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Oxytetracycline production by *Streptomyces rimosus* in solid state fermentation of sweet potato residue

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Sweet potato residue, a starchy agricultural waste, was used as a substrate to produce oxytetracycline by *Streptomyces rimosus* TM-55 in a solid-state fermentation. Oxytetracycline was detected on the third day, reached its maximum value on the sixth day and remained constant to the twentieth day. Optimal conditions for oxytetracycline production were an initial pH of 5.5 to 6.5, supplemented with 20% (w/v) defatted roasted peanut meal, as the sole nitrogen source, 1.0% (w/v) $CaCO_3$ and 2.0% (w/v) $MgSO_4 \cdot 7H_2O$, being incubated at 26 to 35°C for 6 to 7 days. Oxytetracycline reached 12.1 mg/g substrate.

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Introduction

Although more than 5500 antibiotics have been reported, most of them have no economic value due to toxicity or ineffectiveness in clinical use (Demain & Solomon 1982). In Taiwan, most antibiotics, such as tetracyclines, cephalosporins, penicillins and aminoglycosides, are imported and their derivatives are then prepared locally.

Tetracyclines are broad-spectrum antibiotics used in a variety of infections caused by Gram-positive and Gram-negative bacteria, various rickettsias, trachoma, coccidia, amoebae, balantidia and mycoplasma (Chopra & Howe 1978). They are usually produced by submerged culture (Abou-Zeid & Youset 1971) wherein the media composition and culture conditions affect the production (Komatsu *et al.* 1975).

Sweet potatoes are abundantly available in Taiwan. In 1988, the production was 254,791 tons. As starchy materials can be used by a number of microorganisms (Senéz *et al.* 1980; Yang & Chiu 1986; Yang 1988; Yang & Ling 1989) we have investigated the possibility of using sweet potato residue to produce oxytetracycline with *S. rimosus* TM-55 in a solid-state fermentation.

Materials and Methods

Sweet Potato Residue

Sweet potato residue was purchased from the local market in Taiwan, and screened with a 4–16 mesh to remove dust and large aggregates. It contained 14 to 16% moisture, 2.3 to 3.1% crude protein, 2.7 to 3.6% ash, 16 to 18% crude fiber and 65 to 70% carbohydrate.

Test Organism

Streptomyces rimosus TM-55 was provided by Dr C. H. Ku (Taiwan Cyanamide Ltd), and used to produce oxytetracycline. *Bacillus subtilis* ATCC 6633 was used to assay the antimicrobial activity.

On a utilisé des résidus de patates douces, un résidu agricole amylacé, comme substrat pour la production d'oxytétracycline par *Streptomyces rimosus* TM-55 par fermentation en milieu solide. On a détecté l'oxytétracycline le 3ème jour. Celui-ci a atteint sa concentration maximum le 6ème jour et celle-ci est restée constante jusqu'au 20ème jour. Les conditions optimales pour la production d'oxytétracycline sont les suivantes: un pH initial compris entre 5.5 et 6.5, l'ajout de 20% (p/v) de farine d'arachide dégraissée, comme seule source d'azote, 1.0% (p/v) de $CaCO_3$ et 2.0% (p/v) de $MgSO_4.7H_2O$, une température d'incubation de 26 à 35°C pendant 6 à 7 jours. On a atteint 12.1 mg d'oxytetracycline par g de substrat.

Culture Media and Culture Conditions

S. rimosus was cultivated at 26°C on a slant of medium, containing (g/l): soluble starch, 10; yeast extract, 1; beef extract, 1; Tryptose, 2; $FeSO_47H_2O$, 0.1; and agar, 20. The basal solid medium contained (g/l): sweet potato residue, 100; $(NH_4)_2SO_4$, 0.5; rice bran or defatted roasted peanut meal, 20; $CaCO_3$, 1; and NaCl, 0.2. The medium was mixed thoroughly with spores of *S. rimosus* and distilled water and incubated statically in flasks (the depth of medium was about 1.5 to 2.0 cm) at 26°C for 6 to 7 days by stirring once a day. Initial pH of substrate was measured directly with pH meter, while final pH was determined after mixing with 4 vol distilled water.

Submerged fermentation medium contained (g/l): soluble starch, 20; corn steep liquor, 10; $(NH_4)_2SO_4$, 6; $CaCO_3$, 8; NaCl, 5; and soybean oil, 2. The medium was inoculated with spores, incubated at 26°C, and shaken at 250 rev/min for 4 to 15 days.

Determination of Antibiotic

After fermentation, the culture mass was extracted with 4 vol distilled water by shaking at room temperature for 5 min. Further shaking or using larger volumes of water did not increase recovery. Antimicrobial activity of extract was measured by the paper disc method (diam. 8 mm, Toyo Seisakusho Co.) using *B. subtilis* as the test organism in antibiotic medium I (Difco Laboratory, USA) at 30°C. Total oxytetracycline equivalent potency was calculated from the clear zone of a standard curve of oxytetracycline (Sigma Co., USA) in the range of 1.0 μg/ml to 10 mg/ml.

Moisture Content

A sample of culture was dried at 60°C under vacuum for 8 to 12 h until its weight remained constant. The weight difference after drying was considered the moisture content.

Results

Sweet potato residue, distilled water and spores of *S. rimosus* TM-55 were mixed thoroughly and cultivated at 26°C for 6 to 7 days. The oxytetracycline potency was undetectable by the paper disc method. To improve the oxytetracycline production, the following parameters were investigated.

Mechanism of Oxytetracycline Secretion

The time course of oxytetracycline production and substrate pH in solid-state fermentation with 0.5% $(NH_4)_2SO_4$ and 20% rice bran as combined nitrogen

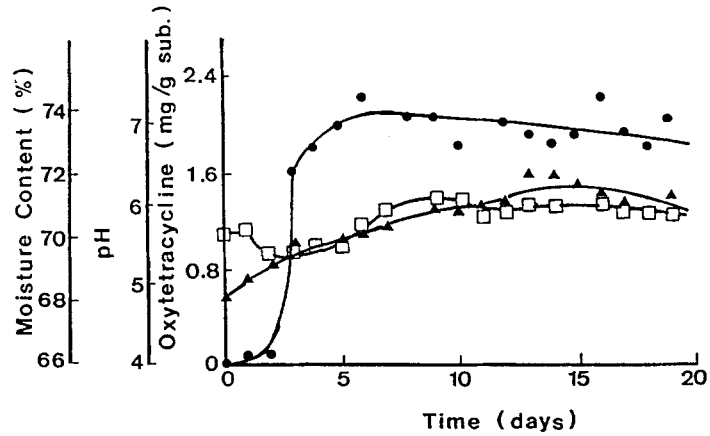

Figure 1. Time course of oxytetracycline production in solid-state fermentation at 26°C with 3.5×10^7 spores per g substrate. The medium contained sweet potato residue, 100 g, as carbon source, and was supplemented with rice bran, 20 g; $(NH_4)_2SO_4$, 0.5 g; $CaCO_3$, 1 g; and NaCl 0.2 g. ●—Oxytetracycline potency; ▲—moisture content; □—pH.

sources is shown in Fig. 1. During fermentation, oxytetracycline was detected on day 3, reached a maximal yield of 2.22 mg/g substrate by day 6 and remained constant to day 20. The moisture content of substrate gradually increased over the first 14 days and then remained approximately constant. Using 0.5% $(NH_4)_2SO_4$ and 20% defatted roasted peanut meal as combined nitrogen sources, oxytetracycline reached a maximal yield of 4.79 mg/g substrate by day 6, remained constant to day 20 and then decreased gradually to 1.58 mg/g substrate by day 40. The change of substrate pH during this incubation was similar to that with 0.5% $(NH_4)_2SO_4$ and 20% rice bran as combined nitrogen sources up to day 20 but it then increased gradually from 6.0 to 7.9 during the next 20 days.

The production of oxytetracycline in submerged fermentation was substantially less than in solid-state fermentation, it reached a maximum after 7 days' cultivation (Fig. 2).

Inoculum Size

In solid-state fermentation each gram of substrate inoculated with 5.0×10^7 spores could yield 2.47 mg of total oxytetracycline equivalent potency. When the inoculum size was greater than 5.0×10^8 spores or less than 5.0×10^6 spores, oxytetracycline production decreased over 7 days' incubation. A heavy inoculum favoured oxytetracycline production up to 4 days' incubation but could not sustain a high production up to 10 days (Fig. 3).

Initial Moisture content

Oxytetracycline production increased when the initial moisture content was increased from 60% to 75% but this was less than 55%, oxytetracycline production was low as the substrate was too dry for cell growth and antibiotic production. At the initial moisture content of 75%, each gram of substrate produced 4.12 mg of total oxytetracycline equivalent potency. At an initial moisture content of 85%, total oxytetracycline equivalent potency was only 2.72 mg/g substrate, due to the adhesion of the substrate preventing gas diffusion.

Initial pH

The optimal pH for oxytetracycline production was between 5.5 and 6.5 (Fig. 4). When the initial pH was lower than 4.2, oxytetracycline could not be detected. Since the original pH of sweet potato residue was between 5.5 and 5.7, there was no necessity to adjust the substrate pH for oxytetracycline production in solid-state fermentation.

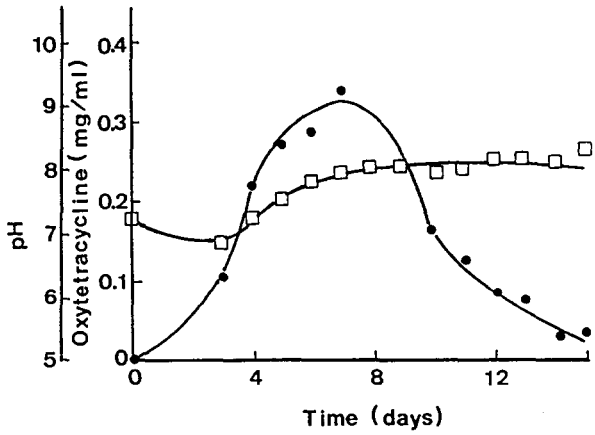

Figure 2. Time course of oxytetracycline production in submerged fermentation at 28°C with 7.0×10^6 spores per ml. ●—Oxytetracycline potency; □—pH.

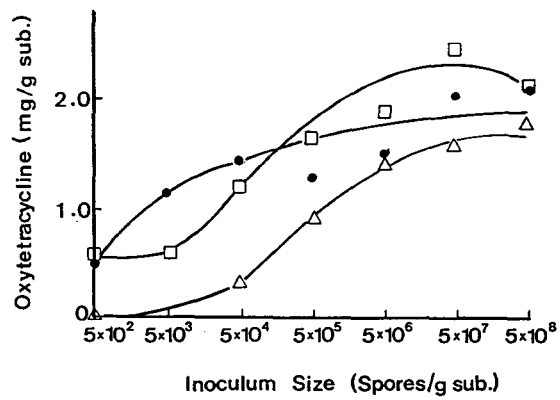

Figure 3. Effect of inoculum size on oxytetracycline production in solid-state fermentation at 26°C. The culture medium was as described in Fig. 1. △—After 4 days' incubation; □—after 7 days' incubation; ●—after 11 days' incubation.

Incubation temperature

Oxytetracycline production was maximal between 26 and 35°C after 7 days' incubation (Fig. 5). After 11 days, however, maximal oxytetracycline production was between 20 and 30°C. Although higher incubation temperatures decreased the incubation time, they inhibited oxytetracycline production.

Particle Size

Sweet potato residue particles do not have regular shapes. Small particles would reduce gas diffusion and heat transfer in the solid substrate, while large particles would decrease the contact area with microbes per unit weight. However, there was no significant difference in oxytetracycline production using particles between 4 and 40 mesh size.

Additional Carbon Source

Maltose added to the solid-state fermentation stimulated oxytetracycline production by 1.4-fold though glucose or soluble starch had little effect, while galactose and sucrose inhibited production by 8.2 and 24%, respectively.

Nitrogen Source

Sweet potato residue contains 65 to 70% carbohydrate and 2.3 to 3.1% crude protein. The C/N ratio of about 65 was too high for cell growth and oxytetracycline production, therefore a nitrogen source supplement was necessary. The effect of nitrogen source on oxytetracycline production and substrate pH is shown in Table 1. All nitrogen sources improved oxytetracycline production. To improve the utilization of agricultural waste, an organic nitrogen source was used to replace the inorganic nitrogen source. Defatted roasted peanut meal, at 20%, gave the best enhancement of oxytetracycline production followed by rice bran, wheat bran, peanut powder and soybean meal. Adding ammonium sulphate, rice bran, wheat bran, peanut powder and soybean meal to defatted roasted peanut meal did not stimulate production any further.

Addition of Precursors or Other Chemicals

The effect of some precursors or other chemicals on oxytetracycline production is shown in Table 2. Histidine stimulated oxytetracycline production by up to 35%, though sodium glutamate, soybean oil and methionine had little effect.

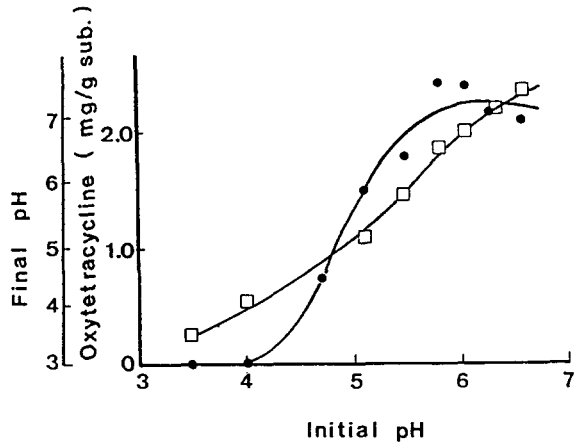

Figure 4. Effect of initial pH on oxytetracycline production in solid-state fermentation at 26° for 7 days. The culture medium was as described in Fig. 1. ●—Oxytetracycline potency; □—final pH.

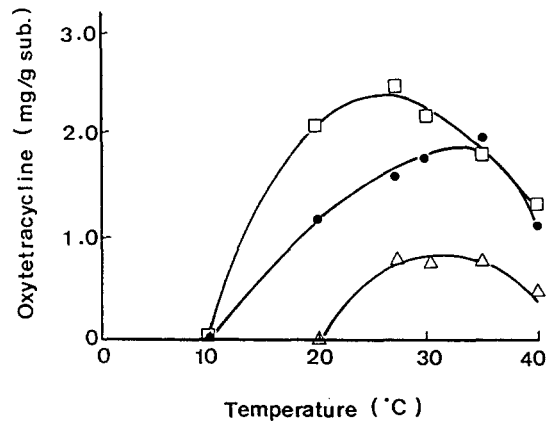

Figure 5. Effect of incubation temperature on oxytetracycline production in solid-state fermentation. The culture medium was as described in Fig.1. △—After 3 days' incubation; ●—after 7 days' incubation; □—after 11 days' incubation.

Table 1. Effect of nitrogen sources on oxytetracycline production.

Nitrogen source	Concentration (% w/w)	Initial pH	Final pH	Oxytetracycline (μg/g substrate)
None		5.95	6.71	386
$(NH_4)_2SO_4$	0.5	5.87	5.80	613
NH_4Cl	0.5	5.76	5.33	741
NH_4NO_3	0.5	5.89	6.47	998
Urea	0.5	6.25	6.09	1591
KNO_3	0.5	5.84	6.84	556
Rice bran	20	5.57	6.73	4515
Wheat bran	20	5.58	5.97	4354
Soybean meal	20	5.25	5.94	3833
Defatted roasted peanut meal	20	5.78	5.63	6372
Raw peanut powder	20	5.75	5.98	3866
Fried peanut powder	20	5.81	5.97	4160
Defatted roasted peanut meal	20			
$(NH_4)_2SO_4$	0.5	5.71	5.53	5903
Defatted roasted peanut meal	10			
Rice bran	10	5.73	6.10	5533
Defatted roasted peanut meal	10			
Wheat bran	10	5.52	6.20	4876
Defatted roasted peanut meal	10			
Soybean meal	10	5.66	6.10	4349
Defatted roasted peanut meal	10			
Raw peanut powder	10	5.69	6.79	4780
Defatted roasted peanut meal	10			
Fried peanut powder	10	5.80	6.00	5707

Culture medium was sweet potato residue 100 g, $CaCo_3$ 1 g, and NaCl 0.2 g with moisture content 70%. The medium was cultivated at 26°C for 7 days.

Table 2. Effect of other chemicals on oxytetracycline production.

Chemicals	Concentration (% w/w)	Initial pH	Final pH	Oxytetracycline (μg/g substrate)
None		5.78	5.63	6372
Sodium acetate	0.5	5.61	5.54	<10
Sodium glutamate	0.5	5.80	6.02	6287
	1.0	5.83	6.20	6471
	2.0	5.79	6.80	6380
Histidine	0.5	5.80	5.78	7546
	1.0	5.82	5.77	8085
	2.0	5.96	5.53	8578
Methionine	0.5	5.61	5.67	6087
	1.0	5.78	5.52	6173
	2.0	5.78	5.45	5885
Soybean oil	0.5	5.65	5.69	6172
	1.0	5.69	5.71	6345
	2.0	5.75	5.80	6589

Culture medium was sweet potato residue 100 g, defatted peanut meal 20 g, $CaCO_3$ 1 g, and NaCl 0.2 g with moisture content 70%. The medium was cultivated at 26°C for 7 days.

Sodium acetate even at 0.5% (w/w) inhibited oxytetracycline production completely.

Inorganic Salts

A supplement of inorganic salts improved oxytetracycline production. Sweet potato residue solid media without $CaCO_3$ was unfavourable for oxytetracycline production. Adding $CaCO_3$ not only regulated the substrate pH but also stimulated oxytetracycline production (Table 3). $MgSO_4 \cdot 7H_2O$ stimulated oxytetracycline production by up to 60% but NaCl, KH_2PO_4 and $MgCl_2 \cdot 7H_2O$ were without significant effect.

Discussion

Solid-state fermentations are distinguished from submerged cultures by microbial growth and product formation occurring at or near the surfaces of solid materials with low moisture content (Mudgett 1986). The moisture content of sweet potato residues used in solid-state fermentation was 60 to 75% and were lower than the water-holding capacity (Yang 1988). These conditions were suitable for aerobic growth of the organism.

During the fermentation, the moisture content of the substrate increased. This could be due to the production of metabolic water of microbes, as has been observed in the spawning of mushroom (Wang 1981) and other processes (Yang *et al.* 1986; Yang 1988). The moisture content of the substrate affected not only cell morphogenesis but also the quality and quantity of antibiotic production (Wang 1983; Yang & Ling 1989).

In addition, substrate pH decreased in the first 3 to 4 days, and then increased

Table 3. Effect of inorganic salts on oxytetracycline production.

Inorganic salt	Concentration (% w/w)	Initial pH	Final pH	Oxytetracycline (μg/g substrate)
None*		4.82	4.87	42
$CaCO_3$*	0.5	5.31	5.11	4378
	1.0	5.46	5.67	6823
	1.5	5.63	6.22	4723
	2.0	5.68	6.50	3429
	3.0	5.74	6.58	2620
NaCl†	0.2	5.47	5.40	6406
	0.5	5.42	5.52	6491
	1.0	5.33	5.48	5912
KH_2PO_4†	0.5	5.36	5.69	4798
	1.0	5.36	5.33	2689
	1.5	5.35	5.20	2439
	2.0	5.38	5.17	1991
$MgSO_4 \cdot 7H_2O$†	0.5	5.47	5.44	5886
	1.0	5.45	5.50	9292
	2.0	5.43	5.38	10881
	3.0	5.41	5.40	6799
$MgCl_2 \cdot 7H_2O$†	0.5	5.47	5.41	5227
	1.0	5.39	5.40	5857
	2.0	5.29	5.31	6767
	3.0	5.29	5.28	6634

* Culture medium was sweet potato residue 100 g, and defatted roasted peanut meal 20 g with moisture content 70%. The medium was cultivated at 26°C for 7 days.
† Supplemented with $CaCO_3$ 1 g/100 g substrate.

gradually. Matsushima *et al.* (1981) indicated that the pH drop during acid protease production might be due to the accumulation of organic acid and the accumulation of sulphate ion following utilization of $(NH_4)_2SO_4$. The pH of the substrate might be maintained using alkaline or appropriate salts (Narahara *et al.* 1982) or other kinds of nitrogen sources (Nishio *et al.* 1981; Yang 1988; Yang & Ling 1989). In oxytetracycline production, an initial substrate pH lower than 4.2 was unfavourable for antibiotic production. Although $CaCO_3$ is frequently added to the medium to counteract excess acidity and to enhance antibiotic production, an abundance might interfere with the extraction process (Yang & Chiu 1986). *S. rimosus* during oxytetracycline production was less sensitive to the substrate moisture content and pH than *S. viridifaciens* during tetracycline production (Yang & Ling 1989), or *Aspergillus niger* for protease production (Yang & Chiu 1986), or amylolytic yeast for protein enrichment (Yang 1988). This property might be useful for scaling-up production.

Oxytetracycline was detected on day 2 of incubation, reached its maximum after 6 days and then decreased gradually afterwards. The mechanism of tetracycline secretion in solid-state fermentation is probably the same as that proposed in submerged fermentation. However, oxytetracycline activity decreased sharply after prolonged incubation in submerged fermentation. This phenomenon might be due to cell autolysis (Okami & Oomura 1979). Oxytetracycline production by solid-state fermentation was more stable than that in submerged fermentation and the product could be temporarily stored without losing activity significantly (Hesseltine 1972).

Nitrogen supplementation enhanced cell growth and oxytetracycline production. Defatted roasted peanut meal, fried peanut powder, raw peanut powder, wheat bran, rice bran and soybean meal gave good oxytetracycline production being consistent with similar results for chlortetracycline production with *S. aureofaciens* (Wang 1958), and tetracycline production with *S. viridifaciens* (Yang & Ling 1989). Combined nitrogen sources did not enhance oxytetracycline production over that of defatted roasted peanut meal alone. Abou-Zeid *et al.* (1981) indicated that peptone or soybean meal was the best organic nitrogen source for oxytetracycline production with *S. rimosus*; urea or KNO_3 was next, while combined nitrogen sources had no effect.

Miller *et al.* (1956) showed that acetate was the precursor of tetracycline, while adding shikimic acid did not improve the biosynthesis of chlortetracycline. Snell *et al.* (1960) indicated that glutamate was a precursor of oxytetracycline, and provided the 2-carboxyl and 4-amino groups in C_2-C_{4a} of the A-ring. This study showed that sodium acetate inhibited the biosynthesis of oxytetracycline. Adding L-histidine, however, improved production 1.9-fold, though sodium glutamate, methionine and soybean oil were without effect.

Niedercorn (1952) showed that salts of calcium, magnesium or both not only adjusted the pH of substrate, but also decreased the toxicity of the antibiotic to the tested organisms, while Nakayama (1977) reported that phosphate salts would inhibit the tetracycline, chlortetracycline and oxytetracycline production in submerged fermentation. In this study, $CaCO_3$ and $MgSO_4 \cdot 7H_2O$ stimulated antibiotic production while NaCl, and KH_2PO_4 did not.

In submerged culture, each ml of culture broth produced more than 12 mg of tetracycline or chlortetracycline when the medium contained sucrose, soybean meal, corn steep liquor and inorganic salts (Smekal & Zajicek 1976). In solid-state fermentation, each gram of substrate produced 4.72 mg of tetracycline (Yang & Ling 1989). In this study, each gram of substrate also produced 12.11 mg of oxytetracycline (Table 4), the product was more stable than that in submerged culture, and the energy input was also less than that in submerged culture. Therefore, production of oxytetracycline in solid-state fermentation might be a feasible process in agricultural waste treatment and utilization.

Table 4. Effect of ingredients on oxytetracycline production in solid-state fermentation with initial moisture content of 68% at 26°C for 7 days.

Medium item*	C-0	M-1	M-2	R-3	R-4	W-5	W-6	S-7	S-8	P-9	P-10	P-11	P-12	P-13	P-14	P-15	P-16
Sweet potato residue (g)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
$(NH_4)_2SO_4$ (g)	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
$CaCO_3$ (g)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.5	1.0	1.0	1.0	1.0	1.0
NaCl (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.2
$MgSO_4 \cdot 7H_2O$ (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	2.0
Histidine (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	2.0
Maltose (g)	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0
Rice bran (g)	0.0	0.0	0.0	20.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wheat bran (g)	0.0	0.0	0.0	0.0	0.0	20.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Soybean meal (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Peanut meal (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Initial pH	5.95	5.87	5.82	5.57	5.61	5.58	5.65	5.25	5.20	4.82	5.46	5.63	5.78	5.71	5.96	5.43	5.90
Final pH	6.71	5.80	5.92	6.73	6.58	5.97	6.01	5.94	6.21	4.87	5.67	6.22	5.63	5.53	5.53	5.38	5.93
Total oxytetracycline equivalent potency (μg/g substrate)	386	613	2410	4514	4454	4354	4511	3833	3238	42	6823	4723	6372	5903	8578	10881	12106

* C—control, without nitrogen source; M—with inorganic nitrogen source; R—with rice bran as nitrogen source; W—with wheat bran as nitrogen source; S—with soybean meal as nitrogen source; P—with defatted roast peanut meal as nitrogen source.
Culture conditions were the same as described in Table 1.

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References

ABOU-ZEID, A.A., EL-DIWANY, A.I. & SHAKER, H.M. 1981 Role of nitrogen sources in fermentative production of oxytetracycline by *Streptomyces rimosus* 93060. *Agricultural Wastes* **3**, 257–265.

ABOU-ZEID, A.A. & YOUSET, B.A. 1971 Fermentative production of tetracyclines. *Indian Journal of Technology* **9**, 430–431.

CHOPRA, I. & HOWE, T.G.B. 1978 Bacterial resistance to the tetracyclines. *Microbiological Reviews* **42**, 707–724.

DEMAIN, A.L. & SOLOMON, N.A. 1982 Industrial microbiology. In *Biotechnology*, pp. 160–177. Taiwan: Yih-Shiuan.

HESSELTINE, C.W. 1972 Solid-state fermentation. *Biotechnology and Bioengineering* **14**, 517–532.

KOMATSU, K.I., MIZUNO, M. & KODAIRA, R. 1975 Effect of methionines on cephalosporin C and penicillin N production by a mutant of *Cephalosporium acremonium*. *Journal of Antibiotics* **28**, 881–888.

MATSUSHIMA, K., HAYAKAWA, M., ITO, M. & STIMADA, K. 1981 Ecological study on the proteolytic enzymes of microorganisms. Part III. Features of the proteolytic enzyme system of hyperacid-productive and non-acid-productive fungi. *Journal of General Applied Microbiology* **27**, 423–426.

MILLER, P.A., MCCORMICK, J.R.D. & DOERSCHUK, A.P. 1956 Studies of chlortetracycline biosynthesis and the preparation of chlotetracycline-C. *Science* **123**, 1030–1031.

MUDGETT, R.E. 1986 Solid state fermentation. In *Manual of Industrial Microbiology and Biotechnology*, eds. Demain, A.L. & Soloman, N.A. pp. 66–83. Washington, DC: American Society of Microbiology.

NAKAYAMA, K. 1977 Regulation of microbial secondary metabolism. *Hakko to Taisha* **36**, 1–33.

NARAHARA, H., KOYAMA, Y., YOSHIDA, T., PICHANGKURA, S., UEDA, R. & TAGUCHI, H. 1982 Growth and enzyme production in a solid-state culture of *Aspergillus oryae*. *Journal of Fermentation Technology* **60**, 311–319.

NIEDERCORN, J.G. 1952 Aureomycin. US Patent 2,609,329. Sept. 2, 1952.

NISHIO, N., KURISU, H. & NAGAI, S. 1981 Thermophilic cellulase production by *Taralomyces* sp. in solid state cultivation. *Journal of Fermentation Technology* **59**, 407–410.

OKAMI, Y. & OOMURA, G. 1979 Production of antibiotic substances. In *Introduction of Antibiotic Production*, pp. 1–49. Tokyo: Kyoritsu Press.

SENÉZ, J.C., RAIMBAULT, M. & DESCHAMPS, F. 1980 Protein enrichment of starchy substrate for animal feeds by solid state fermentation. *World Animal Review* **35**, 36–39.

SMEKAL, F. & ZAJICEK, J. 1976 Fermentation production of tetracycline antibiotics. Czech. 162,200. Feb. 15, 1976. 3pp.

SNELL, J.F., BIRCH, A.J. & THOMPSON, P.L. 1960 Synthesis of tetracycline antibiotics. *Journal of the American Chemical Society* **82**, 2402.

WANG, E.L. 1958 Studies on production of chlortetracycline and chlorine metabolism of *Streptomyces aureofaciens*. *Journal of Antibiotics, Series A* **12**, 31–40.

WANG, H.H. 1981 Metabolic water of mushroom in bottled spawns. *Mushroom Science* **11**, 93–101.

WANG, J.Y. 1983 Effect of moisture content on morphogenesis and antibiotic production of *Cephalosporium acremonium* M 8650 in solid state fermentation. Master's Thesis, Graduate Institute of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan.

YANG, S.S. 1988 Protein enrichment of sweet potato residue with amylolytic yeasts by solid state fermentation. *Biotechnology and Bioengineering* **32**, 886–890.

YANG, S.S. & CHIU, W.F. 1986 Protease production with sweet potato residue by solid state fermentation. *Chinese Journal of Microbiology and Immunology* **19**, 276–288.

YANG, S.S., DURAND, A. & BLACHERE, H. 1986 Protein enrichment of sugar beet residue with conidia of *Trichoderma album* by solid state fermentation. *Chinese Journal of Microbiology and Immunology* **19**, 11–22.

YANG, S.S. & LING, M.Y. 1989 Tetracycline production of sweet potato residue by solid state fermentation. *Biotechnology and Bioengineering* **33**, 1021–1028.

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