *S. coelicolor* A3(2). In contrast, these sequences are not found flanking *dasBC*. Downstream of *dasC*, an ORF (SCO5235) predicted to encode β-*N*-acetylglucosaminidase belonging to family 20 of the glycosylhydrolase classification (10, 11) is adjacent in the same orientation. SCO5235 may be transcribed with *dasBC*, and the product of the ORF would serve for the hydrolysis of $(GlcNAc)_2$, which is taken up via the DasABC system.

In *S. griseus*, DasA is involved in the regulation of morphological differentiation in response to Glc and has been speculated to bind Glc or a Glc derivative (35). Although the DasA proteins of *S. griseus* and *S. coelicolor* A3(2) show only 34% amino acid identity with each other, it would be interesting to investigate the involvement of DasA in the morphological differentiation, as well as to compare the binding specificities of the DasA proteins, of these bacteria.

ACKNOWLEDGMENTS

We thank Yusuke Kameoka at Meiji University for his help in measuring amino sugar concentrations.

A.S. acknowledges a research fellowship (January 2003 to February 2004) from the Japanese Society for the Promotion of Science (JSPS). This work was partly supported by a grant-in-aid for JSPS fellows to A.S. (2003) and subsequently by a Ministry of Education, Science, Sports and Culture grant-in-aid for young scientists (B), grant 17780056 (2005–2006).

REFERENCES

1. **Alting-Mees, M. A., and J. M. Short.** 1989. pBluescript II: gene mapping vectors. Nucleic Acids Res. **17:**9494.
2. **Bentley, S. D., K. F. Chater, A. M. Cerdeno-Tarraga, G. L. Challis, N. R. Thomson, K. D. James, D. E. Harris, M. A. Quail, H. Kieser, D. Harper, A. Bateman, S. Brown, G. Chandra, C. W. Chen, M. Collins, A. Cronin, A. Fraser, A. Goble, J. Hidalgo, T. Hornsby, S. Howarth, C. F. Huang, T. Kieser, L. Larke, L. Murphy, K. Oliver, S. O'Neil, E. Rabbinowitsch, M. A. Rajandream, K. Rutherford, S. Rutter, K. Seeger, D. Saunders, S. Sharp, R. Squares, S. Squares, K. Taylor, T. Warren, A. Wietzorrek, J. Woodward, B. G. Barrell, J. Parkhill, and D. A. Hopwood.** 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature **417:**141–147.
3. **Blaak, H., J. Schellmann, S. Walter, B. Henrissat, and H. Schrempf.** 1993. Characteristics of an exochitinase from *Streptomyces olivaceoviridis*, its corresponding gene, putative protein domains and relationship to other chitinases. Eur. J. Biochem. **214:**659–669.
4. **Blondelet-Rouault, M. H., J. Weiser, A. Lebrihi, P. Branny, and J. L. Pernodet.** 1997. Antibiotic resistance gene cassettes derived from the omega interposon for use in *E. coli* and *Streptomyces*. Gene **190:**315–317.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
6. **Coutinho, P. M., and B. Henrissat.** 1999. Carbohydrate-active enzymes: an integrated database approach, p. 3–12. *In* H. J. Gilbert, G. Davies, B. Henrissast, and B. Svensson (ed.), Recent advances in carbohydrate bioengineering. The Royal Society of Chemistry, Cambridge, United Kingdom.
7. **Dassa, E., and P. Bouige.** 2001. The ABC of ABCs: a phylogenetic and functional classification of ABC systems in living organisms. Res. Microbiol. **152:**211–229.
8. **Delic, I., P. Robbins, and J. Westpheling.** 1992. Direct repeat sequences are implicated in the regulation of two *Streptomyces* chitinase promoters that are subjected to carbon catabolite control. Proc. Natl. Acad. Sci. USA **89:**1885–1889.
9. **Fujii, T., and K. Miyashita.** 1993. Multiple domain structure in a chitinase gene (*chiC*) of *Streptomyces lividans*. J. Gen. Microbiol. **139:**677–686.
10. **Henrissat, B.** 1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. **280:**309–316.
11. **Henrissat, B., and A. Bairoch.** 1993. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. **293:**781–788.
12. **Kawase, T., S. Yokoyama, A. Saito, T. Fujii, N. Nikaidou, K. Miyashita, and T. Watanabe.** 2006. Comparison of enzymatic and antifungal properties between family 18 and 19 chitinases from *S. coelicolor* A3(2). Biosci. Biotechnol. Biochem. **70:**988–998.
13. **Kieser, T., M. J. Bibb, M. J. Buttner, K. F. Chater, and D. A. Hopwood.** 2000. Practical *Streptomyces* genetics. John Innes Foundation, Norwich, United Kingdom.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
15. **MacNeil, D. J., K. M. Gewain, C. L. Ruby, G. Dezeny, P. H. Gibbons, and T. MacNeil.** 1992. Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilizing a novel integration vector. Gene **111:**61–68.
16. **Miyashita, K., and T. Fujii.** 1993. Nucleotide sequence and analysis of a gene (*chiA*) for a chitinase from *Streptomyces lividans* 66. Biosci. Biotechnol. Biochem. **57:**1691–1698.
17. **Miyashita, K., T. Fujii, and A. Saito.** 2000. Induction and repression of a *Streptomyces lividans* chitinase gene promoter in response to various carbon sources. Biosci. Biotechnol. Biochem. **64:**39–43.
18. **Miyashita, K., T. Fujii, and Y. Sawada.** 1991. Molecular cloning and characterization of chitinase genes from *Streptomyces lividans* 66. J. Gen. Microbiol. **137:**2065–2072.
19. **Muth, G., B. Nußbaumer, W. Wohlleben, and A. Pühler.** 1989. A vector system with temperature-sensitive replication for gene disruption and mutational cloning in streptomycetes. Mol. Gen. Genet. **219:**341–348.
20. **Ni, X., and J. Westpheling.** 1997. Direct repeat sequences in the *Streptomyces* chitinase-63 promoter direct both glucose repression and chitin induction. Proc. Natl. Acad. Sci. USA **94:**13116–13121.
21. **Ohno, T., S. Armand, T. Hata, N. Nikaidou, B. Henrissat, M. Mitsutomi, and T. Watanabe.** 1996. A modular family 19 chitinase found in the prokaryotic organism *Streptomyces griseus* HUT6037. J. Bacteriol. **178:**5065–5070.
22. **Orikoshi, H., S. Nakayama, K. Miyamoto, C. Hatano, M. Yasuda, Y. Inamori, and H. Tsujibo.** 2005. Roles of four chitinases (ChiA, ChiB, ChiC, and ChiD) in the chitin degradation system of marine bacterium *Alteromonas* sp. strain O-7. Appl. Environ. Microbiol. **71:**1811–1815.
23. **Rigali, S., H. Nothaft, E. E. Noens, M. Schlicht, S. Colson, M. Müller, B. Joris, H. K. Koerten, D. A. Hopwood, F. Titgemeyer, and G. P. van Wesel.** 2006. The sugar phosphotransferase system of *Streptomyces coelicolor* is

regulated by the GntR-family regulator DasR and links *N*-acetylglucosamine metabolism to the control of development. Mol. Microbiol. **61:**1237–1251.
24. **Rigali, S., M. Schlicht, P. Hokisson, H. Nothaft, M. Merzbacher, B. Joris, and F. Titgemeyer.** 2004. Extending the classification of bacterial transcription factors beyond the helix-turn-helix motif as an alternative approach to discover new *cis*/*trans* relationships. Nucleic Acid Res. **32:**3418–3426.
25. **Robbins, P. W., K. Overbye, C. Albright, B. Benfield, and J. Pero.** 1992. Cloning and high-level expression of chitinase-encoding gene of *Streptomyces plicatus*. Gene **111:**69–76.
26. **Saier, M. H. Jr.** 2000. Families of transmembrane sugar transport proteins. Mol. Microbiol. **35:**699–710.
27. **Saito, A., T. Fujii, and K. Miyashita.** 1999. Chitinase system in *Streptomyces*. Actinomycetologica **13:**1–10.
28. **Saito, A., T. Fujii, T. Yoneyama, and K. Miyashita.** 1998. *glkA* is involved in glucose repression of chitinase production in *Streptomyces lividans*. J. Bacteriol. **180:**2911–2914.
29. **Saito, A., T. Fujii, T. Yoneyama, M. Redenbach, T. Ohno, T. Watanabe, and K. Miyashita.** 1999. High-multiplicity of chitinase genes in *Streptomyces coelicolor* A3(2). Biosci. Biotechnol. Biochem. **63:**710–718.
30. **Saito, A., M. Ishizaka, P. B. F. Jr., T. Fujii, and K. Miyashita.** 2000. Transcriptional co-regulation of five chitinase genes scattered on the *Streptomyces coelicolor* A3(2) chromosome. Microbiology **146:**2937–2946.
31. **Saito, A., and H. Schrempf.** 2004. Mutational analysis of the binding affinity and transport activity for *N*-acetylglucosamine of the novel ABC transporter Ngc in the chitin-degrader *Streptomyces olivaceoviridis*. Mol. Genet. Genomics **271:**545–553.
32. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
33. **Saurin, W., W. Köster, and E. Dassa.** 1994. Bacterial binding protein-dependent permeases: characterization of distinctive signatures for functionally related integral cytoplasmic membrane proteins. Mol. Microbiol. **12:**993–1004.
34. **Schlochtermeier, A., A. Walter, J. Schröder, M. Moormann, and H. Schrempf.** 1992. The gene encoding the cellulase (Avicelase) CelA from *Streptomyces reticuli* and analysis of protein domain. Mol. Microbiol. **6:**3611–3621.
35. **Seo, J., Y. Ohnishi, A. Hirata, and S. Horinouchi.** 2002. ATP-binding cassette transport system involved in regulation of morphological differentiation in response to glucose in *Streptomyces griseus*. J. Bacteriol. **184:**91–103.
36. **Tsujibo, H., M. Kosaka, S. Ikenishi, T. Sato, K. Miyamoto, and Y. Inamori.** 2004. Molecular characterization of a high-affinity xylobiose transporter of *Streptomyces thermoviolaceus* OPC-520 and its transcriptional regulation. J. Bacteriol. **186:**1029–1037.
37. **van Wesel, G. P., J. White, P. Young, P. W. Postma, and M. J. Bibb.** 1997. Substrate induction and glucose repression of maltose utilization by *Streptomyces coelicolor* A3(2) is controlled by *malR*, a member of the *lacI-galR* family of regulatory genes. Mol. Microbiol. **23:**537–549.
38. **Wang, F., X. Xiao, A. Saito, and H. Schrempf.** 2002. *Streptomyces olivaceoviridis* possesses a phosphotransferase system that mediates specific, phosphoenolpyruvate-dependent uptake of *N*-acetylglucosamine. Mol. Genet. Genomics **268:**344–351.
39. **Wilkinson, A. J., and K. H. G. Verschueren.** 2003. Crystal structures of periplasmic solute-binding proteins in ABC transport complexes illuminate their function, p. 187–207. *In* I. B. Holland, S. P. C. Cole, K. Kuchler, and C. F. Higgins (ed.), ABC proteins from bacteria to man. Academic Press, London, United Kingdom.
40. **Xiao, X., F. Wang, A. Saito, J. Majka, A. Schlösser, and H. Schrempf.** 2002. The novel *Streptomyces olivaceoviridis* ABC transporter Ngc mediates uptake of *N*-acetylglucosamine and *N*,*N*′-diacetylchitobiose. Mol. Genet. Genomics **267:**429–439.
41. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.