VOL. 52 NO. 12, DEC. 1999 THE JOURNAL OF ANTIBIOTICS pp. 1077 — 1085

Production of a Family of Kinase-inhibiting Lactones from Fungal Fermentations

ANNE DOMBROWSKI*, ROSALIND JENKINS, SUSAN RAGHOOBAR, GERALD BILLS, JON POLISHOOK, FERNANDO PELÁEZ†, BRUCE BURGESS††, ANNIE ZHAO, LEEYUAN HUANG, YAN ZHANG and MICHAEL GOETZ

Merck Research Laboratories,
P. O. Box 2000, Rahway, NJ 07065, USA
†Merck Sharp & Dohme de España,
S.A. Josefa Valcarcel 38, 28027 Madrid, Spain
††Merck Manufacturing Division,
P. O. Box 2000, Rahway, NJ 07065, USA

(Received for publication April 26, 1999)

During the course of our screening for natural products from fungi, extracts of several cultures were found to make a family of related resorcylic acid lactone compounds, which are potent inhibitors of MEK kinase. Comparative and empirical studies of fermentation conditions improved the titers of the compounds of interest. Striking changes in the ratios and amounts of the major and minor compounds in some cases were achieved by manipulations of media composition.

Evidence has suggested that MEK (MapK/ERK kinase), a mitogen-activated protein kinase which is threonine/tyrosine specific, is important in growth factor signal transduction[1]. Activation of MEK appears to be necessary for cell differentiation and transformation; thus, a specific inhibitor of MEK may have potential as an anti-cancer agent. A family of lactones was found in our natural products screening program to inhibit MEK. The compounds are related to hypothemycin, a known resorcylic acid lactone, reported to be an antifungal agent[2,3]. Recent reports describe several MEK inhibitors: PD098059, which reversed cell transformation[4,5], U0126, an inhibitor of AP-1 transactivation[6], and Ro 09-2210, which had potent anti-proliferative effects[7]. A recent patent[8] covers a kinase inhibitor, similar to the MEK inhibitors mentioned here, which inhibits cell growth.

This paper describes the fermentation of three fungi producing related resorcylic acid lactones. The fungi respond differently to the same fermentation conditions, and the ratios of major and minor compounds sometimes can be manipulated by medium changes. The fermentation processes for two of the cultures were successfully scaled up from shake flasks to 23-liter tanks.

Methods

Origin and Maintenance of Fungi

The fungi used in these studies, listed in Table 1, were maintained as frozen vegetative mycelia at −75°C. The sterile stains were distinguished based on differences in growth rates, colony pigmentation and texture, and microscopic characteristics of hyphal branching when grown on a set of mycological media that included cornmeal agar, oatmeal agar, malt-yeast extract agar, and sterilized wood supported on water agar. Unless stated otherwise, all fungi are deposited in the Merck Microbial Resources Culture Collection.

Media

Seed medium

A 1-ml aliquot of frozen vegetative mycelium was used to inoculate 50 ml seed medium in a 250 ml unbaffled Erlenmeyer flask. The medium composition was (in g/liter): corn steep powder, 2.5; tomato paste, 40.0; oat flour, 10.0; glucose, 10.0 and trace elements solution, 10 ml/liter (consisting of, in g/liter: $FeSO_4 \cdot 7H_2O$, 1.0; $MnSO_4 \cdot H_2O$, 1.0; $CuCl_2 \cdot 2H_2O$, 0.025; $CaCl_2$, 0.1; H_3BO_3, 0.056;

$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.019; $ZnSO_4 \cdot 7H_2O$, 0.2), pH 6.8. The seed cultures were incubated at 25°C on a gyratory shaker (220 rpm, 5.1 cm throw), 85% relative humidity, until adequate biomass was obtained (approximately 4~5 days) for inoculation of the production medium.

Production Media

The following liquid production media were prepared with distilled water, dispensed at 50 ml per 250 ml unbaffled Erlenmeyer flask, and autoclaved. Production flasks were inoculated with 1~2 ml of the seed culture grown as described above and incubated at 22°C, with shaking at 220 rpm.

CYS80 (g/liter): sucrose, 80.0; yellow corn meal, 50.0; yeast extract, 1.0.

MV8 (g/liter): maltose, 75.0; V8 juice, 200 ml/liter; soy flour, 1.0; L-proline, 3.0; MES buffer, 16.2; pH 6.5.

NPF2 (g/liter): glucose, 150.0; urea, 4.0; NZ amine Type A, 4.0; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.25; KCl, 0.25; $ZnSO_4 \cdot 7H_2O$, 0.9; $CaCO_3$, 16.5.

AD2 (g/liter): glucose (autoclaved separately), 150.0; glycerol, 20.0; yeast extract, 4.0; $NaNO_3$, 1.0; monosodium glutamate, 3.0; Na_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 1.0; K-elements, 1.0 ml/liter; $CaCO_3$, 8.0; pH 7.0 before adding $CaCO_3$. K-elements include (g/liter): $FeCl_3 \cdot 6H_2O$, 5.8; $MnSO_4 \cdot H_2O$, 0.1; $CoCl_2 \cdot 6H_2O$, 0.02; $CuSO_4 \cdot 5H_2O$, 0.015; $Na_2MoO_4 \cdot 2H_2O$, 0.012; $ZnCl_2$, 0.02; $SnCl_2 \cdot 2H_2O$, 0.005; H_3BO_3, 0.01; KCl, 0.02; concentrated HCl, 2.0 ml/liter.

LSFAD1 (g/liter): glycerol, 75.0; glucose, 50.0, ardamine PH, 5.0; soybean meal, 5.0; tomato paste, 5.0; sodium citrate, 2.0; $(NH_4)_2SO_4$, 2.0; pH 7.0.

Def2 (g/liter): glycerol, 60.0; monosodium glutamate, 10.0; L-tryptophan, 0.7; NH_4Cl, 3.0; K_2HPO_4, 1.0; $CaCO_3$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.58; MES buffer, 20.0; glucose, 60.0 (autoclaved separately); 50X salts, 20.0 ml/liter; pH 6.0. The 50X salts solution contains (mg/liter): $FeSO_4 \cdot 7H_2O$, 500; $ZnSO_4 \cdot 7H_2O$, 500; $MnSO_4 \cdot H_2O$, 100; $CuSO_4 \cdot 5H_2O$, 50; $CoCl_2 \cdot 6H_2O$, 40.

Fermentation in 23-liter Tanks

Fermenter parameters for the production of L-783,277 were as follows: Temperature, 25°C; agitation, 400 rpm; airflow, 0.5 vvm; pressure, 0.6 bar. The working volume was 14 liters and the inoculum size was 3.6% (v/v). The dissolved oxygen of the medium was maintained at or above 30% relative to saturation throughout the fermentation. The media used in the fermenters were CYS80 or MV8, as described in Methods, with the addition of polyglycol 2000 as antifoam at 1.7 ml/liter.

Fermenter parameters for the production of L-783,279 were as follows: Temperature, 22°C; agitation, 400 to 600 rpm; airflow, 0.4 vvm; pressure, 0.3 bar. The working volume was 15 liters, with an inoculation size of 3.3% (v/v). The glucose was sterilized separately and added to the fermenter in two equal parts. One addition was at 0 hours, and the other addition was at 13 days of incubation. Increases in agitation from 400 to 600 rpm were necessary to maintain oxygen at or above 30% relative to saturation. After four days of cultivation, the pH was controlled using a sterile 10% sulfuric acid solution, to maintain a maximum pH of 7.0. The medium was NPF2, as described in Methods, with the glucose sterilized separately and added as a post-sterile shot. Polyglycol 2000 was used as an antifoam at 3 ml/liter.

Assay Conditions

The fermentations (fungal growth plus broth or solid substrate) were extracted with methylethylketone and assayed initially using MEK, as described by ZHAO *et al.*[9]. The extracts were evaporated to dryness and redissolved in methanol for analysis by HPLC. The HPLC conditions for separation and quantitation of the compounds were: Zorbax RxC8 (DuPont), 4.6×250 mm column; elution at 1 ml/minute with acetonitrile : water (35 : 65); temperature, 50°C; detection at 300 nm with a Knauer UV detector.

Results

Lactone-producing Fungi

The isolation and characterization of these resorcylic acid lactones (shown in Figure 1) are reported by A. ZHAO *et al.*[9]. The taxonomic relationship among the three fungi that produced these compounds is unknown because only a vegetative or an asexual state was observed in culture (Table 1). Morphological analyses as well as the disparate geographic origins indicate the three strains are distinct species. Only one of these strains (MF6275) produced characteristic sporulating structures that could be assigned to the form genus *Phoma*; the other two strains (MF6280, MF6293) did not sporulate in agar culture and could not be identified further.

Fermentation Studies

Dark *vs.* Light

The kinase inhibitory activity was first observed when culture MF6275 was grown in CYS80 production medium in the dark. Subsequently, comparison of metabolite production under dark *vs.* light conditions showed that the

Fig. 1. Chemical structures of lactones.

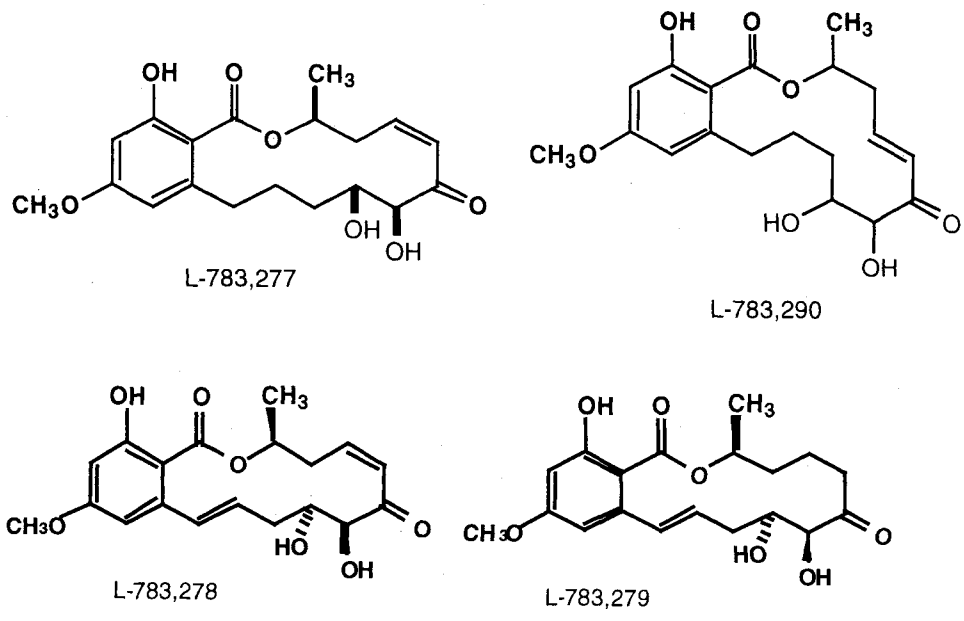


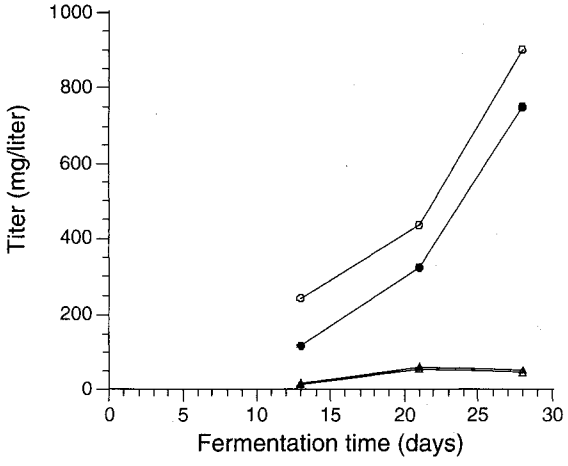
Table 1. Lactone-producing fungi and their geographic origins.

Strain numbers	Identification	Substratum	Location	Compounds made
MF6275 ATCC74403	*Phoma* sp.	fruitbody of *Helvella acetabulum*	Guadalajara, Spain	L-783,277 L-783,290
MF6280	Sterile fungus	soil	Near Tucson, Arizona	L-783,278 L-783,279
MF6293	Sterile fungus	leaf litter of *Guarae guidonia*	El Verde, Puerto Rico	L-783,278 L-783,279

level of production of the major compound L-783,277 was approximately 20% greater in the dark than in the light (Figure 2). On the other hand, the production of the minor compound L-783,290 appeared to be unaffected by the light. Cultures MF6293 and MF6280 did not exhibit this difference in production in a comparison of dark and light

Fig. 2. Production of resorcylic acid lactones by MF6275 in light and dark conditions.

●: L-783,277 (in light), ○: L-783,277 (in dark), △: L-783,290 (in light), ▲: L-783,290 (in dark).



conditions (data not shown).

Since there was no evidence of light-induced isomerization or instability of either L-783,277 or L-783,290 in any solvent tested, the effects of light *vs.* dark on L-783,277 production are presumably due to physiological or biosynthetic rather than chemical causes. Examples of light-regulated genes have been reported in fungi, such as those affecting developmental processes: hyphal branching and conidia formation in *Neurospora*[10,11], fruiting in *Schizophyllum*[12], and spore formation in *Coprinus*[13]. Further work will be necessary to determine if the observed effect of light on production titers in our studies is regulated at the transcription level.

Media Studies

Culture MF6275, which was initially screened on liquid medium, was tested in several other liquid media to find conditions under which higher levels of compound would be produced (Table 2A). Cultures MF6293 and MF6280, on the other hand, were screened initially using a solid vermiculite support over which a nutrient solution had been poured. After activity was detected with these cultures, efforts were made to find liquid media in which the compounds of interest would be made and to improve the titers of the compounds produced (Table 2B and 2C). Although a number of media were tested, some yielded little or no production of the lactones; representatives of the productive media are shown here.

We were unable to identify a single medium that supported the best production of the lactone compounds in all three fungi (Table 2). The best medium for production of the major compound L-783,277 by culture MF6275 appeared to be CYS80, with MV8 nearly as good. AD2 and LSFAD1 yielded good titers of L-783,277 also. The minor compound L-783,290 was made by MF6275 in low amounts in all the media tested. By contrast, for culture MF6293 the best medium for production of both L-783,279 and L-783,278, appeared to be MV8, with very little produced in CYS80. The third culture MF6280 produced L-783,278 best in CYS80 and LSFAD1 media, with MV8 also supporting good production. However, the production of L-783,279 by culture MF6280 was best in LSFAD1. The difference in metabolite production by culture MF6280 based on medium used was of interest. In CYS80 L-783,278 was the major compound, while L-783,279 was the minor compound. In NPF2, AD2 and Def2, L-783,279 was the major compound, while L-783,278 was the minor. The two compounds were produced in more equivalent amounts in LSFAD1 and MV8. Cultures MF6275 and MF6280 were chosen for further studies, as sources of the compounds of interest L-783,277, L-783,278 and L-783,279.

Further Studies with Culture MF6275

Flask Media Volume Studies with MF6275

The effect of varying the volume of medium in shake flasks was examined. Studies with 30, 50 or 70 ml of CYS80 medium in 250 ml flasks indicated that more metabolite was produced at the higher volumes (Figure 3A). This effect was also observed at the 2-liter flask level (Figure 3B), suggesting that low oxygen tensions may favor greater production levels of L-783,277. The minor compound L-783,290 was not much affected by the volume of media in the flasks (250 ml or 2-liter flasks).

Carbon Level Studies with MF6275

Figures 4A and 4B show the effect of varying the carbon levels on production of L-783,277 by MF6275. The best medium for L-783,277 production, CYS80, was prepared with three levels of sucrose: 50, 80 and 100 g/liter (80 g/liter was the standard). MV8 was prepared with three levels of maltose: 50, 75 and 100 g/liter maltose (75 g/liter was the standard). For CYS80, decreasing the amount of sucrose from 80 g/liter to 50 g/liter increased the titer of L-783,277 from 621 mg/liter to 990 mg/liter. However, for MV8, increasing the amount of maltose from 75 g/liter to 100 g/liter improved the titer (from 500 mg/liter to 1450 mg/liter).

Table 2. Effect of medium and incubation time on titers of lactones.

A. **MF6275**	Day 17		Day 21		Day 28	
Media	L-783,277	L-783,290	L-783,277	L-783,290	L-783,277	L-783,290
CYS80	370	2	513	18	621	19
MV8	118	10	572	21	528	<10
AD2	37	33	262	29	482	27
LSFAD1	534	28	431	16	429	40
Def2	<10	<10	<10	<10	<10	<10
NPF2	<10	<10	<10	<10	<10	<10

B. **MF6293**	Day 17		Day 24	
Media	L-783,278	L-783,279	L-783,278	L-783,279
CYS80	0	0	7	0
MV8	72	29	528	45
AD2	0.2	0	130	0

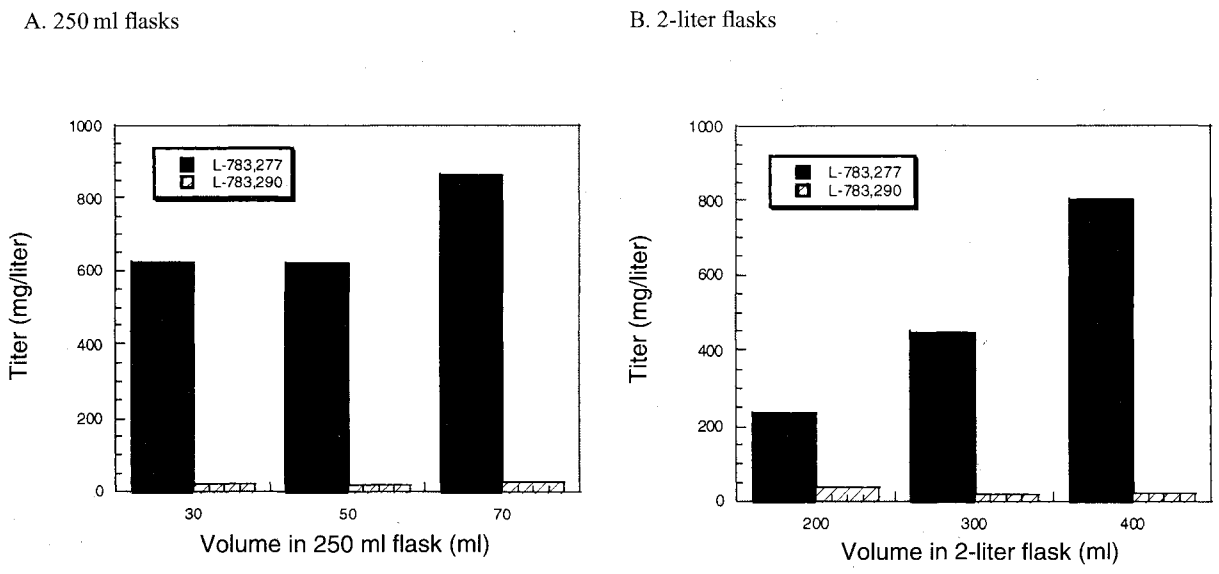
C. **MF6280**	Day 14		Day 22	
Media	L-783,278	L-783,279	L-783,278	L-783,279
CYS80	1172	242	1456	327
MV8	443	398	642	291
AD2	72	382	77	477
LSFAD1	909	421	915	751
Def2	21	144	15	217
NPF2	10	141	32	248

Titers expressed in mg/liter

Scale-up of MF6275 in 23-liter Tanks

To determine that the processes developed for shake flasks were scalable to tanks, MF6275 was grown in two 23-liter tanks, to compare the production of L-783,277 in the two media CYS80 and MV8 media (Figure 5). The culture produced in 23-liter tanks, but the titers were not as high as had been observed in shake flasks, using the parameters noted above. The yield of L-783,277 in CYS80 at the harvest point was 243 mg/liter and in MV8 the titer was 254 mg/liter, although MV8 appeared to peak over 300 mg/liter before declining. In the shake flask controls, which used tank media, CYS80 yielded nearly 500 mg/liter and MV8 was over 300 mg/liter.

Fig. 3. Production by MF6275 in 250 ml and 2-liter flasks.



Further Sudies with Culture MF6280

Carbon Source Substitutions with MF6280

An effort was made to determine a medium in which titers of L-783,279 produced by culture MF6280 were enhanced over L-783,278 titers. This was due to the fact that the isolation of L-783,279, in the presence of large amounts of L-783,278, was difficult. Therefore, we attempted to improve the production of L-783,279, while minimizing the levels of L-783,278 (below 10% of the L-783,279 levels). In the media study above with MF6280 (Table 2C), LSFAD1 yielded the highest L-783,279 titers, but the L-783,278 levels were even higher. In several other media, the L-783,278 levels were elevated as well (CYS80, MV8, and less so in AD2). The data suggested the appropriate media with which to continue development were NPF2 and Def2. These media supported titers of 200~300 mg/liter L-783,279 at 22 days, with ratios of L-783,278/ L-783,279 of 7~12%. Additional carbon/energy source studies were done to determine if the titers and ratio could be further improved. The data in Table 3 indicated that, compared to glucose in the original medium, none of the carbon substitutions tried enhanced the titers or ratios. Fructose showed comparable results, at least for NPF2. Since it is less cost-effective than glucose, the carbon source was not changed.

In subsequent experiments, titers of L-783,279 in NPF2 and the ratios of L-783,278/ L-783,279 appeared to be somewhat better than Def2 (Table 4). Although the titers varied somewhat from one experiment to another, the best L-783,279 titer was 400 mg/liter in NPF2 with a ratio of 9%. NPF2 was chosen for subsequent work with culture MF6280 for L-783,279 production and was used in the 23-liter scale-up.

Scale-up of MF6280 in 23-liter Tanks

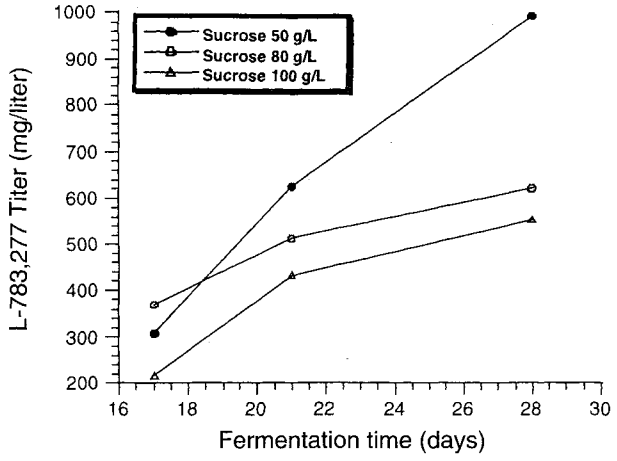
MF6280 was scaled up in 23-liter fermenters, with the objective of maximizing the process for L-783,279, while reducing the levels of L-783,278 below 10% (Figure 6). NPF2 medium was used, with the glucose sterilized separately. One of the tanks yielded up to 800 mg/liter. The levels of L-783,278 were fairly low, but did not meet the 10% objective. The L-783,278 titers were lowest in the early part of the fermentation, but seemed to increase in the later stages.

Discussion

The results reported here show that three fungi, which make a family of related lactones, responded differently to various fermentation conditions. Those conditions, which were best for lactone production by one culture, were not necessarily the ones that were best for production by another culture. MF6275 responded best to CYS80 or MV8 in the dark, while MF6293 preferred MV8 but did poorly

Fig. 4. Carbon level studies in MF6275.

A. CYS medium



B. MV8 medium

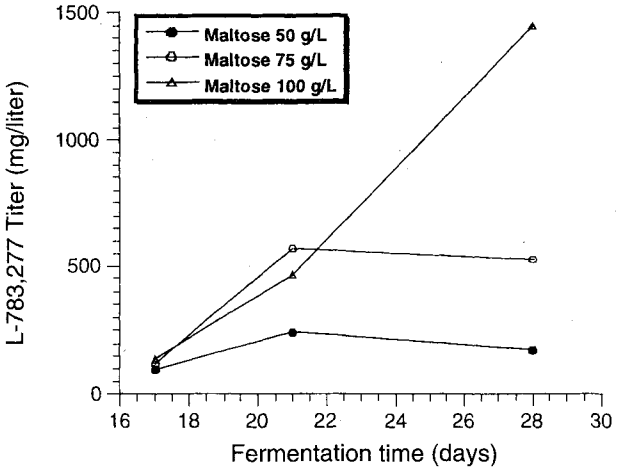


Fig. 5. Production by MF6275 in 23-liter tanks.

○: CYS80 tank, □: MV8 tank, ◇: CYS80 shake flask, ×: MV8 shake flask.

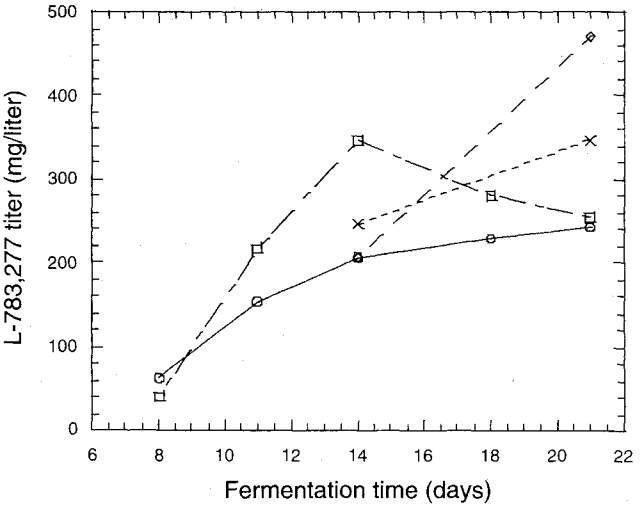


Table 3. MF6280 carbon source substitutions.

Carbon source		L-783,278	L-783,279
NPF2	glucose	38	249
	glycerol	0	<10
	fructose	27	243
	lactose	0	0
	sucrose	<10	80
	mannitol	0	0
Def2	glucose	13	398
	glycerol	<10	28
	fructose	50	170
	lactose	<10	27
	sucrose	21	209
	mannitol	<10	34

Best titers (mg/liter) of 14~28 day samples.

on CYS80. Carbon source manipulation in CYS80 and MV8 further increased the titer of the major compound in MF6275. MF6280 produced the lactone L-783,279 in good titers on CYS80 and MV8, as well as on media LSFAD1 and AD2. However, the best media for the production of L-783,279 by MF6280 had the disadvantage of also supporting high production of L-783,278, which complicated downstream processing. Another medium (NPF2) was chosen for development of this culture in order to minimize the production of L-783,278. Media manipulation in culture MF6280 appeared to greatly influence the ratio of major and minor compounds. This was not true in MF6275: the major compound remained the major compound in all conditions; the minor compound was not much affected by any of the conditions.

A better understanding of the biosynthetic origins of these resorcylic acid lactones would be of interest. There appears to be no precursor-product relationship of one

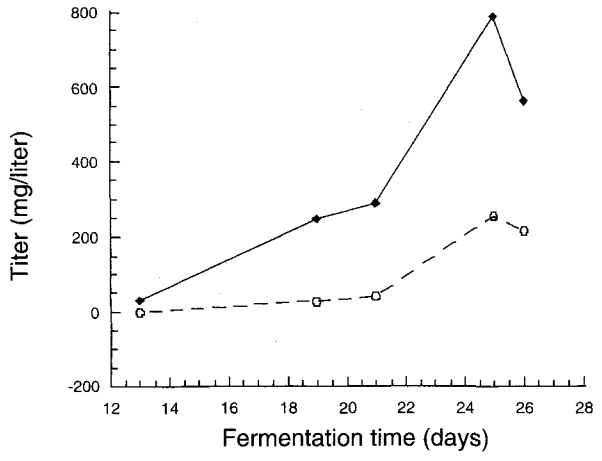
Table 4. Production of MF6280 in NPF2 and Def2.

		Day 14		Day 21		Day 28-30	
	Medium	L-783,278	L-783,279	L-783,278	L-783,279	L-783,278	L-783,279
Expt. 1	NPF2	<10	108	<10	203	<10	319
	Def2	<10	53	<10	215	<10	209
Expt. 2	NPF2	18	222	36	372	38	401
	Def2	44	208	35	331	45	340

Titers expressed in mg/liter.

Fig. 6. Production by MF6280 in 23-liter tanks.

○: L-783,278, ◆: L-783,279.



compound to another, as there were no indications of one compound being produced earlier than the other in time course analyses. Nor did extracts appear to contain compounds in measurable amounts that could be taken as possible intermediates.

There does not seem to be any direct correlation between the media components and the compounds which are produced. The differences in production of resorcylic acid lactones reported here appears to be culture dependent. Culture type A (MF6275) produced compounds L-783,277 and L-783,290; Culture type B (MF6293 and MF6280) produced compounds L-783,278 and L-783,279. Within each type, the distribution of compounds was affected by medium. The carbon sources do not appear to account for the yield differences. However, the overall yields were lower in defined media or partially defined media (Def2, NPF2, AD2). The complex nitrogen sources in the other media CYS80, LSFAD1 and MV8 supported higher titers. Thus, the slow utilization of nitrogen sources appeared to yield higher titers. Yeast extract did not appear to be important as a nitrogen source, however, since AD2 (containing yeast extract) was one of the lower yielding media. While there appeared to be less growth in the defined media in some cases, this may indicate a need for trace nutrients supplied in the complex media. However, differences in growth do not appear to account for all the differences in yields. Perhaps the metabolic pathways or pathways of enzymes involved are different, when various nitrogen sources are used and results in the observed differences in titers. Differences in yields from various types of complex nitrogen sources have been observed elsewhere[14], where it was speculated that the amino acid composition and the molecular weight distributions of proteins and peptides in the complex nitrogen sources are critical for production. Further studies are required to understand the mechanistic basis for the observations reported here.

Acknowledgments

The authors thank PRAKASH MASUREKAR for helpful discussions and WILLIAM STROHL for critical reading of the manuscript.

References

1) COWLEY, S.; H. PATERSON, P. KEMP & C. MARSHALL: Activation of MAP kinase kinase is necessary and sufficient for PC-12 differentiation and for transformation of NIH 3T3 cells. Cell 77: 841~852, 1994
2) AGATSUMA, T., *et al.*: Revised structure and stereochemistry of hypothemycin. Chem. Pharm. Bull. 41: 373~375, 1993
3) NAIR, M. & S. CAREY: Metabolites of Pyrenomycetes XIII[1]: Structure of (+) hypothemycin, an antibiotic macrolide from *Hypomyces trichothecoides*. Tetrahedron Letters 21: 2011~2012, 1980
4) DUDLEY, D. T.; L. PANG, S. DECKER, A. BRIDGES & A. SALTIEL: A synthetic inhibitor of the mitogen-activated protein kinase cascade. Proc. Natl. Acad. Sci. USA 92: 7686~7689, 1995
5) ALESSI, D.; A. CUENDA, P. COHEN, D. DUDLEY & A. SALTIEL: PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase *in vitro* and *in vivo*. J. Biol. Chem. 270: 27489~27494, 1995
6) FAVATA, M.; K. HORIUCHI, E. MANOS, A. DAULERIO, D. STRADLEY, W. FEESER, D. VAN DYK, W. PITTS, R. EARL, F. HOBBS, R. COPELAND, R. MAGOLDA, P. SCHERLE & J. TRZASKOS: Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J. Biol. Chem. 273: 18623~18632, 1998
7) WILLIAMS, D. H.; S. E. WILKINSON, T. PURTON, A. LAMONT, H. FLOTOW & E. J. MURRAY: Ro 09-2210 exhibits potent anti-proliferative effects on activated T cells by selectively blocking MKK activity. Biochemistry 37: 9579~9585, 1998
8) GIESE, N. & N. LOKKER (Cor Therapeutics): Method and compositions for inhibiting protein kinases, US patent 5,728,726, March 17, 1998
9) ZHAO, A.; S. LEE, M. MOJENA, R. JENKINS, D. PATRICK, H. HUBER, M. GOETZ, O. HENSENS, D. ZINK, D. VILELLA, A. DOMBROWSKI, R. LINGHAM & L. HUANG: Resorcylic acid lactones: Naturally occurring potent and selective inhibitors of MEK. J. Antibiotics 52: 1086~1094, 1999
10) LAUTER, F.; U. MARCHFELDER, V. RUSSO, C. YAMASHIRO, E. YATZKAN & O. YARDEN: Photoregulation of cot-1, a kinase-encoding gene involved in hyphal growth in *Neurospora crassa*. Fungal Genetics & Biology 23: 300~310, 1998
11) ARPAIA, G.; J. LOROS, J. DUNLAP, G. MORELLI & G. MACINO: Light induction of the clock-controlled gene ccg-1 is not transduced through the circadian clock in *Neurospora crassa*. Mol. Gen. Genet. 247: 157~163, 1995
12) HORTON, J.; G. PALMER & W. SMITH: Regulation of dikaryon-expressed genes by FRT1 in the basidiomycete *Schizophyllum commune*. Fungal Genetics & Biology 26: 36~47, 1999
13) KERTESZ CHALOUPKOVA, K.; P. WALSER, J. GRANADO, M. AEBI & U. KUES. Blue light overrides repression of aesexual sporulation by mating type genes in the basidiomycete *Coprinus cinereus*. Fungal Genetics & Biology 23: 95~109, 1998
14) MASUREKAR, P.; J. FOUNTOULAKIS, T. HALLADA, M. SOSA & L. KAPLAN.: Pneumocandins from *Zalerion arboricola* II. Modification of product spectrum by mutation and medium manipulation. J. Antibiotics 45: 1867~1874, 1992