A Novel Epimerization System in Fungal Secondary Metabolism Involved in the Conversion of Isopenicillin N into Penicillin N in Acremonium chrysogenum*

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The epimerization step that converts isopenicillin N into penicillin N during cephalosporin biosynthesis has remained uncharacterized despite its industrial relevance. A transcriptional analysis of a 9-kb region located downstream of the pbcC gene revealed the presence of two transcripts that correspond to the genes named cefD1 and cefD2 encoding proteins with high similarity to long chain acyl-CoA synthetases and acyl-CoA racemases from Mus musculus, Homo sapiens, and Rattus norvegicus. Both genes are expressed in opposite orientations from a bidirectional promoter region. Targeted inactivation of cefD1 and cefD2 was achieved by the two-marker gene replacement procedure. Disrupted strains lacked isopenicillin N epimerase activity, were blocked in cephalosporin C production, and accumulated isopenicillin N. Complementation in trans of the disrupted nonproducer mutant with both genes restored epimerase activity and cephalosporin biosynthesis. However, when cefD1 or cefD2 were introduced separately into the double-disrupted mutant, no epimerase activity was detected, indicating that the concerted action of both proteins encoded by cefD1 and cefD2 is required for epimerization of isopenicillin N into penicillin N. This epimerization system occurs in eukaryotic cells and is entirely different from the known epimerization systems involved in the biosynthesis of bacterial β-lactam antibiotics.

The molecular genetics of cephalosporin biosynthesis is an excellent model for secondary metabolism, since considerable information on the enzymeology (reviewed in Refs. 1 and 2), molecular genetics, and gene expression mechanisms (3–5) has accumulated in the last few years. The biosynthesis of cephalosporins by Acremonium chrysogenum and cephemycins by Amycolatopsis (Nocardia) lactamdurans and Streptomyces clavuligerus (reviewed in Refs. 6 and 7) begins with the formation of the tripeptide δ-(L-α-aminoacipetyl)-L-cysteinyl-β-valine (ACV) by the ACV synthetase (8), followed by cyclization of ACV to isopenicillin N (IPN). IPN is later converted into penicillin N by an epimerase activity that has remained uncharacterized so far in A. chrysogenum. After the epimerization step, penicillin N is transformed by a deacetoxycephalosporin C synthase (expandase) into deacetoxycephalosporin C, and finally, deacetoxycephalosporin C is converted into deacetylcephalosporin C (DAC) and cephalosporin C by DAC synthase (hydroxylase) and DAC acetyltransferase, respectively (Fig. 1).

The genes pbcAB (9) and pbcC (10) encoding ACV synthetase and IPN synthase are linked together in chromosome VII in the so-called “early cephalosporin gene cluster” (11). The genes cefEF, encoding the bifunctional expandase-hydroxylase (12), and cefG, encoding the DAC acetyltransferase (13, 14), are linked together in the so-called “late cephalosporin cluster” in chromosome I (11).

The isopenicillin N epimerization step still remains unclear. Demain and co-workers (15, 16) reported that isopenicillin N was converted into penicillin N by extracts of A. chrysogenum, although the epimerizing enzyme was extremely labile (17, 18), preventing purification of the protein. However, the isopenicillin N epimerase has been purified from S. clavuligerus (19, 20) and A. lactamdurans (21), and the cefD gene encoding this protein was cloned from both microorganisms (22, 23). Repeated attempts to clone the homologous cefD gene of A. chrysogenum using as probe the bacterial cefD gene or oligonucleotides based on bacterial epimerase conserved amino acid regions with the A. chrysogenum preferred codon usage were unsuccessful.2 These results suggested that the fungal IPN epimerization system was different from the bacterial one.

In this article, we describe that two genes contain genetic information required for the epimerization step converting isopenicillin N into penicillin N in A. chrysogenum. The corresponding gene products act in a two-protein system described for the first time in antibiotic biosynthesis. The two genes (named cefD1 and cefD2) are completely different from the cefD gene of A. lactamdurans and S. clavuligerus.

EXPERIMENTAL PROCEDURES

Microbial Strains, Media, and Culture Conditions—A. chrysogenum C10 (ATCC 48278), a strain producing high levels of cephalosporin C, was used for the genetic studies. Escherichia coli ESS2321, a β-lactam supersensitive strain, was used for routine cephalosporin bioassays.

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1 The abbreviations used are: ACV, δ-(L-α-aminoacipetyl)-L-cysteinyl-β-valine; IPN, isopenicillin N; DAC, deacetoxycephalosporin C; HPLC, high pressure liquid chromatograph; GITC, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate.

2 S. Gutiérrez and J. F. Martín, unpublished results.
Escherichia coli DH5α and E. coli WK6 were used for plasmid purification (double-stranded or single-stranded DNA, respectively). All A. chrysogenum strains were first grown in seed medium (24) for 48 h. Ten ml of seed culture was used to inoculate 100 ml of defined production medium (MDFA) (24) in 500-ml triple-baffled flasks. The cultures were incubated at 25 °C in a rotary shaker at 250 rpm. The levels of cephalosporin antibiotics were determined by bioassay using E. coli ESS2231 as the test strain in plates with penicillinase from Bacillus cereus UL1 as described previously (13).

Plasmids Containing the cefD1 and/or cefD2 Genes—Several constructions named pCD4.5, pCD1, pCD2, and pCD1+2 were obtained from plasmid pJL43 (9).

pD4.5 was obtained from pJL43 by inserting a 6.8-kb SalI fragment bearing the cefD1, cefD2, and bidirectional promoter region (see Fig. 5). SalI and BglII restriction sites were introduced by in vitro mutagenesis in the cefD1 and cefD2 genes, respectively. Finally, the hygromycin resistance (hph) cassette, subcloned from pAN7-1 (25), was inserted in the BglII site to inactivate the cefD2 gene.

pCD1+2 was obtained by inserting a 5.8-kb BamHI-EcoRV fragment bearing the cefD1 and cefD2 genes into the BamHI-EcoRI site of pC43 (26). pCD1 containing the cefD1 gene was constructed by partial digestion of pCD1+2 with SacI and religation.

pCD2 contains a 3.5-kb BamHI-SalI fragment bearing the cefD2 gene inserted into the BamHI-EcoRI site of pC43.

Transformation of A. chrysogenum—Transformation of A. chrysogenum protoplasts was performed as described previously (27).

DNA Sequencing and Intron Analysis—DNA fragments were subcloned into pBluescript SK+/EBI Data Bank under the accession number AJ507632.

Site-directed Mutagenesis—in vitro mutagenesis was performed with the QuikChange site-directed mutagenesis kit (Stratagene) by following the manufacturer’s instructions. Oligonucleotides A1 (5'-CCGCTTACCGTGACGCAAAC-3') and A2 (5'-GCTTGCGGCTGACGCCAG-3') were used in the formation of a SacI site in the cefD1 gene.

Similarly, a BglII site in the cefD2 gene was obtained with the
FIG. 3. Amino acid sequence of the CefD1 protein. Alignment of the deduced amino acid sequences of the protein encoded by the cefD1 gene of A. chrysogenum and the long chain acyl-CoA synthetases of H. sapiens (vlacs), R. norvegicus (vlacs), and M. musculus (vlacs). Only the identical amino acids are shaded. The consensus motifs A–H of acetyl-CoA ligases with functionally conserved amino acids are overlined.

FIG. 4. Amino acid sequence of the CefD2 protein. Alignment of the deduced amino acid sequences of the protein encoded by the cefD2 gene of A. chrysogenum, with the acyl-CoA racemases of H. sapiens (rm), M. musculus (mcar), and R. norvegicus (p-EP1). Identical amino acids are shaded.
oligonucleotides B1 (5'-GGCGCCATAGATCTGCCAAGAGCATGC-3') and B2 (5'-GCATGCTCTTGGCAGATCTATGGCGCC-3').

Southern and Northern Blotting and DNA Hybridizations—Genomic DNA of A. chrysogenum was isolated as described previously (27). Three g of genomic DNA from A. chrysogenum C10 or its transformants were digested with EcoRI and the fragments were resolved in a 0.7% agarose gel. The gel was blotted onto Hybond-N membranes (Amersham Biosciences) and hybridized with different probes (see "Results") labeled with [32P]dCTP (30).

RNA Isolation and Northern Analysis—Total A. chrysogenum RNA was isolated with the RNeasy Kit (Qiagen). Total RNA was resolved by agarose-formaldehyde gel electrophoresis and blotted onto Hybond-N membranes (Amersham Biosciences) as described by Sambrook et al. (30).

Hybridization probes were labeled with [α-32P]dCTP by nick translation and purified by filtration through Wizard minicolumns (Promega). Hybridizations were carried out as described by Sambrook et al. (30). The intensity of the hybridization bands was determined by using a phosphor imager scanner (Instant Imager; Packard).

RESULTS

Transcript Map of the Region Located Downstream of the pcbC Gene—Since the genes encoding all other proteins involved in CPC biosynthesis are clustered in two separate loci, we hypothesized that the gene encoding the protein(s) involved by Neuss et al. (33). Isopenicillin N and penicillin N (500 μg/ml) were derivatized with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) (2 mg/ml in acetonitrile) for 2 h at pH 8.5 (adjusted with sodium bicarbonate). The HPLC separation was performed in a Beckman System Gold HPLC equipped with a μBondapack C18 column with a mobile phase of methanol/acetonitrile/acetic acid/water (36:7:2:55) with a flow of 1.2 ml/min.

IPN Epimerase Assay—The IPN epimerase assay was performed as described by Lubbe et al. (34). IPN epimerase activity was determined by measuring the in vitro conversion of isopenicillin into penicillin N. The results were quantified by a coupled reaction by determining the conversion of IPN into cephalosporin C (CPC). Cell-free extracts were prepared from A. chrysogenum cultures grown for 72 h. The enzymatic activity was expressed in units/mg of protein. One unit is the activity forming 1 ng of CPC/min in the coupled assay. The specific activity is given as units/mg of protein. Total protein concentration in the cell extract was measured by the Bradford assay.
mass of 71 kDa. The encoded protein showed similarity to long chain acyl-CoA synthetases (Fig. 3), particularly those from *Homo sapiens* (26.3% identical amino acids), *Rattus norvegicus* (25.5% identity), and *Mus musculus* (25.5% identity) (38). The ORF1-encoded protein has all the characteristic motifs of the acyl-CoA ligases involved in the activation (usually through an adenylation step) of the carboxyl group of fatty acids or amino acids (39).

ORF2 consists of 1146 nucleotides, and it is interrupted by the presence of one intron. RT-PCR studies using RNA from 48-h cultures of *A. chrysogenum* C10 confirmed the presence of the intron. A DNA band of the expected size (92 bp), assuming the splicing of the intron, was obtained by RT-PCR. The nucleotide sequence of the amplified DNA band showed that the intron had been removed at the splicing sites corresponding to nucleotides 64–157 numbered from the ATG translation initiation codon. ORF2 encoded a protein of 383 amino acids with a deduced molecular mass of 41.4 kDa. The encoded protein showed (Fig. 4) similarity to *α*-methyl-acyl-CoA racemases, particularly those from *H. sapiens* (42.1% identical amino acid) *M. musculus* (39.4% identity) and *R. norvegicus* (39.3%) (40, 41).

**Targeted Inactivation of ORF1 and ORF2 Results in Mutants Blocked in Cephalosporin Production—**To study the role of the proteins encoded by ORF1 and ORF2 in cephalosporin C biosynthesis, targeted inactivation of both genes was performed with the double marker technique (42) developed for targeted inactivation in *A. chrysogenum* (43).

To perform targeted inactivation of these genes, plasmid pD4.5 was constructed (Fig. 5A); this plasmid carries an incomplete ORF2 (obtained by insertion of the hygromycin resistance cassette) and ORF1 inactivated by a frameshift mutation introduced in *vitro*. Plasmid pD4.5 contains also the bleomycin resistance marker (Fig. 5).

Plasmid pD4.5 was transformed into *A. chrysogenum* C10, and transformants were selected by the resistance to hygromycin B. The transformants were screened for those clones showing a hyg<sup>R</sup> phle<sup>S</sup> phenotype, indicating that double recombination had occurred. Thirty-three transformants of the 250 clones tested showed the hyg<sup>R</sup> phle<sup>S</sup> phenotype. All of these 33 transformants were tested for cephalosporin production by the agar plug method. Nine transformants showed a drastic reduction in cephalosporin production, and seven additional transformants showed no cephalosporin production at all as compared with *A. chrysogenum* C10, suggesting that targeted inactivation had occurred in the mutants.

To confirm that targeted inactivation took place at the right position, the seven nonproducing transformants and two others with drastic reduction in cephalosporin production were analyzed by Southern blot. *A. chrysogenum* C10 and one transformant (Fig. 5B, lane 8) with no reduction in cephalosporin production were used as controls. The DNA of all strains was digested with *SalI* and hybridized with a probe internal to the bidirectional promoter region located between ORF1 and ORF2 (Fig. 5A). Results showed that the *A. chrysogenum* C10 (Fig. 5B, lane 11) hybridized with a genomic DNA band of 6.8 kb; however, in transformants with no cephalosporin production (Fig. 5B, lanes 3–7, 9, and 10), the 6.8-kb hybridization band was converted into a band of 3.8 kb, as expected, by a canonical double recombination (Fig. 5A). In transformants with a drastic reduction in cephalosporin production (Fig. 5B, lanes 1 and 2) the 6.8 kb is converted into a 5.0-kb hybridization signal, suggesting that a noncanonical recombination process had occurred at the ORF1-ORF2 locus. By contrast, in the transformant with no reduction in cephalosporin production (Fig. 5B, lane 8), two hybridization bands of 3.8 and 6.8 kb were present.
suggesting an ectopical integration of the pD4.5 plasmid. To study in more detail the effect of targeted inactivation of the ORF1 and ORF2, three transformants named TD167, TD189, and TD234 (lanes 3, 5, and 10 in Fig. 5B) were selected.

The Disrupted Transformants TD167, TD189, and TD234 Lacked Isopenicillin Epimerase Activity—To study whether proteins encoded by ORF1 and ORF2 were involved in the conversion of isopenicillin N into penicillin N, the epimerase activity was measured in cell extracts of three disrupted transformants (TD167, TD189, and TD234) and the parental strain A. chrysogenum C10. Results showed that the epimerase activity was absent in the ORF1-ORF2-disrupted strains, both at 72 and 96 h, whereas the A. chrysogenum C10 strain had a high level of IPN epimerase activity (92 units/mg of protein) at 72 h. These results clearly indicated that the proteins encoded by the ORF1 and ORF2 are involved in the epimerization step in cephalosporin C biosynthesis. Thus, the corresponding genes have been named cefD1 and cefD2 according to standard /H9252-lac-tam gene nomenclature, since the designation cefD is used to describe the bacterial epimerase gene (44).

The TD167, TD189, and TD234 Disrupted Strains Accumulate Isopenicillin N—If cefD1 and cefD2 encode the IPN epimerase, the TD167, TD189, and TD234 mutants should accumulate isopenicillin N. Results showed (Fig. 6A) that no cephalosporins were detected by bioassay in any of the disrupted strains, whereas in the same culture conditions high cephalosporin production was observed in either A. chrysogenum C10 or in the nondisrupted transformant. Analysis by HPLC of the culture broth supernatant (Fig. 6B) showed that the disrupted strains formed no traces of cephalosporin C, whereas in A. chrysogenum C10 cephalosporin C is produced.

The culture filtrates from the three disrupted strains, TD167, TD189, and TD234, and the parental A. chrysogenum C10 were treated with GITC (see “Experimental Procedures”), and the derivatized compounds were resolved by HPLC. Results showed that in A. chrysogenum C10 culture broth the peaks corresponding to both isopenicillin N and penicillin N were present (Fig. 7). However, in the three disrupted strains, the peak corresponding to penicillin N is absent, and at the same time there is an accumulation of isopenicillin N, confirming the previous evidence indicating that disrupted strains are blocked in isopenicillin N epimerase.

Complementation of Both cefD1 and cefD2 Mutations Is Required for Restoration of Epimerase Activity—To elucidate whether both the acyl-CoA synthetase and the acyl-racemase encoded by cefD1 and cefD2 or just one of them were involved in the conversion of isopenicillin N into penicillin N, a series of complemented strains lacking one or both of these genes was created.

For this purpose, plasmids pCD1, pCD2, and pCD1+2 bearing the cefD1, cefD2, or both genes, respectively, were constructed (Fig. 8). These three plasmids were transformed sep-
Fig. 8. Southern analysis showing the complementation in trans of the disrupted TD189 mutant with pCD1 and pCD2. A, plasmid pCD1 containing the cefD1 gene. Southern blot hybridization of SacI-digested genomic DNA from five transformants, untransformed A. chrysogenum C10, and mutant TD189, using as probe the 1-kb HindIII-EcoRV internal to the bidirectional promoter of cefD1 and cefD2. Lane 1, TCD1-28; lane 2, TCD1-32; lane 3, TCD1-38; lane 4, TCD1-49; lane 5, TCD1-90; lane 6, TD189 (disrupted mutant); lane 7, A. chrysogenum C10. The sizes of the hybridization bands are indicated on the right. B, plasmid pCD2 containing the cefD2 gene. Southern blot hybridization of ClaI + SpeI-digested genomic DNA from five transformants, untransformed A. chrysogenum C10, and mutant TD189 with the same probe internal to the bidirectional promoter of cefD1 and cefD2 genes. Lane 1, TCD2-28; lane 2, TCD2-32; lane 3, TCD2-38; lane 4, TCD2-49; lane 5, TCD2-90; lane 6, TD189 (disrupted mutant); lane 7, A. chrysogenum C10. The sizes of the hybridization bands are indicated on the right. C, plasmid pCD1+2 containing the cefD1 and cefD2 genes. Southern blot hybridization is shown of ClaI + SpeI-digested genomic DNA from three transformants, untransformed A. chrysogenum C10, and mutant TD189, with the same labeled probe internal to the bidirectional promoter. Lane 1, TCD1-24; lane 2, TCD1-15; lane 3, TCD2-28; lane 4, TD189; lane 5, A. chrysogenum C10. The sizes of the hybridization bands are indicated on the right. The 24-kb hybridization band corresponded to the endogenous band in the gene-disrupted clone TD189.

Table 1
Isopenicillin N epimerase activity in transformants TCD1+2, TCD2, and TCD2 derived from TD189 by complementation with cefD1+2, cefD1 and cefD2 genes, respectively

| Strain         | Genotype                        | Epimerase activity (units/mg of protein) |
|----------------|---------------------------------|------------------------------------------|
| A. chrysogenum | Wild type                       | 92                                       |
| A. chrysogenum | TD189 cefD1, cefD2::hph         | 0                                        |
| A. chrysogenum | TCD1-15                         | 105                                      |
| A. chrysogenum | TCD1-8 cefD2::hph               | 0                                        |
| A. chrysogenum | TCD2-49 cefD1                   | 0                                        |

strains), indicating that the insert of the plasmid pCD1 was intact.

Similarly, five transformants (TCD2) obtained with the pCD2 plasmid were selected, and their DNA was digested with a mixture of SpeI and ClaI and hybridized with the same probe (1 kb of the promoter region) as above. Results showed (Fig. 8B) a hybridization band of 5 kb in several transformants, indicating that the insert of plasmid pCD2 is intact. The large 24-kb hybridization band corresponded to the endogenous hybridizing band in the cefD1-cefD2 disrupted clone (TD189; lane 5), whereas the wild type A. chrysogenum gave a 20-kb hybridization band.

Finally, the DNA of three transformants (TCD1+2) obtained with the pCD1+2 plasmid was digested with a mixture of SpeI and ClaI and hybridized with the same 1-kb probe as above. Results showed (Fig. 8C) that the probe hybridized with a band of 7.3 kb, indicating that the insert of the plasmid pCD1+2 was integrated in an intact form. The 24-kb hybridization band corresponded to the endogenous hybridizing band in the cefD1-cefD2-disrupted clone (TD189).

To study which of the two ORFs were responsible for the epimerization step, the IPN epimerase activity was measured in three representative transformants TCD1, TCD2, and TCD1+2. Results showed (Table 1) that only in A. chrysogenum TCD1+2
transformants (where cefD1 and cefD2 are present) was the epimerase activity restored. In transformants TCD1 and TCD2, where just one of the genes was functional, no epimerase activity was detected. These results indicate that both cefD1 and cefD2 proteins are indeed involved in the epimerization of isopenicillin N into penicillin N.

Cephalosporin Production in TCD1, TCD2, and TCD1/H110012 Transformants—Cephalosporin C fermentations with three of the TCD1, TCD2, and TCD1/H110012 transformants were performed. Results showed (Fig. 9A) that in transformants TCD1-2, the cephalosporin production was restored to levels similar to those of A. chrysogenum C10. In TCD1 transformants (Fig. 9B), a very small amount of cephalosporins production was detected (~1% of cephalosporin produced in A. chrysogenum C10), whereas in TCD2 transformants (Fig. 9B), no cephalosporin was produced at all. HPLC analysis (Fig. 9C) confirmed that TCD1-2 transformants produced authentic cephalosporin C as in A. chrysogenum C10.

**DISCUSSION**

D-Amino acids are very frequent components of nonribosomal peptides (45, 46) and their derivatives (e.g., β-lactams). Very little is known about the mechanism of amino acid epimerization that give rise to the D-configuration. In many cases, epimerization is performed by an epimerization domain integrated into the large modular peptide synthetases (8, 47), whereas in other cases, the D-amino acids are formed by separate epimerases (e.g., the D-alanine component of cyclosporin and HC toxin) (48).

In this work, we report that the epimerization step converting isopenicillin N into penicillin N in A. chrysogenum is catalyzed by a two-protein system never described before in the biosynthesis of antibiotics or other secondary metabolites. These proteins are encoded by two genes expressed in opposite orientation from a bidirectional promoter region: cefD1, which shows high similarity to long chain acyl-CoA synthetases (39),
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Our results show that the product of both genes cefD1 and cefD2 are necessary for full epimerase activity and for cephalosporin biosynthesis, which supports the proposed activation and epimerization model. Finally, the required hydrolysis of the CoA thioesters has been reported to occur in a nonstereoselective manner by different thioesterases (56).

The Streptomyces clavuligerus and Amycolatopsis lactamurans isopenicillin N epimerases appear to work by an entirely different mechanism, since they are single proteins with a molecular weight of 59,000 (for the A. lactamurans) or 63,000 (for the S. clavuligerus enzyme) that catalyze a pyridoxal phosphate-dependent removal of the amino group at C-2 of the α-aminoacidipyl chain, followed by reintroduction in the D-configuration (19, 21).

**REFERENCES**

1. Demain, A. L. (1983) in Antibiotics Containing the β-Lactum Structure (Demain, A. L., and Solomon, N. A., eds) pp. 189–228, Springer-Verlag New York Inc., New York.

2. Martin, J. F., and Liras, P. (1989) Adv. Biochem. Eng. Biotechnol. 39, 153–187.

3. Aharanowitsz, Y., Cohen, G., and Martin, J. F. (1992) Annu. Rev. Microbiol. 46, 461–495.

4. Martin, J. F., Gutiérrez, S., and Demain, A. L. (1997) in Fungal Biotechnology (Arne, T., ed.) pp. 93–127, Chapman and Hall, Weinheim, Germany.

5. Martin, J. F. (2000) J. Bacteriol. 182, 2355–2362.

6. Liras, P. (1999) Antonie Leeuwenhoek 75, 109–124.

7. Martin, J. F. (1998) Appl. Microbiol. Biotechnol. 50, 1–15.

8. Martin, J. F. (2000) J. Antibiot. (Tokyo) 53, 1008–1021.

9. Gutiérrez, S., Diez, B., Alvarez, E., and Martin, J. F. (1991) J. Bacteriol. 173, 2354–2365.

10. Samson, S. M., Belagaje, R., Blankenship, D. T., Chapman, J. L., Perry, D., Skatrud, P. L., van Frank, R. M., Abraham, E. P., Baldwin, J. E., Queener, S. W., and Ingolia, T. D. (1985) Nature 318, 191–194.

11. Gutiérrez, S., Fierro, F., Casqueiro, J., and Martin, J. F. (1999) Antonie Leeuwenhoek 75, 81–94.

12. Samson, S. M., Chapman, J. L., Belagaje, R., Queener, S. W., and Ingolia, T. D. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5705–5709.

13. Baldwin, J. E., Kepping, J. W., Singh, P. D., and Vallojo, C. A. (1981) Biochem. J. 194, 649–663.

14. Jayatilake, G. S., Huddleston, J. A., and Abraham, E. P. (1981) Biochem. J. 194, 645–647.

15. Jones, S. E., Westlake, D. W. S., and Wolfe, S. (1985) Can. J. Microbiol. 31, 1526–1531.

16. Usui, S., and Yu, C. A. (1989) Biochim. Biophys. Acta. 999, 78–85.

17. Laiz, L., Liras, P., Castro, J. M., and Martin, J. F. (1990) J. Gen. Microbiol. 136, 663–671.

18. Kocavec, V., Tobin, M. B., and Miller, J. R. (1990) J. Bacteriol. 172, 3952–3958.

19. Coque, J. R., Liras, P., and Martin, J. F. (1993) Mol. Gen. Genet. 236, 453–458.

20. Shen, Y. Q., Wolfe, S., and Demain, A. L. (1986) Bio/Technology 4, 61–64.

21. Punt, P. J., Dingemans, M. A., Kuyvenhoven, A., Soede, R. D., Fowellis, P. H., and van den Houdel, C. C. A. (1990) Gene (Amst.) 93, 101–109.

22. Gutiérrez, S., Marcos, A. T., Casqueiro, J., Kosalovkova, K., Fernández, F. J., Velasco, J., and Martin, J. F. (1999) Microbiology 145, 317–324.

23. Gutiérrez, S., Diez, B., Alvarez, E., Barredo, J. L., and Martin, J. F. (1991) Mol. Gen. Genet. 225, 56–64.

24. Henikoff, S. (1984) Gene (Amst.) 28, 351–359.

25. Sanger, F., Nicken, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467.

26. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

27. Kosalovkova, K., Marcos, A. T., and Martin, J. F. (2001) J. Ind. Microbiol. Biotechnol. 27, 252–258.

28. Neuss, N., Berry, D. M., Kupka, J., Demain, A. L., Queener, S. W., Buckworth, D. C., and Hukstet, L. S. (1982) J. Antibiot. 35, 580–584.

29. Ludwig, C., Wolfe, S., and Demain, A. L. (1986) Appl. Microbiol. Biotechnol. 23, 367–368.

30. Ullan, R. V., Liu, G., Casqueiro, J., Gutiérrez, S., Bareuolo, O., and Martin, J. F. (2002) Mol. Gen. Genet. 267, 673–683.

31. Velasco, J., Gutiérrez, S., Fernández, F. J., Marcos, A. T., Arenós, C., and
37. Velasco, J., Gutiérrez, S., Casqueiro, J., Fierro, F., Campoy, S., and Martín, J. F. (2001) Appl. Microbiol. Biotechnol. 57, 300–306
38. Uchiyama, A., Aoyama, T., Kamijo, K., Uchida, Y., Kondo, N., Orii, T., and Hashimoto, T. (1996) J. Biol. Chem. 271, 30360–30365
39. Turgay, K., Krause, M., and Marahiel, M. A. (1992) Mol. Microbiol. 6, 529–546
40. Reichel, C., Brugger, R., Bang, H., Geisslinger, G., and Brune, K. (1997) Mol. Pharmacol. 51, 76–582
41. Schmitz, W., Helander, H. M., Hiltunen, J. K., and Conzelmann, E. (1997) Biochem. J. 326, 883–889
42. Mansour, S. L., Thomas, K. R., and Capecchi, M. R. (1988) Nature 336, 348–352
43. Liu, C., Casqueiro, J., Bañuelos, O., Cardoza, R. E., Gutiérrez, S., and Martin, J. F. (2001) J. Bacteriol. 183, 1765–1772
44. Martin, J. F., Ingolia, T. D., and Queener, S. W. (1990) in Molecular Industrial Mycology (Leong, S. A., and Berka, R., ed) pp. 149–195, Marcel Dekker, New York
45. Kleinkauf, H., and von Dohen, H. (1996) Eur. J. Biochem. 236, 335–351
46. Martin, J. F., Gutiérrez, S., and Aparicio, J. F. (2000) in Encyclopedia of Microbiology (Lederberg, J. ed) pp. 213–237, Academic Press, Inc., San Diego, CA
47. Stachelhaus, T., and Marahiel, M. A. (1995) FEMS Microbiol. Lett. 125, 3–14
48. Cheng, Y.-Q., and Walton, J. D. (2000) J. Biol. Chem. 275, 4906–4911
49. Schmitz, W., Fingerhut, R., and Conzelmann, E. (1994) Eur. J. Biochem. 222, 313–323
50. Schmitz, W., Albers, C., Fingerhut, R., and Conzelmann, E. (1995) Eur. J. Biochem. 231, 815–822
51. Ackman, R. G., and Hansen, R. P. (1967) Lipids 2, 357–362
52. Fingerhut, R., Schmitz, W., and Conzelmann, E. (1993) J. Inherited Metab. Dis. 16, 591–594
53. van Veldhoven, P. P., Croes, K., Asselbergs, S., Herdewijn, P., and Mannaerts, G. P. (1996) FEBS Lett. 388, 80–84
54. Schmitz, W., and Conzelmann, E. (1997) Eur. J. Biochem. 244, 434–440
55. Caldwell, J., Hutt, A. J., and Fourner-Gigleux, S. (1988) Biochem. Pharmacol. 37, 105–114
56. Knihinicki, R. D., Day, R. O., and Williams, K. M. (1991) Biochem. Pharmacol. 42, 1905–1911
57. Shieh, W. R., and Chen, C. S. (1993) J. Biol. Chem. 268, 3487–3493
A Novel Epimerization System in Fungal Secondary Metabolism Involved in the Conversion of Isopenicillin N into Penicillin N in *Acremonium chrysogenum*

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