Bot. Bull. Acad. Sin. (1993) **34:** 363-372

Studies of the production of Neihumicin by *Micromonospora neihuensis* Wu

Rong Yang Wu
Institute of Botany, Academia Sinica, Taipei, Taiwan 115, Republic of China

(*Received January 11, 1993; Accepted July 10, 1993*)

Abstract. Strain NH3-1 of *Micromonospora neihuensis* Wu produces a new antibiotic, named Neihumicin. Previous studies (Wu *et al.*, 1988) showed its cytotoxic effects *in vitro* against KB tissue culture cells as well as its antifungal activity against *Saccharomyces cerevisiae* ATCC 9763. The optimum conditions for cell growth and antibiotic production from strain NH3-1 were studied by formulating media for spore, seed, and antibiotic production cultures. On agar plates, the best colony growth and sporulation occurred on tryptone yeast glucose medium. In liquid cultures, the mycelia grew well in enriched media including yeast extract, malt extract (ISP-2), tomato paste oatmeal (TPO), peptone-potato glycerol (PPG), tryptone yeast glucose (TYG), and oatmeal (ISP-3) media. The enriched media also supported good antibiotic production. A temperature of 25°C, agitation at 200 rpm, and supplementation with vitamins B_{12} and C were found to provide optimal conditions.

Key words: Antibiotic; Antifungal; *Micromonospora*; Neihumicin.

Introduction

A newly isolated *actinomycete* species, *Micromonospora neihuensis* Wu showed unique taxonomic characteristics (Wu *et al.*, 1988), and produced antibiotics showing cytotoxic effects *in vitro* against KB tissue culture cells (ED_{50} 0.94 μg/ml) and antifungal activities against *Saccharomyces cerevisiae* ATCC 9763. An antibiotic named Neihumicin was isolated from *M. neihuensis* mycelial cakes by extraction with 90% methanol followed by crystallization in a mixture of methanol and ethanol (1:1 v/v). Subsequent studies based on UV, IR, NMR, MS, and X-ray crystallographic analysis determined the structure of Neihumicin (Yang *et al.*, 1988) as (Z)-3, (Z)-6-dibenzylidene-2-methoxy-3,6-dihydropyrazin-5-one (Fig. 1).

To prepare for the mass production of Neihumicin, optimal culture conditions, particularly media, for spore, seed, and antibiotic production by *M. neihuensis* Wu were investigated.

Materials and Methods

Strains

The antibiotic producing strain NH3-1 of *M. neihuensis* Wu had been isolated from Nei-Hu soil (Wu *et al.*, 1988). Stock culture was maintained on tryptone yeast glucose (TYG) agar at 28°C for 14 to 21 days, and stored at 4°C in a refrigerator.

Media and Cultivation

Three kinds of media were formulated to study the optimal culture conditions for sporulation, seeding and fermentation, according to Seino (1985), Komagata

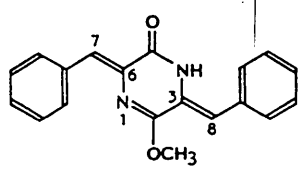

Fig. 1. Structure of Neihumicin

(1986), Waksman (1967), Wu (1984) and Wu *et al.* (1983, 1988).

Ten sporulation media were used for spore formation on agar plate culture (Table 1). Typically, each agar plate was inoculated with 0.1 ml of spore suspension in normal saline prepared from an agar slant of stock culture, followed by incubation at 28°C for three weeks. At the end of each week, the cell morphology, sporulation, and growth characteristics were observed microscopically (Nikon 104 light microscope) according to Arai (1976), Waksman (1967), Sykes and Skinner (1973), Alexander (1961), Wu *et al.* (1988) and Buchanan and Gibbons (1974).

Nine media were formulated for seed cultures (Table 2). Each culture contained 50 ml of medium in a 125 ml Erlenmeyer flask. The medium pH was adjusted to 7.0 before sterilization. Each flask was inoculated with 0.1 ml of spore suspension and cultivated at 28°C, 120 rpm for 5 days. Culture pH, cell weight, and antibiotic potency were measured daily.

Eighteen media were formulated for antibiotic production (Table 3). Each culture contained 50 ml of medium in a 125 ml Erlenmeyer flask. The medium pH was adjusted to 7.0 before sterilization. Each flask was inoculated with spore suspension from a stock culture slant. Seed culture was grown on TYG liquid seed medium at 28°C, 120 rpm for 4 days. Subsequently, each production culture was inoculated with 2 % (v/v) seed, followed by incubation at 20, 25, or 35°C, with agitation at 80, 120, or 200 rpm, respectively, for 6 days. A 5 ml quantity of culture broth was sampled daily for pH and antibiotic potency measurement.

Table 1. *Composition of sporulation media**

Components	Medium designation**								
	WA	V-8A	OA	OYA	ISP-4	TYSA	PCA	CYCA	TYGA
Agar-agar (g)	20	20	20	20	20	20	20	20	20
V-8 juice (ml)	-	50	-	-	-	-	-	-	-
$CaCO_3$ (g)	-	9.3	-	-	2	-	-	-	-
Quaker white oats (g)	-	-	3	20	-	-	-	-	-
KNO_3 (g)	-	-	0.2	-	-	-	-	-	-
K_2HPO_4 (g)	-	-	0.5	-	1	-	-	-	1
$MgSO_4$-$7H_2O$ (g)	-	-	0.2	-	1	-	-	-	-
Yeast extract (g)	-	-	-	1	-	0.3	-	2	3
Soluble starch (g)	-	-	-	-	10	1-2	10	-	-
NaCl (g)	-	-	-	-	1	-	5	-	-
$(NH_4)_2SO_4$ (g)	-	-	-	-	2	-	-	-	-
Trace salt (ml)***	-	-	-	-	1	-	-	-	-
$FeSO_4$-$7H_2O$ (g)	-	-	-	-	-	-	-	-	-
$MnCl_2$-$4H_2O$ (g)	-	-	-	-	-	-	-	-	-
$ZnSO_4$-$7H_2O$ (g)	-	-	-	-	-	-	-	-	-
Corn steep liquor (g)	-	-	-	-	-	-	5	-	-
Peptone (g)	-	-	-	-	-	-	5	-	-
$CaCl_2$ (g)	-	-	-	-	-	-	0.5	-	-
Czapek Dox (g)	-	-	-	-	-	-	-	33.4	-
Casein (g)	-	-	-	-	-	-	-	6	-
Tryptone (g)	-	-	-	-	-	-	-	-	5
Glucose (g)	-	-	-	-	-	-	-	-	10
KH_2PO_4 (g)	-	-	-	-	-	-	-	-	1

* Each medium contains listed components in 1 liter distilled water.

** WA: white agar, V-8A: 1/20 V-8 juice agar, OA: oatmeal agar, OYA: OA-yeast extract agar, ISP-4: mineral salt starch agar, TYSA: thin-yeast starch agar, PCA: peptone corn agar, CYCA: Czapek-Dox yeast extract casein agar, TYGA: tryptone yeast extract glucose agar.

***Trace salt solution contains 0.1 g each of $ZnSO_4$-$7H_2O$, $MnCl_2$-$4H_2O$, and $FeSO_4$-$7H_2O$ in 100 ml distilled water.

Table 2. *Composition of seed media**

Components	Medium designation**								
	ISP-2	TPO	CD	PPG	GT	PY	TYG	MAM	ISP-3
Yeast extract (g)	4	-	-	-	-	3	3	-	-
Malt extract (ml)	10	-	-	-	-	-	-	-	-
Dextrose (g)	4	-	-	-	-	-	-	-	-
Tomato paste (g)	-	20	-	-	-	-	-	-	-
Oatmeal (g)	-	20	-	-	-	-	-	-	-
$NaNO_3$ (g)	-	-	3	-	-	-	-	-	-
K_2HPO_4-$3H_2O$ (g)	-	-	1	0.5	0.5	-	1	51.6	-
$MgSO_4$-$7H_2O$ (g)	-	-	0.5	-	0.5	-	-	0.5	-
KCl (g)	-	-	0.5	-	-	-	-	-	-
$FeSO_4$-$7H_2O$ (mg)	-	-	10	10	10	-	-	-	-
Sucrose (g)	-	-	30	-	-	-	-	-	-
Potato (g)	-	-	-	100	-	-	-	-	-
Glycerol (ml)	-	-	-	5	15	-	-	-	-
Peptone (g)	-	-	-	2	-	5	-	-	-
NaCl (g)	-	-	-	0.5	0.5	-	-	-	-
Asparagine (g)	-	-	-	-	1	-	-	1	-
Tyrosine (g)	-	-	-	-	0.5	-	-	-	-
2M $Ca(NO_3)_2$ (ml)	-	-	-	-	-	1	-	-	-
Tryptone (g)	-	-	-	-	-	-	5	-	-
Glucose (g)	-	-	-	-	-	-	10	20	-
NH_4NO_3 (g)	-	-	-	-	-	-	-	2	-
Succinic acid (g)	-	-	-	-	-	-	-	0.5	-
Quaker white oats (g)	-	-	-	-	-	-	-	-	20

* Each medium contains listed components in 1000 ml distilled water, except MAM (900 ml).

**ISP-2: yeast extract malt extract medium, TPO: tomato-paste oatmeal medium, CD: Czapek's solution, PPG: potato peptone glycerol medium, GT: glycerol tyrosine medium, PY: peptone yeast medium, TYG: tryptone yeast extract glucose, MAM: minimal actinomycete medium, ISP-3: oatmeal medium.

Growth Factors

Seed cultures in TYG medium were supplemented with 10 mg/l of one of vitamins B_1, B_2, B_6, B_{12}, C, or folic acid, to test their affect on antibiotic production.

Antibiotic Activity Test

Antibiotic activity was analyzed using the paper disc method (Code of Federal Regulations, 1981) with *Saccharomyces cerevisiae* ATCC 9763 as the indicator. The antibiotic potency of a culture broth was presented as the diameter of the inhibition zones.

Scanning Electron Microscopy

The plate cultures of sporulation media were sampled for scanning electron microscopy preparation according to Wu *et al.* (1988). The cell morphology and sporulation were observed using a Zeiss DSM 950 scanning electron microscope according to Arai (1976), Waksman (1976), Sykes and Skinner (1973), Alexander (1961), Wu *et al.* (1988), and Buchanan and Gibbons (1974).

Results

Spore Formation

Growth characteristics on agar plates agreed with previous reports (Wu *et al.*, 1988). As shown in Table 4, the best media for sporulation was TYGA, whereas the worst were WA, OA, and OYA. On TYGA, strain NH3-1 grew into colonies of 2 to 3 mm diameter and were light yellow to yellow in color. Colonies revealed irregular and convex surfaces. Scant aerial mycelia were observed in all the media used. Under the microscope, the TYGA culture showed rapid formation of a considerable number of single spores at the tip of the sporophores after 1 week of cultivation. After 3 weeks of cultiva-

Table 3. *Composition of production media**

Components	Medium designation**								
	SA	GC	GM	GG	FM	PDB	YS	OA	CM
Sucrose (g)	10	—	—	—	—	—	—	—	—
Corn meal (g)	12	20	—	—	—	—	—	—	—
NaCl (g)	3	5	—	0.5	—	—	—	—	—
$MgSO_4$-$7H_2O$ (g)	0.5	—	—	0.5	—	—	—	—	—
KH_2PO_4 (g)	0.1	—	—	—	—	—	—	—	—
Glycerol (ml)	—	15	—	15	—	—	—	—	—
Peptone (g)	—	5	45	—	—	—	—	—	—
Glucose (g)	—	—	25	—	20	—	—	—	—
Casein (g)	—	—	4	—	—	—	—	—	0.2
Molasses (g)	—	—	10	—	—	—	—	—	—
$CaCO_3$ (g)	—	—	2	—	1	—	—	—	0.2
Na-glutamate (g)	—	—	—	5	—	—	—	—	—
K_2HPO_4 (g)	—	—	—	0.5	—	—	—	—	0.2
$FeSO_4$ (mg)	—	—	—	0.01	—	—	—	—	5
Beef extract (g)	—	—	—	—	3	—	—	—	—
Fish meal (g)	—	—	—	—	20	—	—	—	—
Cellulose (g)	—	—	—	—	1	—	—	—	—
Yeast extract (g)	—	—	—	—	5	—	2	—	0.1
Potato (g)#	—	—	—	—	—	200	—	—	—
Dextrose (g)	—	—	—	—	—	10	—	—	—
Soluble starch (g)	—	—	—	—	—	—	10	—	—
Oatmeal (g)	—	—	—	—	—	—	—	20	—
Trace salt (ml)***	—	—	—	—	—	—	—	1	—
NH_4NO_3 (g)	—	—	—	—	—	—	—	—	0.2
Urea (g)	—	—	—	—	—	—	—	—	0.1
Casamino acid (g)	—	—	—	—	—	—	—	—	0.1
Cellulose powder (g)	—	—	—	—	—	—	—	—	20
Peptone (g)	10	—	—	—	3	2	—	2	—
Dextrose (g)	40	—	—	—	—	—	—	—	—
Nutrient broth (g)	—	8	—	—	—	—	—	—	—
Tryptone (g)	—	—	5	—	—	—	—	—	—
Yeast extract (g)	—	—	3	—	5	2	—	—	4
Glucose (g)	—	—	10	—	10	10	—	—	4
K_2HPO_4 (g)	—	—	1	0.5	—	—	—	0.5	—
KH_2PO_4 (g)	—	—	1	—	—	—	—	—	—
Glycerol (ml)	—	—	—	15	—	—	—	5	—
Asparagine (g)	—	—	—	1	—	—	—	—	—
Tyrosine (g)	—	—	—	0.5	—	—	—	—	—
$MgSO_4$-$7H_2O$ (g)	—	—	—	0.5	—	—	—	—	—
$FeSO_4$ (mg)	—	—	—	10	—	—	—	10	—
NaCl (g)	—	—	—	0.5	—	—	—	0.5	—
Hydrolysed casein (g)	—	—	—	—	—	0.5	—	—	—
Tomato paste (g)	—	—	—	—	—	—	20	—	—
Oatmeal (g)	—	—	—	—	—	—	20	—	—
Potato (g)#	—	—	—	—	—	—	—	100	—
Malt extract (g)	—	—	—	—	—	—	—	—	10

Broth from boiling potato for 20 min.

* Each medium contains listed components in 1 liter distilled water.

**SA: sucrose corn meal medium, GC: glycerol corn medium, GM: glucose molasses medium, GG: glycerol glutamate medium, FM: fish meal medium, PDB: potato dextrose broth medium, YS: yeast starch medium, OA: oatmeal medium, CM: cellulose medium, SM: Sabouraud medium, NB: nutrient broth medium, TYG: tryptone yeast glucose medium, GT: glycerol tyrosine medium, PY: peptone yeast extract medium, CM-1: CM-1 medium, TPOA: tomato paste oatmeal, PPG: potato peptone glycerol medium, YM: yeast extract malt extract medium.

***Trace salt solution contains 0.1 g each of $ZnSO_4$-$7H_2O$, $MnCl_2$-$4H_2O$ and $FeSO_4$-$7H_2O$, in 100 ml distilled water.

Table 4. *Growth characteristics of strain NH3-1 on various sporulation media incubated at 28°C for 3 weeks*

	Sporulation media									
	WA	V-8A	OA	OYA	ISP-4	TYSA	TPCA	PCA	CYCA	TYGA
Colony growth*	Poor 0.5 mm	Slight 0.5 mm	Poor 0.5 mm	Poor 0.5 mm	Little 0.5-1 mm	Slight 0.5 mm	Moderate 1 mm	Moderate 1 mm	Good 2 mm	Good 2-3 mm
Color	White	Light yellow	White	White	Light yellow	White	Light yellow	Yellow	Yellow	Yellow
Aerial mycelia	Scant	Scant	Scant	Scant	Scant	Scant	Scant	Scant	Scant	Scant
Spore	Scant	Few	Poor	Poor	Few	Few	Moderate	Moderate	Good	Abundant

*The diameter (mm) of the largest single colony.

tion, matured spores and sporophores grew in monopodially arranged clusters (Fig. 2). On ISP-4 agar culture, various small sizes of unmatured spores and globular bodies were grown on short sporophores (Fig. 3). This spore morphology suggests that strain NH3-1 belongs to the genus *Micromonospora*.

Selection of Seed Media

Flask cultures of eight seed media were inoculated with spore suspensions from the four best and four worst sporulation media (TYGA, PCA, ISP-4, TYSA, WA, V-8A, OYA, and PCA). Results shown in Table 5 suggest that ISP-2, TPO, TYG, and ISP-3 were the best seed media, they were used for subsequent studies.

Strain NH3-1 grew rapidly in ISP-2, TPO, TYG, and ISP-3 media with the formation of mycelial clumps (1 to 2 mm diameter) and the growth peaked in 3 to 4 days. The mycelia clumps on PPG medium were larger (3 to 5 mm diameter) but fewer. After 4 to 5 days, the cultures on TYG and ISP-2 media became pink in color, possibly due to the diffusion of reddish pigments (Wu *et al.*, 1988).

In seed cultures, cell growth was affected significantly by the inoculum. Typically, inocula from highly sporulating agar slants, e.g. PCA and TYGA, supported better cell growth, even in poorly growing flask cultures on CD, GT, and MAM media. Antibiotic production correlated with cell growth. Poor growth on CD, GT, and MAM media led to low antibiotic potency, while rapidly growing cultures yielded high antibiotic potency (excepting the culture on PPG medium).

Optimum Culture Conditions

Selection of Fermentation Media

The antibiotic production varied on the 18 fermentation media tested (Fig. 4a,b,c). After 5 days of cultivation, cultures on PY, GM, and TYG media produced the highest antibiotic potency. In these highly productive cultures, the antibiotic production increased rapidly from day 1 to day 3 and then leveled off. Poorly producing cultures, such as those on FM and GT, showed a lag of antibiotic production.

Effect of Temperature

Strain NH3-1 was cultivated in each of the fermentation media at 20, 25, and 35°C, at 120 rpm. The results suggested that 25°C is the optimal cultivation temperature for antibiotic production. At 25°C the antibiotic potency maximized in 2 to 3 days (Fig. 4a,b,c), reaching a maximum of 21 mm on GM medium, whereas at 20°C a longer lag phase was observed. The production maximized in 3 to 4 days with lower antibiotic potency (Fig. 5a,b,c). The lowest antibiotic production was measured at 35°C (Fig. 6a,b,c). Cultures grown at 35°C typically showed a longer lag phase, lower production rate and lower potency (e.g. 14 mm on GM medium) compared with those grown at 20°C.

Effect of Agitation

Agitation enhanced antibiotic production irrespective of the medium used. Typically, cultures grown at 200 rpm (Fig. 7a,b,c) showed higher production rate, longer production phase, and higher antibiotic potency than did those grown at 120 rpm (Fig. 4a, 4b and 4c). For example, for PY medium at 200 rpm, an antibiotic potency of 23.3 mm was measured (Figs. 7c, 9), which is higher than that for PY medium at 120 rpm (19.5 mm). At 80 rpm, poor antibiotic production occurred even on PY medium (Fig. 8).

Effect of Vitamins

Supplementation of 10 mg/l of vitamins B_1, B_2, B_6, B_{12}, C, and folic acid promoted antibiotic production in liquid cultures (Fig. 10). Typically, the antibiotic potency increased rapidly in 3 days. The effect of vitamins varied, however, with vitamins B_{12} and C showing the greatest effect on the antibiotic production.

Discussion

The sporulation, cell growth, and antibiotic production of *Micromonospora neihuensis* NH3-1 are affected by culture conditions, particularly media. On

Table 5. *Cell weight and antibiotic potency of strain NH3-1 grown on various seed media incubated at 28°C, 120 rpm for 5 days*

Media for inoculum	Seed media								
	ISP-2	TPO	CD	PPG	GT	PY	TYG	MAM	ISP-3
	Cell Weight (%)*/Antibiotic Potency (mm)								
WA	7/12	10/8	0.2/0	7/0	1.6/0	16/0	8/7	0/0	23/13
V-8A	11/9	11/12	1/0	13/0	1.7/8	9/0	8.3/11	0/0	21/12
OYA	8/13	10.2/0	1/0	4/0	1/0	11/0	13/0	2/0	26/0
PCA	7/8	10/7.3	2/0	12/0	5/0	10/0	9/0	3/0	30/7.5
TYGA	8/13	12/12	1.6/0	4/0	3.6/14	30/0	36/9	1/0	78/16
TPCA	8/16	15/12	1/0	11/0	1.4/0	20/0	11/15	1/0	48/13
ISP-4	11/9	11/11	1.2/0	10/0	2/0	14/0	30/13	1.2/0	36/10
TYSA	6.6/12	11/13	1.4/0	9/0	2/0	15/0	16/14	0.8/0	36/13

*Cell weight is presented as % of packed cell volume, after centrifugation at 12,000 g for 20 min.

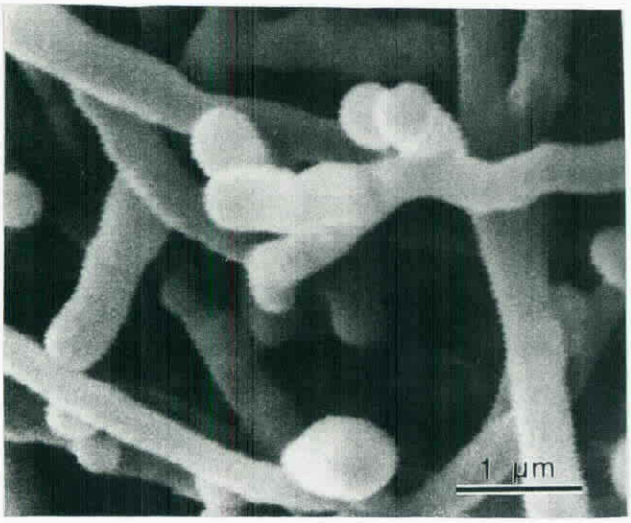

Fig. 2. Scanning electron micrograph of strain NH3-1 cultured on TYG agar at 28°C for 3 weeks.

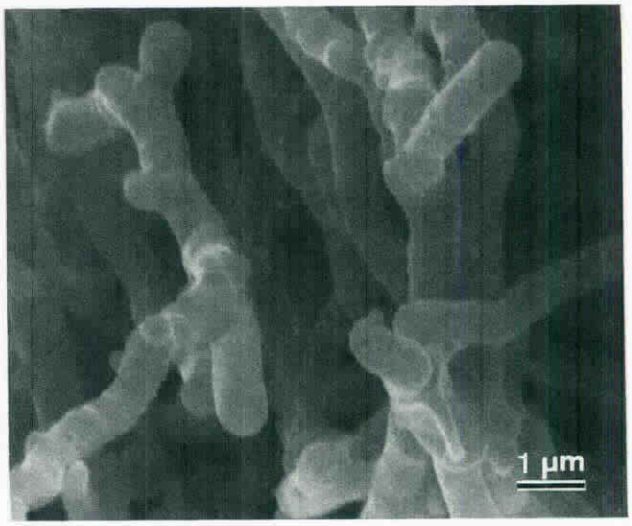

Fig. 3. Scanning electron micrograph of strain NH3-1 cultured on ISP-4 agar at 28°C for 3 weeks.

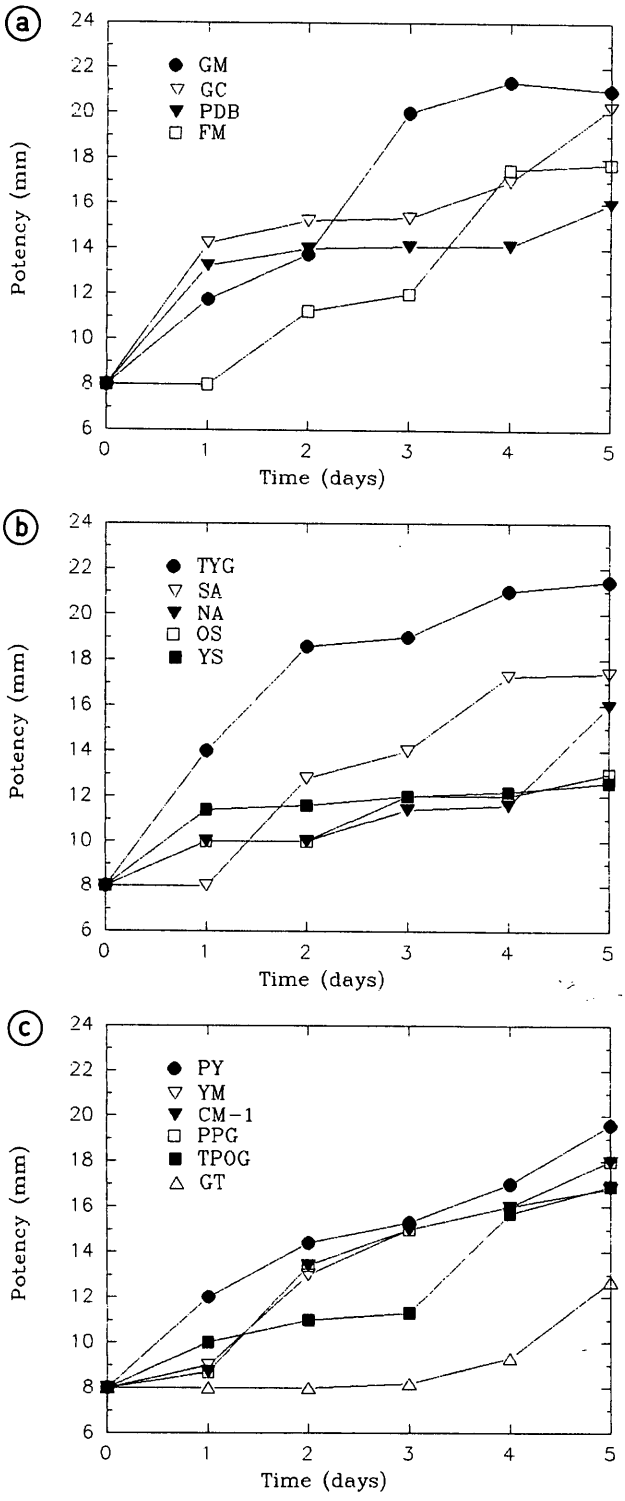

Fig. 4. Antibiotic production from *M. neihuensis* NH3-1 on various production media grown at 25°C, 120 rpm.

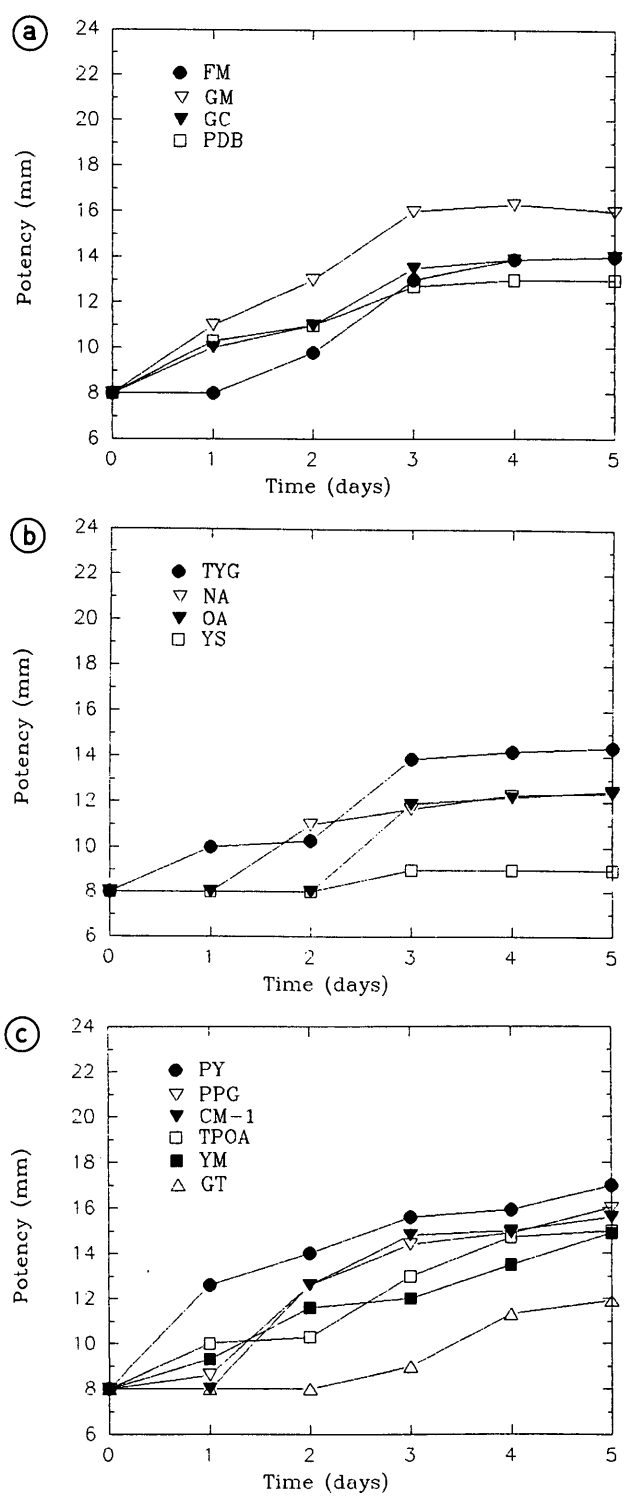

Fig. 5. Antibiotic production from *M. neihuensis* NH3-1 on various production media grown at 20°C, 120 rpm.

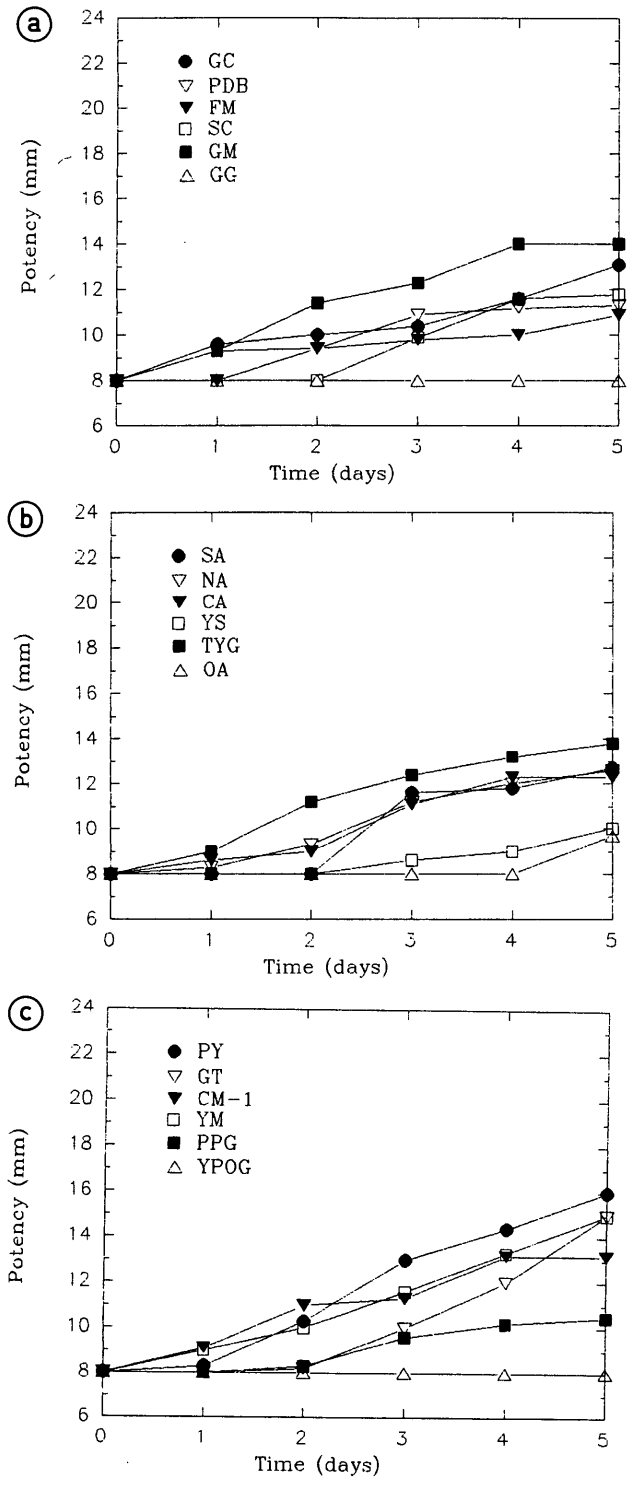

Fig. 6. Antibiotic production from *M. neihuensis* NH3-1 on various production media grown at 35°C, 120 rpm.

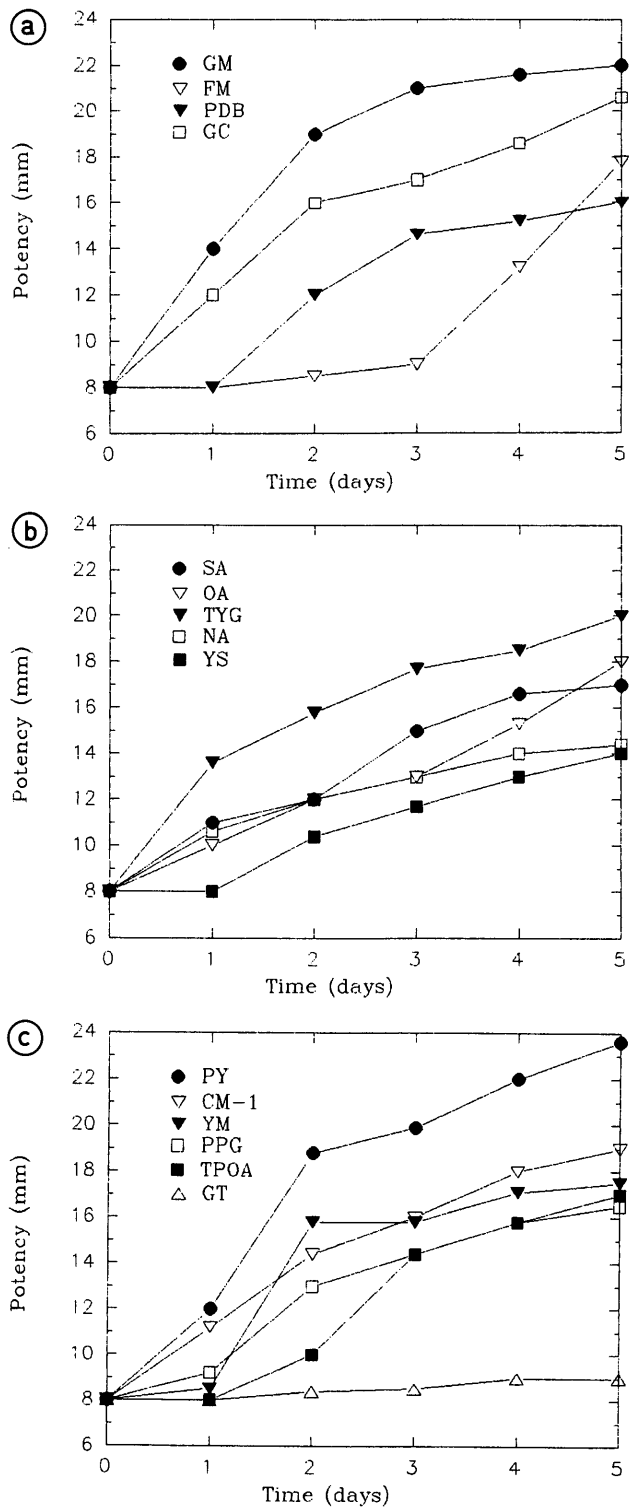

Fig. 7. Antibiotic production from *M. neihuensis* NH3-1 on various production media grown at 25°C, 200 rpm.

agar plate culture, strain NH3-1 grew and sporulated better on media with better nutrition supply, such as tryptone yeast extract glucose (TYG) and Czapek Dox yeast extract casein (CYC) media. In comparison, on media such as OA, ISP-4, and TYSA, the low content of carbon and nitrogen sources correlate with lower spore production. These results agree with a previous report (Funashi *et al.*, 1990) that *Micromonospora* BA 06108 grows better in yeast extract malt agar (ISP-2) and in oatmeal agar (ISP-3) which contain high levels of carbon and nitrogen sources. This sporulation dependence

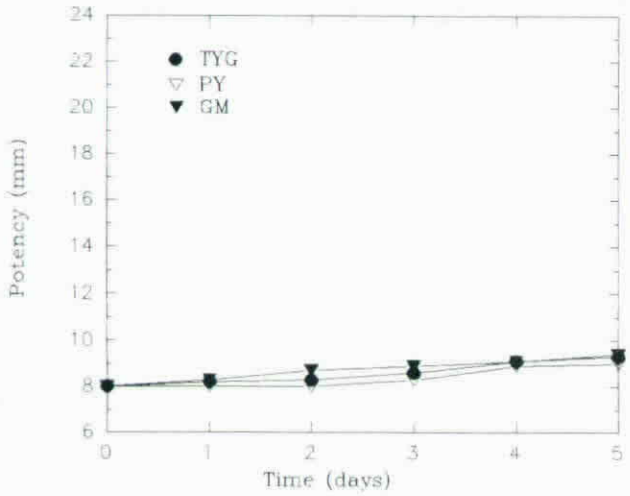

Fig. 8. Antibiotic production from *M. neihuensis* NH3-1 on various production media grown at 25°C, 80 rpm.

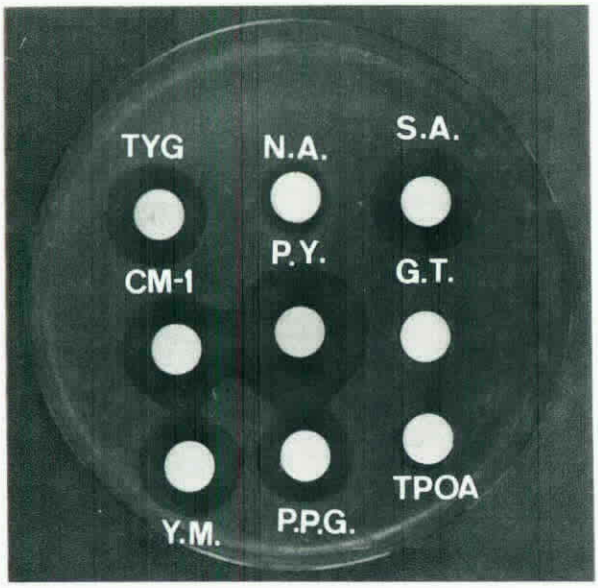

Fig. 9. Antibiotic potency of *M. neihuensis* NH3-1 on various production media grown at 28°C, 200 rpm for 5 days.

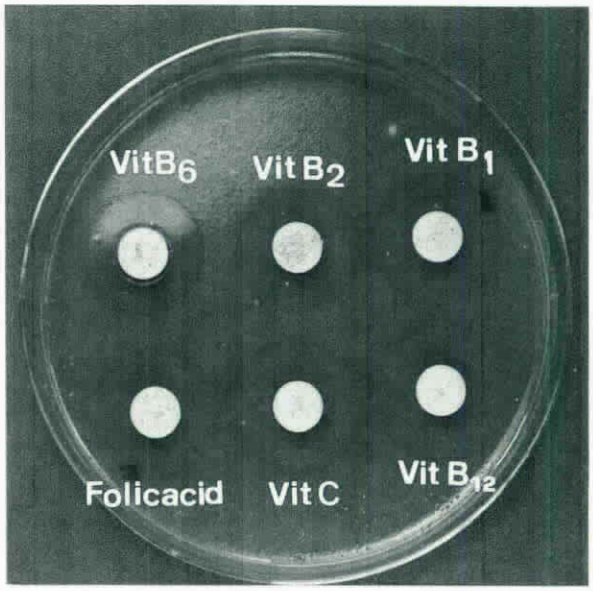

Fig. 10. Affect of supplementation of vitamins and folic acid on antibiotic potency.

on media is distinguished from that of most other actinomycetes, which generally form spores on media with lower nutrition levels (Seino, 1985). The difference between *Micromonospora* and other *actinomycetes* remains to be studied.

The best antibiotic production occurred in cultures using peptone yeast extract (PY), TYG, and glucose molasses (GM) media, which also contain organic carbon and nitrogen sources such as peptone, casein, and glucose. These results are in agreement with those of Kawamura *et al.* (1981).

In conclusion, *M. neihuensis* NH3-1 requires enriched nutrients for growth and antibiotic production. Glucose appears to be the best carbon source, and the best nitrogen source is peptone or casein. The stimulative affects of vitamins B_{12} and C on antibiotic production await further study.

Acknowledgements. The author wishes to thank the National Science Council, ROC, for their financial support (grant number NSC 78-0211-B001-50).

Literature Cited

Alexander, M. (Ed.) 1961. Introduction to soil microbiology. John Wiley, New York.

Arai, T. (Ed.) 1976. Actinomyces. The boundary microorganisms. Toppan Company Limited, Tokyo.

Buchanan, R.E. and N. E. Gibbons (Ed.) 1974. Bergey's manual of determinative bacteriology. 8th edition. Williams & Wilkins Co., Baltimore.

Code of Federal Regulations. 1981. Title 21. Food and drug. Parts 300 to 499, U.S. Government Printing Office, Washington D.C.

Funashi, K., K. Kawamura, F. Satoh, M. Hiramatsu, M. Hagiwara, and M. Okanish. 1990. New analogues of rosarmacin isolated from a *Micromonospora* strain. I. Taxonomy, fermentation, isolation and physico-chemical and biological properties. J. Antibiotics. **43:** 938-947.

Kawamura, Y., Y. Yasuda, and M. Mayama. 1981. Isolation of L-2-(1-methyleyclopropyl) glycine from *Micromonospora miyakonesis* sp nov. I. Taxonomic studies on the producing microorganism. J. Antibiotics. **43:** 367-369.

Komagata, K. 1986. Japan collection of microorganisms JCM catalog of strains. Third edition.

Seino, A. 1985. 放線菌の同定實驗法。日本放線菌研討會。

Sykes, G. and F.A. Skinner (Ed.) 1973. Actinomycetales. Characteristics and practical importance. Academic Press, New York.

Waksman, S. A. (Ed.) 1967. The actinomycetes. A summary of current knowledge. The Ronald Press Company, New York.

Wu, R.Y., M. C. Shiao, and H. M. Lee. 1983. Studies on the *Streptomyces* SC4: Chemical formulation of antibiotic SC4-X. Bot. Bull. Acad. Sin. **24:** 71-87.

Wu, R.Y. 1984. Studies on the *Streptomyces* SC4. II. Taxonomic and biological characteristics of *Streptomyces* strain SC4. Bot. Bull. Acad. Sin. **25:** 112-123.

Wu, R. Y., L. M. Yang, T. Yokoi, and K. H. Lee. 1988. Neihumicin, a new cytotoxic antibiotic from *Micromonospora neihuensis*. I. The producing organism, fermentation, isolation and biological properties. J. Antibiotics. **41:** 481-487.

Yang, L. M., R. Y. Wu., A. T. McPhail, T. Yokoi, and K. H. Lee. 1988. Neihumicin, a new cytotoxic antibiotic from *Micromonospora neihuensis*. II. Structural determination and total synthesis. J. Antibiotics. **41:** 488-493.

Yokoi, T., L. M. Yang, T. Yokoi, R. Y. Wu, and K. H. Lee. 1988. Neihumicin, a new cytotoxic antibiotic from *Micromonospora neihuensis*. III. Structural-activity relationships. J. Antibiotics. **41:** 494-501.

內湖黴素之生產條件的研究

吳榮洋

中央研究院　植物研究所

放線菌 NH 3-1 菌株是由內湖的土壤中分離而得之抗生素產生菌，內湖黴素是新的抗腫瘤抗生素，它可由此菌株之菌體經甲醇萃取後，純化而得。由試管試驗得知內湖黴素對 KB cell 有 cytotoxic 的作用，同樣的對黴菌 Saccharomyces cervisiae ATCC 9763 亦有抑制生長之作用。依菌學分類得知 NH3-1 是 *Micromonospora* 屬之一新菌種，故命名爲 *Micromonospora neihuensis* Wu sp. nov。由各種光譜及 X 光線結晶分析結果得知其化學構造是 (Z)-3, (Z)-6-dibenzylidene-2-methoxy-3,6-dihydropyrazin-5-one。爲準備內湖黴素將來的大量培養，已找出有效的芽孢生長培養基，種菌培養基，醱酵培養基及培養條件。NH3-1 菌株的最佳芽孢培養基是 Tryptone-yeast extract-agar (TYG)。最佳的種菌培養基是 ISP-2, TPO, PPG, TYG 及 ISP-3 等。NH3-1 菌株在這些培養基中能生出較優良的種菌。在 18 種醱酵培養基中以 peptone-extract 可得最高的抗生素產率。而最適條件是用 peptone-extract 培養基於 25°C, 200 RPM 下培養五天。維生素有促進抗生素的生合成作用，特別是維生素 B_{12} 與維生素 C 能使 NH 3-1 菌株於短時間內產生多量抗生素。