SUPPLEMENTAL RESULTS

Expression of the asuD1-D3 Operon in S. lividans. To confirm the *asuD1-D3* involvement in C_5N moiety formation, the *asuD1-D3* operon was cloned into pIJ622 under the control of the constitutive *ermE** promoter to generate pAS9A1 and expressed in *S. lividans*. A novel brown metabolite was detected in the culture, but further attempts to identify the compound have failed so far. Feeding ferulic acid to this strain, a new compound was obtained with a molecular weight of 290.097. MALDI-TOF MS-MS analysis of the TLC purified compound revealed an identical fragmentation pattern as that reported for feruloyl-*N*-(3-hydroxycyclopent-2-enolone)acrylamide (2880-II), which carries a C_5N moiety attached to the carboxyl group of ferulic acid (Supplemental Fig. 2) (1). 2880-II was not formed when any one of the *asuD1*, *asuD2* or *asuD3* was omitted from the expression cassette.

SUPPLEMENTAL METHODS

Fatty Acid Analysis. The mycelia of the fermentation cultures were collected, washed three times with 10% glycerol and mixed with 3 mL MeOH:$CHCl_3$ (2:1) in a glass homogenizer. The mixture was separated by centrifugation, and the supernatant was further rinsed three times with 1 mL of water. The organic layer was extracted, hydrolyzed and the methyl esters of the fatty acids were prepared (2,3). The GC-MS analysis was conducted on a Trace Gas Chromatograph (Thermo Quest Inc. Schaumburg, IL, USA) using a 30 m x 0.25 mm DB-225 column with a 0.15 mm film thickness (J&W Scientific, Folsom, CA). The flow rate of the carrier gas ultrapure helium was 1.5 ml/min and the GC program was as follows: 60°C for 1 min; 15°C/min to 200°C; 5°C/min to 260°C and hold for 10min. The peak areas were recorded by XCalibur-Qual Browser software (Thermo Quest Inc. Schaumburg, IL, USA). The signals of the fatty acid methyl esters were identified by comparison with authentic samples purchased from Sigma, NuCheck Prep.

Construction and Screening of the Genomic Library. Molecular biology procedures and DNA manipulations were carried out according to standard protocols. The cosmid genomic library of *S. nodosus* subsp. *asukaensis* was constructed by cloning the *Sau*3AI partially digested genomic DNA into pOJ446, an *E. coli*-S*treptomyces* shuttle vector. Ligation mixtures were further packaged with the Gigapack III Gold Packaging kit (Stratagene) and transfected into *E. coli* SURE strain (Stratagene). Two ^{32}P-labled DNA probes, the *chcA* and *asuD2* genes, were used for screening the constructed cosmid library.

Mutant Constructions. To construct mutants by antibiotic resistance gene insertion, the target gene-containing DNA fragment was cloned into the *Bam*HI site of the pGM160 vector (4). The internal coding region with or without the junction region were deleted or replaced with either the 1.4 kb apramycin resistance gene or the 0.8 kb kanamycin resistance gene from pOJ446 or pBluescript, respectively (4) (Supplemental Fig. 4). The truncated plasmid clones were delivered by polyethylene glycol-based transformation into *S. nodosus* subsp. *asukaensis*, and integrated into the chromosome forced by incubating the transformants at 39°C with continuing selection for thiostrepton resistance. One or two rounds of streaking at 39°C in the absence of thiostrepton allowed the identification of thiostrepton-sensitive strains, in which the targeted genes were replaced with the antibiotic resistance gene. The resulting recombinants were further confirmed by Southern hybridization analysis (Supplemental Fig. 4), except for the mutant *asuA2*, which was verified by chromosome walking and genomic sequencing.

To construct the *asuE1* mutant, the 1.5 kb upstream and downstream regions, including 100 bp of *asuE1*, were amplified by PCR and tandem cloned in the pHGF9050 vector. The four oligonucleotide primers used, AsuE1-1 to -4, are listed in Supplemental Table 3. The resulting pART1334 was transformed into *E. coli* ET12567/pUZ8002 and conjugated with *S. nodocus* subsp. *asukaensis* wild type (4). The apramycin resistant recombinants resulting from the homologous recombination between the plasmid and the wild type *S. nodosus* were further

selected, subcultured in MS liquid medium for 1-2 rounds, and screened for apramycin sensitive recombinants derived from a second crossover event. The double crossover recombinant was confirmed by Southern hybridization analysis (Supplemental Fig. 4).

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