A54145, A NEW LIPOPEPTIDE ANTIBIOTIC COMPLEX: FACTOR CONTROL THROUGH PRECURSOR DIRECTED BIOSYNTHESIS

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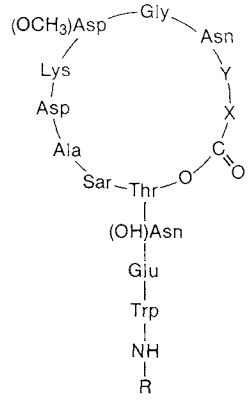
A54145 is a complex of new lipopeptide antibiotics produced by *Streptomyces fradiae*. Eight factors, containing four similar peptide nuclei in combination with three different fatty acid acyl side chains, have been isolated from the natural fermentation and characterized. The nuclei differ only in valine/isoleucine and glutamate/3-CH_3-glutamate substitutions at one or both of two locations on the peptide ring. Prior deacylation of all four nuclei with *Actinoplanes utahensis* had permitted chemical reacylation of each nucleus with new fatty acid acyl chains for structure-activity relationship studies. In an effort to induce the native biosynthesis of preferred factors or analogs by *S. fradiae*, the effect of fatty acid precursors on the fermentation was examined. Many fatty acids were extremely toxic to *S. fradiae*, which limited experiments to slow, continuous feeding of the lipids in stirred bioreactors that were equipped for on-line respiration analysis by mass spectrometry. These studies determined that precursing with aliphatic fatty acids of various chain lengths did enhance the biosynthesis of factors containing specific fatty acid acyl side chains. Caprate, for example, increased the *n*-decanoyl-containing factors from the natural level of ~14% to ~80%. The percentage of factors containing branched-chain fatty acid acyl substituents was also increased, in shaken-flask studies, by enriching the medium with valine or isoleucine. These amino acids additionally enhanced the percentage of nuclei containing either valine or isoleucine.

A54145 is a complex of new acidic lipopeptide antibiotics produced by *Streptomyces fradiae*. Four different cyclic peptide nuclei, varying only in valine/isoleucine and/or glutamate/3-CH_3-glutamate substitutions at one or both of two locations, are produced. The *N*-terminus of each nucleus is acylated with either an *iso*-decanoyl (*i*C_{10}), *n*-decanoyl (*n*C_{10}), or undecanoyl (*a*C_{11}) lipid side chain (Fig. 1)[1]. After 185 hours, the percentage of each nucleus typically produced by the natural fermentation in stirred bioreactors is as follows: A=34%, B=56%, C=1~2% and F=9%. After the same time period, the percentage of factors typically containing each fatty acid acyl chain is as follows: *i*C_{10}=74~78%, *n*C_{10}=13~15% and *a*C_{11}=9~11%[2]. Deacylation of the factor complex with *Actinoplanes utahensis* produced the cyclic peptide nuclei, which were then individually isolated and chemically reacylated with various acyl chains for SAR studies[3]. This complex technique suggested a need for direct biosynthesis of the desired factors in order to eliminate the deacylation-reacylation requirement. A21978C, another lipopeptide antibiotic complex, had previously been subjected to a similar deacylation-reacylation procedure to produce daptomycin[4], an A21978C analog now undergoing clinical evaluation. However, subsequent studies of the A21978C fermentation had shown that biosynthesis of factors containing specific alkanoyl side chains could be regulated by precursing the fermentation with fatty acids[5]. In an effort to similarly induced the native biosynthesis of preferred A54145 factors and/or analogs by *S. fradiae*, the effect of fatty acid and amino acid precursors was examined. This paper describes studies on regulating formation of the A54145 antibiotics *via* precursor directed biosynthesis.

Fig. 1. Structure of the A54145 factor complex.



Factor	MW	X	Y	R
A	1,643	Ile	Glu	8-Methylnonanoyl (iC_{10})
A_1	1,643	Ile	Glu	*n*-Decanoyl (nC_{10})
B	1,657	Ile	3-MethylGlu	*n*-Decanoyl (nC_{10})
B_1	1,657	Ile	3-MethylGlu	8-Methylnonanoyl (iC_{10})
C	1,657	Val	3-MethylGlu	8-Methyldecanoyl (aC_{11})
D	1,657	Ile	Glu	8-Methyldecanoyl (aC_{11})
E	1,671	Ile	3-MethylGlu	8-Methyldecanoyl (aC_{11})
F	1,629	Val	Glu	8-Methylnonanoyl (iC_{10})

Materials and Methods

Microorganism

Submerged culture stocks of NRRL 18160, a nitrosoguanidine-induced mutant of NRRL 18158, classified as a strain of *S. fradiae*[2], were preserved by storage in the vapor phase of liquid nitrogen.

Fermenter Inoculum

Fermenter inoculum was prepared by introducing liquid nitrogen stocks into wide-mouth 250-ml Erlenmeyer flasks containing 50 ml of a medium composed of glucose 1%, potato dextrin 3%, soybean flour 2%, cotton-seed flour 2% and $CaCO_3$ 0.2%. These flasks were incubated at 25°C for 48 hours on a gyratory shaker orbiting at 250 rpm in a 5-cm diameter circle. The resulting mycelial suspension was used directly to provide a 1%-level of inoculum to flask fermenters. When larger volumes of inoculum were required for stirred bioreactors, the culture was transferred serially into 400 ml of the same medium in wide-mouth 2-liter flasks and incubated for an additional 24 hours under the same conditions except that the shaker board was inclined at a 10° angle from the horizontal.

Fermenters

All bioreactors, both stirred vessels and shaken flasks, were incubated for 7~8 days at 25°C. Stirred bioreactors were fully baffled vessels of conventional design with two 6-bladed turbine impellors, a total capacity of 165 liters and an approximate 1 : 1 height-diameter ratio for the 115 liters of medium. The medium was sterilized through the application of 22~25 heating units by the F_0 method[6]. Dissolved oxygen (DO) levels were monitored with a galvanic sensor under an internal head pressure of 0.34 atmospheres. DO was controlled at 40% of air saturation by computer regulation of agitation and/or air flow rates. The pH was controlled at 6.8~7.0 with aqueous H_2SO_4 and NH_4OH. Exhaust gas streams were monitored with a Perkin-Elmer MGA-1200 mass spectrometer interfaced with a Hewlett-Packard computer.

Medium CSV; employed for stirred vessels; contained SAG 471 (Union Carbide) 0.02%, polypropylene glycol (MW 2,000) 0.01%, glucose 0.3%, soybean flour 3.0%, blackstrap molasses 1.0%, and ferrous

ammonium sulfate 0.06% in tap water, adjusted to pH 7.0 with aqueous NaOH prior to autoclaving. A continuous glucose feed, at the rate of $2.5 \sim 3.0 \times 10^{-1}$ mg/ml/hour, was initiated ~20 hours post-inoculation, prior to depletion of the glucose initially incorporated into the medium.

Because continuous feeds and chemostat pH control are normally impractical in shaken flasks, a modification of the CSV medium was employed for flask studies. This medium (DSF) contained glucose 3.0%, soybean flour 2.5%, blackstrap molasses 0.5%, ferrous ammonium sulfate 0.06%, and $CaCO_3$ 0.4%.

Lipids

Lipids were purchased from Aldrich Chemical Company, Inc. and were generally of 96~99.5% purity. They were presented to *S. fradiae* as intermittent droplets of liquid whose flow rate was regulated by computer control of peristalic pumps. Lipid feeds were initiated at a mean rate of about $2 \sim 3 \times 10^{-1}$ μmol/ml/hour when oxygen uptake reached the rate of 0.3~0.35 mmol/liter/minute. The lipid feeds were then increased to a mean rate of approximately $5 \sim 6 \times 10^{-1}$ μmol/ml/hour at 42~48 hours.

A54145 Factor Identification/Quantitation

The A54145 factors were individually identified and quantitated by direct analytical HPLC comparison of fermentation broth supernatants with authentic standards as previously described[2].

Results and Discussion

Caprate Precursing

The natural fermentation, in both the low-yielding SBF[7] and high-yielding CSV media, produced a preponderance of factors possessing the branched-chain iC_{10} acyl units. However, chemical reacylation of individual A54145 nuclei with various fatty acid chains had shown that factors possessing the straight-chain nC_{10}, nC_{11} and nC_{12} acyl units possessed the lowest MIC values vs. *Staphyloccus aureus*[7]. Therefore, an nC_{10} lipid, decanoic acid, was selected for the initial feeding trial. Decanoic acid, however, is a solid at normal room temperatures, melting at 31~32°C, and in insoluble in aqueous media. Further, the A54145 fermentation was conducted at the lower temperature of 25°C. Because esters of fatty acids often melt at lower temperatures and are generally less toxic, the ethyl ester of decanoate, ethyl caprate, which melts at < -15°C, was substituted for the free acid.

Preliminary studies with shaken flask fermenters indicated ethyl caprate was quite toxic to *S. fradiae*. Low levels present at inoculation prevented growth of the culture. Low level batch additions at 48 hours post-inoculation, when the logarithmic growth phase had been completed, terminated the fermentation. An alternate strategy to avoid toxic levels, such as continuous addition of caprate at a rate not exceeding the metabolic uptake rate by *S. fradiae*, appeared to be required[5]. That strategy was impractical in shaken flasks. However, it was readily implemented in stirred bioreactors with the additional advantage of on-line respiration data for use in initiating and/or adjusting feed rates to avoid or correct the toxicity reactions associated with over-feeding. It was subsequently determined that without this information, maximum precursor-product effects resulting from acceptably high lipid feed rates could not be achieved.

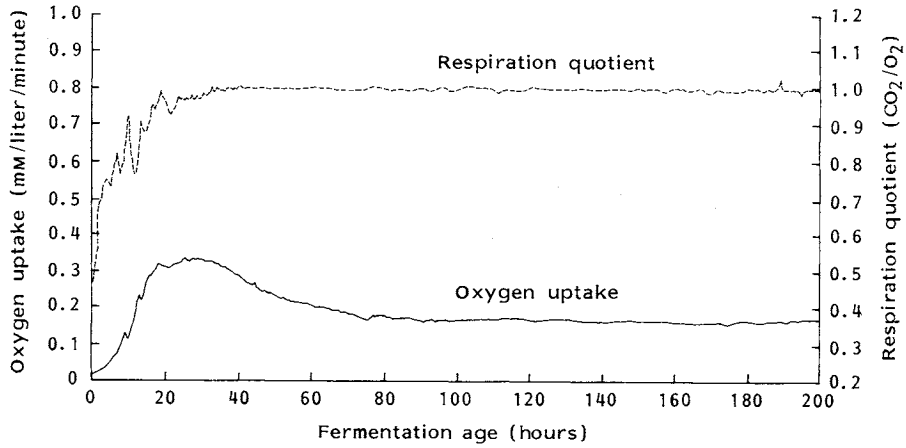
Continuous caprate feeds in stirred bioreactors essentially reversed the iC_{10}-nC_{10} ratio and markedly reduced the aC_{11} content of A54145 in both the SBF and CSV media (Table 1). These data indicated the exogenous nC_{10} lipid did affect the secondary metabolism of *S. fradiae*, resulting in formation of increased amounts of factors containing nC_{10} acyl units. Potential regulation of factor biosynthesis in the A54145 fermentation by lipid precursing was thus established. Incorporation of the precursor into the acyl chains of A54145, calculated on a molar basis from the lipid consumed and the lipid content of the A54145 factors synthesized as determined by HPLC analytical assays, was 1.51% (Table 2).

Table 1. Effect of caprate precursing on A54145 yields and percentage of factors containing each acyl chain.

Medium	Lipid precursor	Total A54145 (μg/ml)	% of factors containing each acyl chain		
			iC_{10}	nC_{10}	aC_{11}
SBF	—	97	68	20	12
	nC_{10}[a]	180	20	79	1
CSV	—	1,410	78	13	9
	nC_{10}	2,230	16	82	2

[a] Ethyl ester.

Fig. 2. Oxygen uptake and respiration quotient profiles of unprecursed A54145 fermentation.



Respiratory Response of *S. fradiae* to Caprate Precursing

Oxygen uptake (OU) and respiration quotient (RQ) profiles of the control A54145 fermentation, which is glucose based, are shown in Fig. 2. Similar profiles displaying the dual effects of a standard caprate feed, which increased OU and lowered the RQ to a mean value approximately intermediate between the calculated value for either glucose or caprate alone, are shown in Fig. 3.

Table 2. Calculated incorporation of caprate precursor into A54145 acyl chains by *Streptomyces fradiae*.

Precursor fed	Ethyl caprate
Total quantity fed	61.84 μmol/ml
Unmetabolized caprate residue	None detected
Antibiotic yield:	
Control fermentation	1,410 μg/ml
Precursed fermentation	2,155 μg/ml
nC_{10} acyl chain content of factors:	
Control fermentation	20.44 μg/ml
Precursed fermentation	180.36 μg/ml
Calculated molar incorporation of caprate into nC_{10} acyl chains of A54145	1.51%

Precursing with Other Lipids

The successful incorporation of caprate into A54145 acyl chains suggested feeding other lipids to determine the range of alkyl homologs useful for modifing the ratio of known acyl chains or producing factors containing new acyl chain analogs. Accordingly, other lipids containing two to 18 carbon atoms were examined (Table 3). The RQ for each lipid was calculated on the basis of complete oxidation when serving as the sole carbon source for *S. fradiae*. The observed RQ value of the unprecursed, glucose-based fermentation was typically ~1.0, identical with the calculated value for glucose metabolism. The calculated RQ for every lipid tested, with the exception of acetate, ranges from 0.7~0.88. Exclusively lipid metabolism would be expected to generate RQs in the same range. However, co-metabolism of glucose with the lipid

Fig. 3. Oxygen uptake and respiration quotient profiles of A54145 fermentation precursed with ethyl caprate.

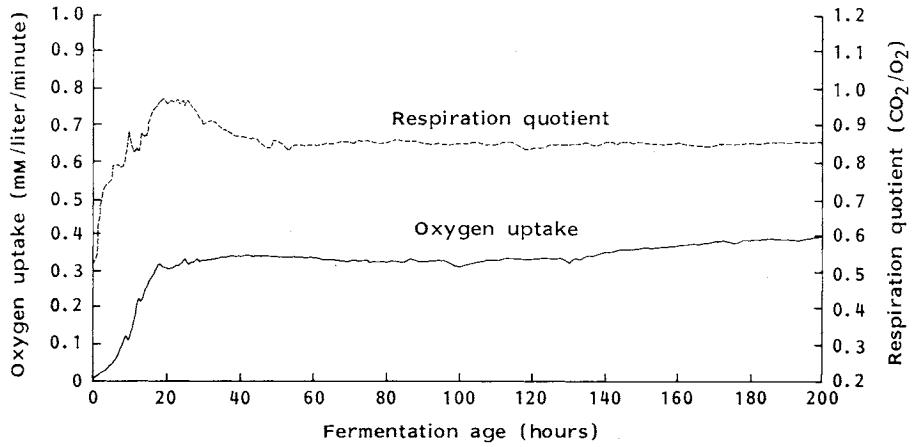


Table 3. Effect of various lipid precursors on biosynthesis of A54145 acyl chains by *Streptomyces fradiae*.

Lipid precursor[a]	RQ[b]		Total A54145 (%)	% of factors containing various acyl chains						
				Known			New			Unknown
	Calcd	Obsd		iC_{10}	nC_{10}	aC_{11}	C_6	C_8	C_9	
—	1.0	1.0	100[c]	76	14	10				
Acetate[d]	1.0	1.0	104	73	15	11				
Propionate[d]	0.88	0.96	28	69	22	8				
Butyrate	0.8	0.93	49	28	58	15				
Hexanoate	0.75	0.83	56	2	2		96			
Caprylate	0.73	0.8	84	17	9	5		69		
Nonanoate	0.72	0.85	95						100	
Caprate[e]	0.71	0.86	158	16	82	2				
Undecanoate	0.76	0.9	136	11	3	26			33	27
Undecylenate	0.71	0.87	153	27	56	2				15
Laurate[f]	0.71	0.9	154	43	54	3				
Tridecanoate[g]	0.7	0.76	64	36	19	5			40	
Myristate[f]	0.7	0.81	207	10	85	5				
Oleate	0.7	0.9	142	49	48	3				

[a] Free acid unless otherwise noted.
[b] Respiration quotient: Calcd is calculated value based on complete oxidation when serving as solecarbon source, Obsd is norminal observed value.
[c] 1,410 μg/ml.
[d] Sodium salt.
[e] Ethyl ester.
[f] Methyl ester.
[g] Liquified in methyl oleate, 1 : 1.

would be expected to modify the RQ to a higher value between 1.0 and the calculated value for the test lipid, the exact value depending upon the ratio of the lipid-glucose metabolic balance.

The RQ for all lipids tested, except acetate, whose RQ is indistinguishable from glucose, confirmed lipid-glucose co-metabolism. Additional confirmation was provided by quantitative assays of residual glucose and lipid; and by OU rates, which in all cases, including acetate, increased with initiation and decreased upon withdrawal of the lipid feed. Except for acetate, every lipid tested either altered the normal ratio of fatty acyl units attached to the nuclei, affected the total quantity of A54145 synthesized, or both.

The $C_3 \sim C_9$ homologs decreased total A54145 levels, with the decrease becoming progressively less pronounced as the carbon chain length increased. $C_{10} \sim C_{18:1}$ lipids, with the exception of tridecanoate, increased total A54145 levels by approximately $50 \sim 100\%$. Pronounced increases in the percentage of factors containing nC_{10} acyl units were common, being produced by butyrate, caprate, undecylenate, laurate, myristate and oleate. New factors containing previously undiscovered C_6, C_8 and C_9 acyl units resulted from precursing with hexanoate (C_6), caprylate (C_8) and nonanoate (C_9).

Only one lipid, undecanoate (nC_{11}), substantially increased the percentage of branched-chain aC_{11} acyl groups. Additionally, it produced large amounts of factors containing C_9 acyl chains, presumably through the loss of a single two-carbon unit from the aliphatic chain by β-oxidation, although α and ω oxidation have also been reported in streptomycetes[8]. It further produced several unknowns which, on the basis of their HPLC retention times, may have contained longer chain acyl units.

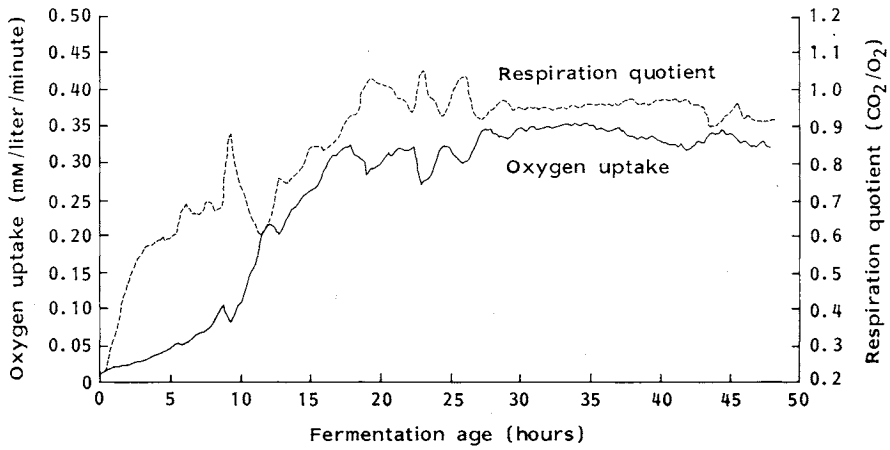
The effect of tridecanoate (C_{13}), which melts at 41°C, may have been tempered by it's dilution in an equal volume of methyl oleate, which was necessary to achieve it's liquefaction. Nevertheless, tridecanoate also produced substantial amounts of factors containing C_9 acyl chains, presumably likewise through the loss of carbon atoms by β-oxidation. In this case, however, the four carbons lost would represent two sets of two-carbon units rather than one set as observed with undecanoate.

Respiratory Response of *S. fradiae* to Excessive Lipid Free Rates

A number of the lipid precursors other than caprate were toxic to *S. fradiae*. Some were apparently more toxic, others less toxic, although individual toxicity levels were not precisely established. Our experimental goal was to feed all precursors at approximately the same level at a rate near, but not exceeding, the consumption rate by *S. fradiae* in order to maximize the potential response to each lipid.

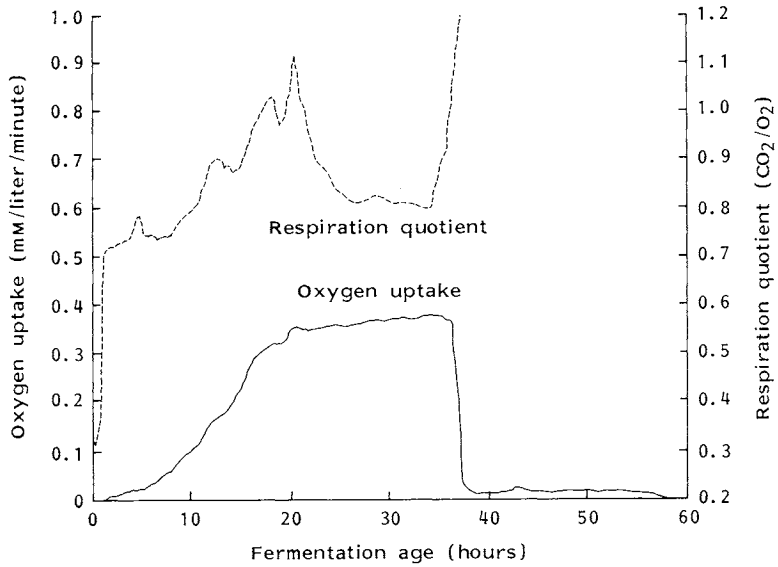
An example of the rapid and significant respiratory response to various levels of octanoic acid is shown in Fig. 4. When the initial feed was begun at 19:00 hours at the slightly elevated rate of 4×10^{-1} μmol/ml/hour, *S. fradiae* responded with increased OU and a declining RQ indicative of lipid metabolism. However, at 23:00 hours the OU began to drop precipitously while the RQ simultaneously ascended. At 23:23 hours the octanoic acid feed was terminated. Within minutes, OU again began to

Fig. 4. Respiratory response of *Streptomyces fradiae* to octanoic acid feed rates.



Octanoic acid feed initiated at 19:00 hours at the rate of 4×10^{-1} μmol/ml/hour. Feed terminated at 23:23 hours. Feed reinitiated at 24:30 hours at a rate of 2×10^{-1} μmol/ml/hour, increased at 41:26 hours to a rate of 4.5×10^{-1} μmol/ml/hour.

Fig. 5. Respiratory response of *Streptomyces fradiae* to excessive feed rate of methyl laurate.



ascend while the RQ again indicated a transition to lipid metabolism. The small excess of octanoic acid in the medium was apparently consumed by about 24:30 hours, at which time the OU began to decline while the RQ again shifted upward. The octanoic acid feed was reestablished at 25:42 hours, but at 50% of the original rate. OU and RQ again responded, and stabilized as the feed was continued. At 41:26 hours the feed rate was then increased to 4.5×10^{-1} μmol/ml/hour, to which the culture again responded positively and the new OU and RQ values stabilized for the remainder of the fermentation.

Fig. 5 shows the results of overfeeding methyl laurate at a rate only slightly above the consumption rate. The toxic lipid accumulated gradually, until metabolism was poisoned, as indicated by precipitous changes in OU and RQ. However, the feed was not terminated, as in the previous example, but was allowed to continue. The reduced catabolic rate hastened the accumulation of the toxic substrate, increasing the rate of metabolic inhibition and speeding demise of the culture. Even when the feed rate was terminated at 50 hours, the accumulated level of the toxic substrate in the medium did not allow metabolic recovery.

Comparison of Ester, Alcohol, Aldehyde and Non-oxygenated Aliphatic C_{10} Compounds

The dual effect of ethyl caprate on acyl chain ratios and total A54145 biosynthesis suggested testing other types of aliphatic C_{10} compounds to determine whether alcohols, aldehydes or non-oxygenated hydrocarbons were also metabolized by *S. fradiae*. Both the C_{10} aldehyde and alcohol were metabolized and altered the acyl chain ratios in a manner similar to caprate (Table 4). However, only the alcohol compared favorably with the ester in regard to increasing total synthesis of A54145. No increase was observed with decyl aldehyde. Neither decane, a paraffinic alkane hydrocarbon, nor 1-decene, the chemically more reactive alkene, were metabolized. Therefore, as expected, they did not alter the acyl chain ratios. Both hydrocarbons, however, exhibited unusually strong toxicity to *S. fradiae* and depressed total A54145 biosynthesis.

Effect of Amino Acid Enrichment

Biosynthesis of the cyclosporins, a group of neutral cyclic oligopeptides produced by *Tolypocladium*,

Table 4. Comparative effects of C_{10} ester, aldehyde, alcohol and non-oxygenated hydrocarbons on A54145 yields and acyl chain biosynthesis.

Compound fed	RQ[a]		Total A54145 (%)	A54145 acyl chains		
	Calcd	Obsd		iC_{10}	nC_{10}	aC_{11}
—	1.0	1.0	100[b]	76[c]	14	10
Ethyl caprate	0.71	0.86	158	16	82	2
Decyl aldehyde	0.69	0.87	106	20	80	0
Decyl alcohol	0.67	0.86	157	22	75	3
Decane	0.65	1.02	37	78	14	7
1-Decene	0.67	1.0	72	77	16	7

[a] Respiration quotient: Calcd is calculated value based on complete oxidation when serving as sole carbon source, Obsd is nominal observed value.
[b] 1,410 μg/ml in control medium with standard glucose feed.
[c] % of total.

Table 5. Effect of amino acid enrichment on biosynthesis of A54145 nuclei and acyl chains in shaken flasks.

Amino acid	Addition level (M)	Total antibiotic (%)	Nuclei[a]				Acyl chains[a]		
			A	B	C	F	iC_{10}	nC_{10}	aC_{11}
—		100[b]	50	39	1	10	65	18	17
L-Valine	0.03	32	32	20	**2**	**54**	**98**	0	2
L-Leucine	0.02	56	42	40	2	**16**	**76**	7	17
L-Isoleucine	0.03	73	**48**	**47**	2	0	16	18	**66**
L-Glutamic acid	0.02	85	**49**	39	1	**11**			
L-Aspartic acid	0.005	134	56	34					

[a] % of total antibiotic produced.
[b] Medium DSF, 550 μg/ml.
Bold underlined indicates area of anticipated effect based on structure of nuclei and acyl chains.

is known to be strongly influenced by the extracellular supply of amino acid precursors in the fermentation medium[9].

A21978C, produced by *Streptomyces roseosporus*, is another lipopeptide antibiotic complex which has structural similarities to A54145. However, all A21978C factors possess the same cyclic peptide core, differing only in the fatty acyl chain attached to the *N*-terminal amino acid. The availability of amino acids to serve as fatty acid primers for A21978C affects fatty acyl group biosynthesis and consequently determines the relative quantities of individual A21978C factors produced by the culture[10]. We postulated that if an analogous effect occurred with the A54145 fermentation, the relative abundance of acyl chains attached to the nuclei, which we had shown to be alterable by lipid precursors, could also be altered by amino acid precursors. In addition, because A54145 contains four nuclei that differ only in valine/isoleucine and/or glutamate/3-CH_3-glutamate substitutions, amino acid precursors could additionally affect the ratio of nuclei synthesized. Both the type and ratio of acyl chains and nuclei synthesized were strongly influenced by precursing with valine and isoleucine (Table 5). Valine virtually eliminated factors containing fatty acyl chains other than iC_{10} and significantly increased the level of the valine-containing "F" nucleus. Isoleucine conversely increased the level of factors containing aC_{11} acyl chains and suppressed synthesis of the "F" nucleus, apparently replacing the valine with isoleucine and simultaneously replacing glutamate with the 3-CH_3-glutamate analog. Leucine exerted the same affects as valine, but to a lesser degree. Although glutamate is contained in the "A" and "F" nuclei, glutamate did not increase the levels of either nucleus.

Each of these precursors depressed the total quantity of A54145 produced while aspartate, which constitutes four amino acid residues in each of the peptide nuclei, increased total antibiotic biosynthesis.

Acknowledgments

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References

1) HUNT, A. H.; R. E. CHANCE, M. G. JOHNSON, J. L. OCCOLOWITZ, J. S. MYNDERSE, D. S. FUKUDA & H. A. KIRST: A54145, a complex of new cyclic lipopeptides containing unusual amino acids. Structural studies. Program and Abstracts of the 28th Intersci. Conf. on Antimicrob. Agents Chemother., No. 969, p. 281, Los Angeles, Oct. 23～26, 1988
2) BOECK, L. D.; H. R. PAPISKA, R. W. WETZEL, J. S. MYNDERSE, D. S. FUKUDA, F. P. MERTZ & D. M. BERRY: A54145, a new lipopeptide antibiotic complex: Discovery, taxonomy, fermentation and HPLC. J. Antibiotics 43: 587～593, 1990
3) FUKUDA, D. S.; P. J. BAKER, M. DEBONO, R. M. MOLLOY & J. S. MYNDERSE: A54145, a new lipopeptide antibiotic complex—Deacylation and reacylation. Program and Abstracts of the 28th Intersci. Conf. on Antimicrob. Agents Chemother., No. 970, p. 281, Los Angeles, Oct. 23～26, 1988
4) BOECK, L. D.; D. S. FUKUDA, B. J. ABBOTT & M. DEBONO: Deacylation of A21978C, an acidic lipopeptide antibiotic complex, by *Actinoplanes utahensis*. J. Antibiotics 41: 1085～1092, 1988
5) HUBER, F. M.; R. L. PIEPER & A. J. TIETZ: Dispersal of insoluble fatty acid percursors in stirred bioreactors as a mechanism to control antibiotic factor distribution. Biotechnology Processes. *In* Conference Proceeding. *Ed.*, C. S. HO, pp. 249～253, Aiche, 1987
6) BOECK, L. D.; R. W. WETZEL, S. C. BURT, F. M. HUBER, G. L. FOWLER & J. S. ALFORD, Jr.: Sterilization of bioreactor media on the basis of computer-calculated thermal input designated as F_0. J. Ind. Microbiol. 3: 305～310, 1988
7) BOECK, L. D.; R. W. WETZEL, D. S. FUKUDA & J. S. MYNDERSE: A54145, a new lipopeptide antibiotic complex—Factor control through precursor directed biosynthesis. Abstracts of the Society for Indust Microbiol, 38: 4, No. P-42, p. 40, Chicago, Aug. 7～12, 1988
8) SHERMAN, M. M.; S. YUE & C. R. HUTCHINSON: Biosynthesis of lasalocid A. Metabolic interrelationships of carboxylic acid precursors and polyether antibiotics. J. Antibiotics 39: 1135～1143, 1986
9) TRABER, R.; H. HOFMANN & H. KOBEL: Cyclosporins—New analogues by precursor directed biosynthesis. J. Antibiotics 42: 591～597, 1989
10) ZMIJEWSKI, M. J., Jr.; B. BRIGGS & J. OCCOLOWITZ: Role of branched chain fatty acid precursors in regulating factor profile in the biosynthesis of A21978 C complex. J. Antibiotics 39: 1483～1485, 1986