

REFERENCES

- Altschul, S., Gish, W., Miller, W., Myers, E. and Lipman, D. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403-410.
- Aparicio, J. F., Colina, A. J., Ceballos, E. and Martin, J. F. 1999. The biosynthetic gene cluster for the 26-membered ring polyene macrolide pimarinin. A new polyketide synthase organization encoded by two subclusters separated by functionalization genes. J. Biol. Chem. 274: 10133-10139.
- Ausubel, F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. 2002. Short protocols in molecular biology : A compendium of methods from current protocols in molecular biology. U.S.A. :John wiley & Sons.
- Badyal, D. and Dadhich, A. P. 2001. Cytochrome P450 and drug interactions. Indian Journal of Phamacology. 33: 248-259.
- Baxevanis, A. and Quellette, F. B. F. 2001. Bioinformatic : a practical guide to the analysis of genes and protiens. U.S.A. :A John wiley & sons.
- Bate, N., Butler, A., Gandecha, A. and Cundliffe, E. 1999. Multiple regulatory genes in the tylosin biosynthetic cluster of *Streptomyces fradiae*. Chem. Biol. 6: 617-624.
- Betlach, M., Kealey, J., Ashley, G. and McDaniel, R. 1998. Characterization of the macrolide P-450 hydroxylase from *Streptomyces venezuelae* which converts narbomycin to picromycin. Biochemistry. 37: 14937-14342.
- Brautaset, T., Sekurova, O., Sletta, H., Ellingsen, T., Strlm, A., Valla, S. and Zotchev, S. 2000. Biosynthesis of the polyene antifungal antibiotic nystatin in *Streptomyces noursei* ATCC 11455: analysis of the gene cluster and deduction of the biosynthetic pathway. Chem. Biol. 7: 395-403.
- Brook, T. D. 1974. Biology of Microorganisms (2nd edition). Englewood Cliffs, New Jersey :Prentice-Hall.

- Caffrey, P., Lynch, S., Flood, E., Finnan, S. and Oliynyk, M. 2001. Amphotericin biosynthesis in *Streptomyces nodosus*: deductions from analysis of polyketide synthase and late genes. Chem. Biol. 8: 713-723.
- Carreras, C., Frykman, S., Ou, S., Cadapan, L., Zavala, S., Woo, E., Leaf, T., Carney, J., Burlingame, M., Patel, S., Ashley, G. and Licari, P. 2002. *Saccharopolyspora erythraea*-catalyzed bioconversion of 6-deoxyerythronolide B analogs for production of novel erythromycins. J. Biotechnol. 92: 217-228.
- Chakrabarti, R. and Schutt, C. 2002. Novel sulfoxides facilitate GC-rich template amplification. Biotechniques. 32: 870-872.
- Cupp-Vickery, J. and Poulos, T. 1995. Structure of cytochrome P450eryF involved in erythromycin biosynthesis. Nat. Struct. Biol. 2: 144-153.
- Cupp-Vickery, J. and Poulos, T. 1997. Structure of cytochrome P450eryF: substrate, inhibitors, and model compounds bound in the active site. Steroids. 62: 112-116.
- Degtyarenko, K. and Archakov, A. 1993. Molecular evolution of P450 superfamily and P450-containing monooxygenase systems. FEBS Lett. 332: 1-8.
- Demain, A. 1999. Pharmaceutically active secondary metabolites of microorganisms. Appl. Microbiol. Biotechnol. 52: 455-463.
- Dickens, M. and Strohl, W. 1996. Isolation and characterization of a gene from *Streptomyces* sp. strain C5 that confers the ability to convert daunomycin to doxorubicin on *Streptomyces lividans* TK24. J. Bacteriol. 178: 3389-3395.
- Faust, B., Hoffmeister, D., Weitnauer, G., Westrich, L., Haag, S., Schneider, P., Decker, H., Kunzel, E., Rohr, J. and Bechthold, A. 2000. Two new tailoring enzymes, a glycosyltransferase and an oxygenase, involved in biosynthesis of the angucycline antibiotic urdamycin A in *Streptomyces fradiae* Tu2717. Microbiology. 146: 147-154.
- Felsenstein, J. 1995. PHYLIP (Phylogeny Inference Package), version 3.57c. Department of Genetics, University of Washington, Seattle.

- Fish, S. and Cundliffe, E. 1997. Stimulation of polyketide metabolism in *Streptomyces fradiae* by tylosin and its glycosylated precursors. Microbiology. 143: 3871-3876.
- Graziani, E., Cane, D., Betlach, M., Kealey, J. and McDaniel, R. 1998. Macrolide biosynthesis: a single cytochrome P450, PicK, is responsible for the hydroxylations that generate methymycin, neomethymycin, and picromycin in *Streptomyces venezuelae*. Bioorg. Med. Chem. Lett. 8: 3117-3120.
- Guengerich, F. 1991. Reactions and significance of cytochrome P-450 enzymes. J. Biol. Chem. 266: 10019-10022.
- Guengerich, F. 2003. Cytochromes P450, drugs, and diseases. Mol. Interv. 3: 194-204.
- Hasemann, C., Kurumbail, R., Boddupalli, S., Peterson, J. and Deisenhofer, J. 1995. Structure and function of cytochromes P450: a comparative analysis of three crystal structures. Structure. 3: 41-62.
- Hasler, J., Estabrook, R., Murray, M., Pikuleva, I., Waterman, M., Capdevila, J., Holla, V., Helvig, C., Falck, J. R., Farrell, G., Kaminsky, L. S., Spivack, S., Boitier, E. and Beaune, P. 1999. Human cytochromes P450. Molecular Aspects of Medicine. 20: 1-137.
- Hopwood, D. 1979. Genetics of antibiotic production by actinomycetes. J. Nat. Prod. 42: 596-602.
- Hopwood, D. 1997. Genetic contributions to understanding polyketide synthases. Chem. Rev. 97: 2465-2498.
- Hopwood, D. 2004. Cracking the polyketide code. PLoS Biology. 2: 0166-0169.
- Hopwood, D. and Sherman, D. 1990. Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. Annu. Rev. Genet. 24: 37-66.
- Hranueli, D., Peri, N., Borovicka, B., Bogdan, S., Cullum, J., Waterman, P. G. and Hunter, I. 2001. Molecular biology of polyketide biosynthesis. Food. Technol. biotechnol. 39: 203-213.

- Ikeda, H., Nonomiya, T., Usami, M., Ohta, T. and Omura, S. 1999. Organization of the biosynthetic gene cluster for the polyketide anthelmintic macrolide avermectin in *Streptomyces avermitilis*. PNAS. 96: 9509-9514.
- Katz, L. and Donadio, S. 1993. Polyketide synthesis: prospects for hybrid antibiotics. Annu. Rev. Microbiol. 47: 875-912.
- Khosla, C. 1997. Harnessing the biosynthetic potential of modular polyketide synthases. Chem. Rev. 97: 2577-2590.
- Kieser, T., Bibb, M., Buttner, M. J., Chater, K. F., and Hopwood, D. A. 2000. Practical Streptomyces genetics. The John Innes Foundation, Norwich, U.K.
- Kneller, D., Cohen, F. and Langridge, R. 1990. Improvements in protein secondary structure prediction by an enhanced neural network. J. Mol. Biol. 214: 171-182.
- Lamb, D., Ikeda, H., Nelson, D., Ishikawa, J., Skaug, T., Jackson, C., Omura, S., Waterman, M. and Kelly, S. 2003. Cytochrome p450 complement (CYPome) of the avermectin-producer *Streptomyces avermitilis* and comparison to that of *Streptomyces coelicolor* A3(2). Biochem. Biophys. Res. Commun. 307: 610-619.
- Leadlay, P., Staunton, J., Oliynyk, M., Bisang, C., Cortes, J., Frost, E., Hughes-Thomas, Z., Jones, M., Kendrew, S., Lester, J., Long, P., McArthur, H., McCormick, E., Oliynyk, Z., Stark, C. and Wilkinson, C. 2001. Engineering of complex polyketide biosynthesis-insights from sequencing of the monensin biosynthetic gene cluster. J. Ind. Microbiol. Biotechnol. 27: 360-367.
- Leys, D., Mowat, C. G., McLean, K. J., Richmond, A., Chapman, S. K., Walkinshaw, M. D. and Munro, A. W. 2003. Atomic structure of mycobacterium tuberculosis CYP121 to 1.06 Å reveals novel features of cytochrome P450. J. Biol. Chem. 278: 5141-5147.
- Li, R., Khaleeli, N. and Townsend, C. A. 2000. Expansion of the clavulanic acid gene cluster: identification and in vivo functional analysis of three new genes required for

- biosynthesis of clavulanic acid by *Streptomyces clavuligerus*. J. Bacteriol. 182: 4087-4095.
- Lozano, M. J. F., Remsing, L. L., Quiros, L. M., Brana, A. F., Fernandez, E., Sanchez, C., Mendez, C., Rohr, J. and Salas, J. A. 2000. Characterization of two polyketide methyltransferases involved in the biosynthesis of the antitumor drug mithramycin by *Streptomyces argillaceus*. J. Biol. Chem. 275: 3065-3074.
- Miles, C., Ost, T., Noble, M., Munro, A. and Chapman, S. 2000. Protein engineering of cytochromes P-450. Biochim. Biophys. Acta. 1543: 383-407.
- Nelson, D., Kamataki, T., Waxman, D., Guengerich, F., Estabrook, R., Feyereisen, R., Gonzalez, F., Coon, M., Gunsalus, I. and Gotoh, O. 1993. The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. DNA Cell Biol. 12: 1-51.
- Olano, C., Moss, S., Brana, A., Sheridan, R., Math, V., Weston, A., Mendez, C., Leadlay, P., Wilkinson, B. and Salas, J. 2004. Biosynthesis of the angiogenesis inhibitor borrelidin by *Streptomyces parvulus* Tu4055: insights into nitrile formation. Mol. Microbiol. 52: 1745-1756.
- Olano, C., Rodriguez, A., Michel, J., Mendez, C., Raynal, M. and Salas, J. 1998. Analysis of a *Streptomyces antibioticus* chromosomal region involved in oleandomycin biosynthesis, which encodes two glycosyltransferases responsible for glycosylation of the macrolactone ring. Mol. Gen. Genet. 259: 299-308.
- Omer, C., Lenstra, R., Little, P. J., Dean, C., Tepperman, J. M., Leto, K. J., Romesser, J. A., O'Keefe, D. P. 1990. Genes for two herbicide-inducible cytochromes P-450 from *Streptomyces griseolus*. J. bacteriol. 172: 3335-3345.
- Omura, T. 1999. Forty years of cytochrome P450. Biochem. Biophys. Res. Commun. 266: 690-698.

- Palaniappan, N., Kim, B. S., Sekiyama, Y., Osada, H. and Reynolds, K. A. 2003. Enhancement and selective production of phoslactomycin B, a protein phosphatase II a inhibitor, through identification and engineering of the corresponding biosynthetic gene cluster. J. Biol. Chem. 278: 35552-35557.
- Parajuli, N., Basnet, D., Chan Lee, H., Sohng, J. and Liou, K. 2004. Genome analyses of *Streptomyces peucetius* ATCC 27952 for the identification and comparison of cytochrome P450 complement with other *Streptomyces*. Arch. Biochem. Biophys. 425: 233-241.
- Patel, N. V. and Omer, C. A. 1992. Phenobarbital and sulfonylurea-inducible operons encoding herbicide metabolizing cytochromes P-450 in *Streptomyces griseolus*. Gene. 112: 67-76.
- Perez-Zuniga, F., Seco, E., Cuesta, T., Degenhardt, F., Rohr, J., Vallin, C., Iznaga, Y., Perez, M., Gonzalez, L. and Malpartida, F. 2004. CE-108, a new macrolide tetraene antibiotic. J. Antibiot (Tokyo). 57: 197-204.
- Pfeifer, B. A. and Khosla, C. 2001. Biosynthesis of polyketides in heterologous hosts. Microbiol. Mol. Biol. Rev. 65: 106-118.
- Poulos, T. 1995. Cytochrome P450. Curr. Opin. Struct. Biol. 5: 767-774.
- Poulos, T., Finzel, B. and Howard, A. 1987. High-resolution crystal structure of cytochrome P450cam. J Mol Biol. 195: 687-700.
- Rawlings, B. 2001. Type I polyketide biosynthesis in bacteria (part B). Nat. Prod. Rep. 18: 231-281.
- Schwecke, T., Aparicio, J., Molnar, I., Konig, A., Khaw, L., Haycock, S., Oliynyk, M., Caffrey, P., Cortes, J., Lester, J., Bohm, G., Staunton, J. and Leadlay, P. 1995. The biosynthetic gene cluster for the polyketide immunosuppressant rapamycin. PNAS. 92: 7839-7843.

- Shen, B. 2003. Polyketide biosynthesis beyond the type I, II and III polyketide synthase paradigms. Curr. Opin. Chem. Biol. 7: 285-295.
- Stassi, D., Donadio, S., Staver, M. and Katz, L. 1993. Identification of a *Saccharopolyspora erythraea* gene required for the final hydroxylation step in erythromycin biosynthesis. J. Bacteriol. 175: 182-189.
- Sun, Y., Hegamyer, G. and Colburn, N. 1993. PCR-direct sequencing of a GC-rich region by inclusion of 10% DMSO: application to mouse c-jun. Biotechniques. 15: 372-374.
- Suwa, M., Sugino, H., Sasaoka, A., Mori, E., Fujii, S., Shinkawa, H., Nimi, O. and Kinashi, H. 2000. Identification of two polyketide synthase gene clusters on the linear plasmid pSLA2-L in *Streptomyces rochei*. Gene. 246: 123-131.
- Thompson, J., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673-4680.
- Trower, M., Lenstra, R., Omer, C., Buchholz, S. and Sariaslani, F. 1992. Cloning, nucleotide sequence determination and expression of the genes encoding cytochrome P-450_{soy} (soyC) and ferredoxin_{soy} (soyB) from *Streptomyces griseus*. Mol. Microbiol. 6: 2125-2134.
- Walczak, R. J., Dickens, M. L., Priestley, N. D. and Strohl, W. R. 1999. Purification, properties, and characterization of recombinant *Streptomyces* sp. strain C5 DoxA, a cytochrome P-450 catalyzing multiple steps in doxorubicin biosynthesis. J. Bacteriol. 181: 298-304.
- Walsh, C., Chen, H., Keating, T., Hubbard, B., Losey, H., Luo, L., Marshall, C., Miller, D. and Patel, H. 2001. Tailoring enzymes that modify nonribosomal peptides during and after chain elongation on NRPS assembly lines. Curr. Opin. Chem. Biol. 5: 525-534.
- Walsh, C., Losey, H. and Freil Meyers, C. 2003. Antibiotic glycosyltransferases. Biochem. Soc. Trans. 31: 487-492.

- Walsh, C. T. 2004. Polyketide and nonribosomal peptide antibiotics: modularity and versatility. Science. 303: 1805-1810.
- Watanabe, I., Nara, F. and Serizawa, N. 1995. Cloning, characterization and expression of the gene encoding cytochrome P-450sca-2 from *Streptomyces carbophilus* involved in production of pravastatin, a specific HMG-CoA reductase inhibitor. Gene. 163: 81-85.
- Xue, Y. and Sherman, D. 2001. Biosynthesis and combinatorial biosynthesis of pikromycin-related macrolides in *Streptomyces venezuelae*. Metab. Eng. 3: 15-26.
- Xue, Y., Wilson, D., Zhao, L., Liu, H. and Sherman, D. 1998a. Hydroxylation of macrolactones YC-17 and narbomycin is mediated by the pikC-encoded cytochrome P450 in *Streptomyces venezuelae*. Chem. Biol. 5: 661-667.
- Xue, Y., Zhao, L., Liu, H. W. and Sherman, D. H. 1998b. A gene cluster for macrolide antibiotic biosynthesis in *Streptomyces venezuelae*: Architecture of metabolic diversity. PNAS. 95: 12111-12116.
- Zhang, Q. and Sherman, D. 2001. Isolation and structure determination of novamethymycin, a new bioactive metabolite of the methymycin biosynthetic pathway in *Streptomyces venezuelae*. J. Nat. Prod. 64: 1447-1450.



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APPENDIX A

Culturing media

1. Non sporulating agar, 1 liter

Soluble starch	20 g
Yeast extract	4 g
Casamino acid	20 g

2. Luria-Bertani (LB) medium, 1 liter

Bacto tryptone	10 g
Yeast extract	5 g
NaCl	5 g

3. Oatmeal agar, 1 liter

Oatmeal	20 g
Trace salt solution	1 ml
Agar	18-20 g
Adjust with 1 N NaOH to pH 7.2	

Boil oatmeal in a water , autoclave before add trace salt solution

4. Trace salt solution (100 ml)

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.1 g
$\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$	0.1 g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.1 g

APPENDIX B

Dot Blot hybridization buffers

1. Standard hybridization buffer

5x SSC (0.075M Sodium citrate, 0.75M NaCl)

N-lauroylsarcosine, 0.1% (W/V)

SDS, 0.02% (W/V)

1X blocking reagent

2. Buffer 1 (Maleic acid buffer)

0.1 maleic acid

0.15 M NaCl

Adjust with solid NaOH to pH 7.5

3. Buffer 2 (Blocking solution)

1X blocking solution in maleic acid buffer

4. Buffer 3 (Detection buffer)

0.1M Tris-HCl

0.1M NaCl

50 mM MgCl₂

Adjust with NaOH to pH 9.5

5. Washing buffer

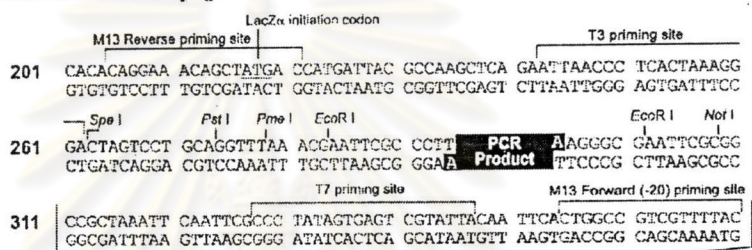
0.3% Tween 20 (V/V)

maleic acid buffer

APPENDIX C

Map of pCR[®]4-TOPO[®]

pCR[®]4-TOPO[®] Map The map below shows the features of pCR[®]4-TOPO[®] and the sequence surrounding the TOPO[®] Cloning site. Restriction sites are labeled to indicate the actual cleavage site. The complete sequence of pCR[®]4-TOPO[®] is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (page 28).



Comments for pCR[®]4-TOPO[®]
3956 nucleotides

lac promoter region: bases 2-216

CAP binding site: bases 95-132

RNA polymerase binding site: bases 133-178

Lac repressor binding site: bases 179-199

Start of transcription: base 179

M13 Reverse priming site: bases 205-221

LacZ α -*ccdB* gene fusion: bases 217-810

LacZ α portion of fusion: bases 217-497

ccdB portion of fusion: bases 508-810

T3 priming site: bases 243-262

TOPO[®] Cloning site: bases 294-295

T7 priming site: bases 328-347

M13 Forward (-20) priming site: bases 355-370

Kanamycin promoter: bases 1021-1070

Kanamycin resistance gene: bases 1159-1953

Ampicillin (*bla*) resistance gene: bases 2203-3063 (c)

Ampicillin (*bla*) promoter: bases 3064-3160 (c)

pUC origin: bases 3161-3834

(c) = complementary strand

BIOGRAPHY

Miss Sittinee Saenmee was born on May 15th, 1979 in Lopburi. She graduated with the Bachelor Degree of Science in Department of Genetics, Faculty of Science, Kasetsart University in 2000. Then, she has been a graduate student in the Master's Degree in Biotechnology program, Faculty of Science, Chulalongkorn University since 2000.



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