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OXYTETRACYCLINE PRODUCTION IN COCONUT WATER

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ABSTRACT

Oxytetracycline (OTC) was produced on a very simple novel medium of coconut water. Buffering and corn steep liquor addition increased the antibiotic content to 145 μg/ml OTC after 5 days of fermentation. The producer organism is a local streptomyces isolate NIST S-70-24B. The fermentation brew did not exhibit vitamin B_{12} activity (*E. coli* factor). The antibiotic was extracted from the brew and identified as oxytetracycline by comparing its physical and chemical properties with the three tetracyclines.

A parallel study on chlortetracycline (CTC) production by *Streptomyces aureofaciens* NRRL 2209 gave a CTC yield of 0.09 μg/ml after 72 hours of fermentation and a vitamin B_{12} activity of 0.14 μg/ml.

Introduction

Oxytetracycline or commonly called Terramycin* is an antibiotic derived from *Streptomyces rimosus*. It belongs to the family of tetracycline antibiotics (compound consisting of 4 connecting rings with various group substitutions).

Terramycin is primarily bacteriostatic although high concentrations can be bactericidal. It is active against a wide range of bacterial infections caused by Gram positive and Gram negative organisms, certain viral and protozoan organisms in both human and animals. It has also found application in the fortification of animal feeds, in crop protection and food preservation.

The tetracyclines are generally produced by fermentation processes but details of media composition for industrial production are usually well-guarded secrets. However from a review one can conclude that the productive organisms assimilate lactose and glucose as carbon sources, soybean oil meal, distillers soluble, modified milk and other animal proteins as well as corn steep liquor, as sources of nitrogen, growth factors and mineral salts. Most of these culture media ingredients

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are not locally available, and coconut water a natural substance might be a good substitute because of its well balanced chemical components including the presence of vitamins, amino acids, mineral salts and growth factors. By using a local strain of Streptomyces S-70-24B, a proven Terramycin producer, instead of *S. rimosus,* (a foreign isolate), as the productive strain, this study was done to find whether coconut water can be used for oxytetracycline production. The present paper describes the results obtained in shake flasks fermentation experiments using coconut water for the production of oxytetracycline.

Materials and Methods

Microorganisms

A. Antibiotic producers. (1) NIST Isolate S-70-24B a streptomyces species obtained from a soil sample from Pampanga after dilution and tetracycline addition. This streptomycete is closely related or similar to *S. rimosus* (Lat *et al*; *in press*). It is comparable to oxytetracycline producing streptomycetes namely *S. armillatus, S. platensis, S. sayamaensis* and *S. rimosus* in its cultural, physiological and biochemical properties and morphological characteristics. (2) *Streptomyces aureofaciens* NRRL-2209 a lyophilized culture obtained from the Northern Regional Research Laboratories in Peoria, Illinois, U.S.A.

B. Test organisms used for antibiotic activity and assay: *Micrococcus pyogenes* var. *aureus* (ATCC 6538-P), *Bacillus subtilis* (ATCC 6633), *B. cereus* var. *mycoides* (ATCC 9634), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Alcaligenes faecalis,* Salmonella gallinarum,* Sarcina lutea* (ATCC 9341), *M. flavus,* Candida albicans,* Saccharomyces cereviseae** and *Fusarium moniliforme.**

C. Test organisms for determining vitamin B_{12} (*E. coli*) activity. *E. coli* M-500 an *E. coli* mutant.

Strain selection

The organisms, S-70-24B and *S. aureofaciens* 2209 were plated out on agar medium (Emerson agar) and incubated at room temperature. When colonies were fully developed, certain areas of the plates were cautiously flooded with a 24 hour broth culture of *E. coli.* The colony producing the biggest inhibition zone was selected as a high antibiotic producer.

Culture media

Coconut water was obtained from mature nuts. Before it was used, it was subjected to the following physico-chemical treatments: 1. base precipitation, 2.

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*Percentage of total amino acids.
*Local strain.

oxidative treatment with perchloric acid, 3. potassium permanganate addition, 4. charcoal adsorption, 5. ion exchange and 6. buffering with 0.2 M monobasic and 0.2 M dibasic sodium phosphate solution (Table 1). For the preparation of media for fermentation, other ingredients were dissolved in the treated coconut water or distilled water. Dilute NaOH or HCl solutions were used to adjust the pH if needed. Other constituents added consisted of corn steep liquor, copra meal, bijon (rice noodles) effluent, spent brewers grain, at a concentration of 3 percent and urea 1%. (Sevilla-Santos, 1984)

Table 1. Preparation of phosphate buffered coconut water (Colowick and Kaplan 1955)

Solution A (ml)	*Solution B (ml)*	*Resulting pH values*	
		Initial	*Before inoculation**
92.0	8.0	5.5	5.3
87.7	12.3	5.7	5.4
81.5	18.5	5.9	5.6
73.5	26.5	6.15	5.9
62.5	37.5	6.4	6.2
51.0	49.0	6.6	6.2
37.0	61.0		
28.0	72.0		

Soln A – 0.2M sol'n of monobasic sodium phosphate (27.8 g in 1000 ml coconut H_2O)
Soln B – 0.2M sol'n of dibasic sodium phosphate (53.65 g of $Na_2HPO_4.7H_2O$ in 1000 ml coconut water)
Add A and B and dilute to a total of 200 ml

Inoculum preparation

Two types of inoculum were tried. Inoculum A consists of an aqueous spores and mycelial suspension prepared by suspending a 7-day old agar slant culture of the producer organism in about 5 ml of sterile distilled water. Inoculum B is a preformed inoculum which takes a longer time to prepare. The 7-day old culture was first inoculated unto the seed medium (Emerson broth) and incubated by shaking for 24 to 48 hours at 30°C. A 5% to 10% of the preformed inoculum was added aseptically to the fermentation media.

Shake flask fermentation

For antibiotic production a 5% inoculum B (preformed) was added to 500 ml flask containing 100 ml of the specified culture media. Incubation was carried out at room temperature (± 30°C) in reciprocating shaker with a 1½ inch displacement at 120 strokes per minute or in Lab-line Orbit Environ shaker at 120 revolution per minute.

When inoculum A was used, the entire suspended contents of one agar slant was poured aseptically into a shake flask.

Determination of antimicrobial activity

The antimicrobial spectrum of sampled brew for S-70-24B and *S. aureofaciens* shaken cultures was determined by the agar dilution method using both agar cups and stainless steel cylinders. Standard curves were prepared from dilutions of pure oxytetracyclines and chlortetracycline powders (Grove and Randall, 1955).

Determination of vitamin B_{12} (E. coli) activity

This was determined in the sampled brew using the modified method of Harrison *et al.* 1951 and *E. coli* mutant as the test organism.

Isolation of terramycin from S-70-24B fermentation broth

There are two methods of recovering the antibiotic from the fermentation brew. One is by adsorption, the other by extraction. The procedure of Stoudt (1963) which is the second method was followed (Fig. 2) in isolating terramycin from the fermentation brew using phosphate buffered coconut water as the fermentation medium. That of Sobin *et al.* (1950) (Fig. 3) was used in isolating terramycin from the fermentation brew produced on soybean meal 2.5%, cornsteep liquor 9%, glycerin 1% and sodium chloride 0.25% with 1% calcium carbonate.

Results and Observations

Selection of S-70-24B as the producer organism

Seven isolates namely S-70-24B, OTC (1), OTC 1 (4), OTC 1 (7), OTC 1 (10), OTC 1 (13) and OTC 111 (1) from the NIST Antibiotic Section were revived. The choice was made from among the isolates which gave inhibition zones of 30.0 ± 2mm *B. cereus* var. *mycoides* plates (1982 assay). Among the isolates subcultured in Emerson agar two failed to grow and two were fast-growing, one of the latter was S-70-24B. The remaining three grew only after five days of incubation at room temperature.

Fermentation studies by shake flask in Emerson broth shows that S-70-24B was the best isolate for antibiotic production as shown in Table 2.

Thus, S-70-24B was chosen as the organism for oxytetracycline production.

Strain selection

To obtain a high-yielding strain for oxytetracycline fermentation, the two colonies which produced big zones of inhibition on the agar plate were picked out and inoculated to liquid media and incubated on a shaker. Isolate 1 and Isolate 3

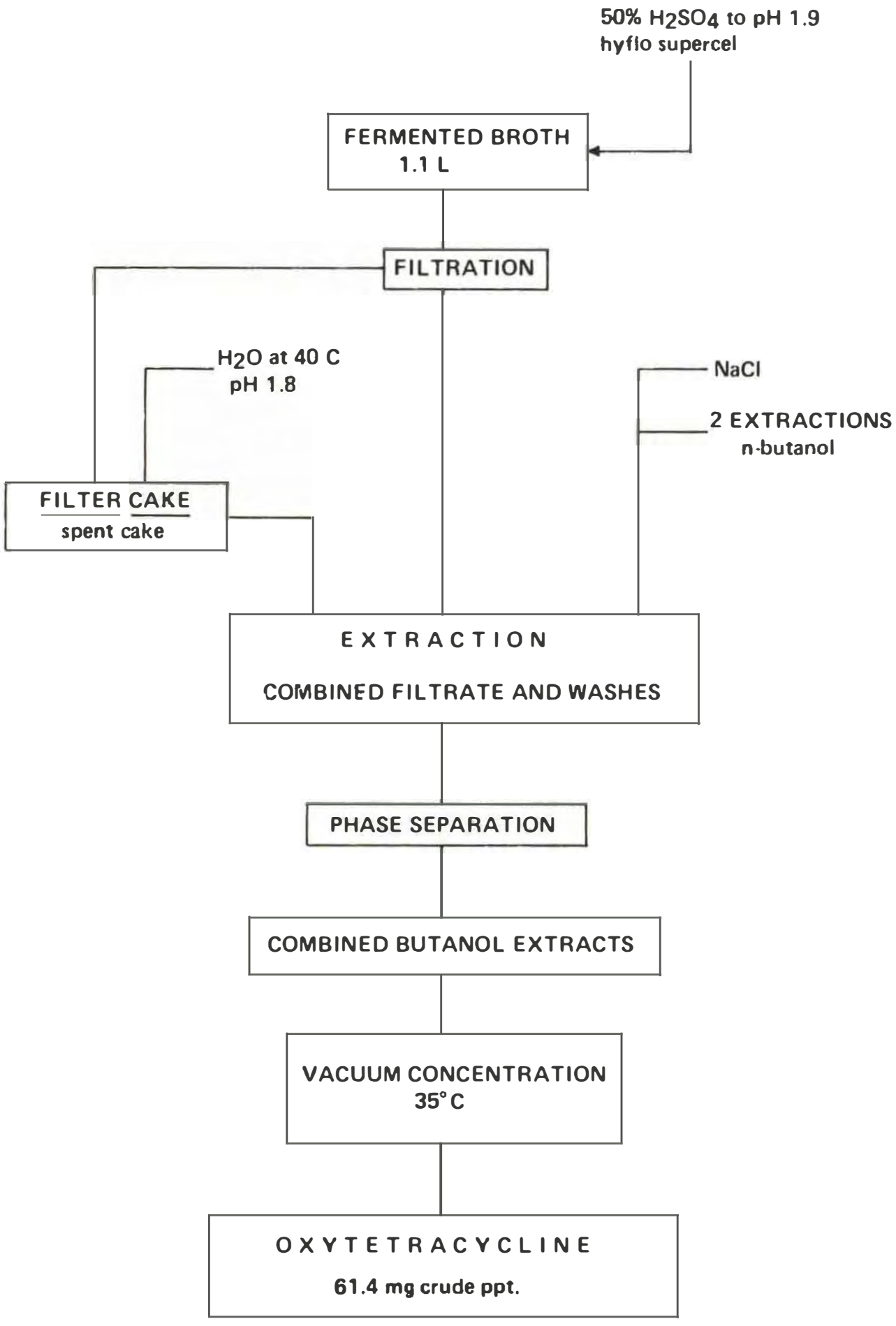

Fig. 2. Recovery of oxytetracycline, Stoud *et al.*, 1963.

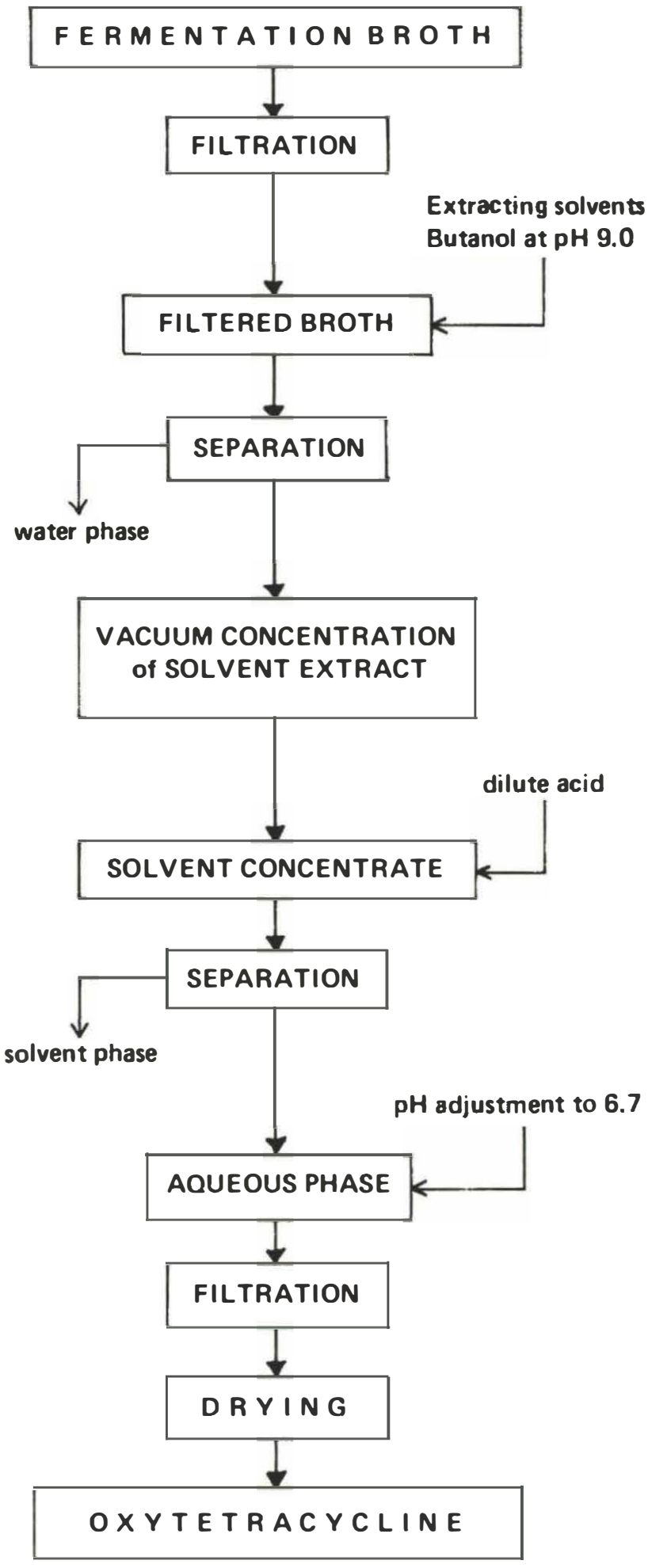

Fig 3. Oxytetracycline extraction process, Sobin *et al.*, 1950.

Table 2. Antibiotic activity of oxytetracycline-producing *Streptomyces* culture in Emerson broth

Streptomyces isolate	*Zones of inhibition (mm)*	
	Trial 1	*Trial 2*
S-70-24B	30.2	23.0
OTC 1 (1)	25.2	21.3
OTC 1 (4)	23.2	17.2
OTC 1 (13)	23.4	21.9
OTC 1 (10)	12.3	10.8

produced 29.0 and 28.0 μg/ml of oxytetracycline while the parent stock culture gave only 23.4 μg/ml. Isolate 1 was selected as the better antibiotic producer.

Shake-flask fermentation of S-70-24B in coconut water

As the fermentation media for shake-flask cultures (untreated) coconut water was used. A week-old brew was tested against six test microorganisms . It was shown that the antibiotic substance produced had a broad spectrum of activity as shown in Table 3.

Table 3. Antibiotic spectra of S-70-24B grown by fermentation in untreated coconut water

Test organisms	*Zone of inhibition* (mm)*
Bacillus cereus var. *mycoides*	28.1
Bacillus subtilis	28.4
Sarcina lutea	24.1
Escherichia coli	19.0
Candida albicans	19.4
Fusarium moniliforme	nil

*Average of three flasks

Shake-flask fermentation in base-treated coconut water

Coconut water after partial and complete base precipitation was compared with untreated coconut water as a culture medium for oxytetracycline production. The results from partial base precipitation does not differ so much from the untreated coconut water in terms of inhibition zones. After complete base precipitation, the medium was found unsuitable for antibiotic production. Perhaps the constituents of coconut water which enhance the production of the antibiotic

substances may have been removed by complete precipitation. The antibiotic activity of the partial and complete base precipitation is shown in Table 4.

Table 4. Antibiotic activity of S-70-24B growth on treated and untreated coconut water

Coconut water treatment	*Zone of inhibition* (mm)* *B. cereus* var. *mycoides* *Trial 1*	*Trial 2*
Untreated	15.6	28.1
Base precipitation (partial)	13.5	27.6
Base precipitation (complete)	8.0	10.5

*Average of 3 flasks

Shake-flask fermentation of S-70-24B in buffered coconut water

Coconut water which was buffered by addition of monobasic and dibasic sodium phosphate to pH values ranging from 5.3 to 6.4 was tried as fermentation medium. The inoculum used was one agar slant culture per 50 ml medium in 250 ml flask. Incubation was on a shaker. Table 5 shows the results of sampling on the 1st, 2nd and 3rd day of incubation.

Table 5. Antibiotic activity of S-70-24B grown on buffered coconut water

pH values *Before inoculating*	*before autoclaving*	*Zone of inhibition* (mm)* *B. cereus* var. *mycoides* *Day 1 (1:3)*	*2 (1:9)*	*3 (1:9)*
5.3	5.5	21:0 (9.6)	19.4 (18)	23.3 (54)
5.4	5.6-5.7	20.4 (8.1)	19.0 (17)	23.9 (63.0)
5.6	5.8-5.9	21.9 (12.0)	19.5 (18)	24.22 (68.4)
5.9	6.0-6.1	20.3 (7.8)	18.0 (12.6)	21.92 (36.0)
6.2	6.2-6.3	8.7 (0.4)	17.4 (10.8)	20.77 (26.1)
6.4	6.4-6.6		16.3 (8.1)	20.4 (24.3)

*Average of 3 trials
Figures in parenthesis represents antibiotic content in μg/ml.

Shake-flask fermentation of S-70-24B antibiotic

For antibiotic production, S-70-24B prefers a wide range of initial pH from 5.5 to 5.9. The highest antibiotic activity of 70 μg/ml was obtained on the third day of fermentation. Fig. 4 presents the data from shake flask fermentation, show-

ing antibiotic production, pH and fermentation time using buffered coconut water with various pH values. An initial pH ranging from 5.5 to 5.9 gave the same trend of the antibiotic production of 70 μg/ml in 3 days time.

Shake-flask fermentation in nitrogen supplemented coconut water

Since coconut water has only about 0.5% protein, an inorganic nitrogen in the form of $(NH_4)_2HPO_4$ was added to the coconut water medium. The result of the second trial which gave the same trend as in the first experiment is given in Table 6. Submerged fermentation in shake flasks containing 0.1% $(NH_4)_2HPO_4$ in coconut water gave the best inhibition of 24.8 mm equivalent to 20 μg/ml of oxytetracycline after 120 hours of fermentation. This is higher than that obtained in using plain coconut water as the fermentation medium.

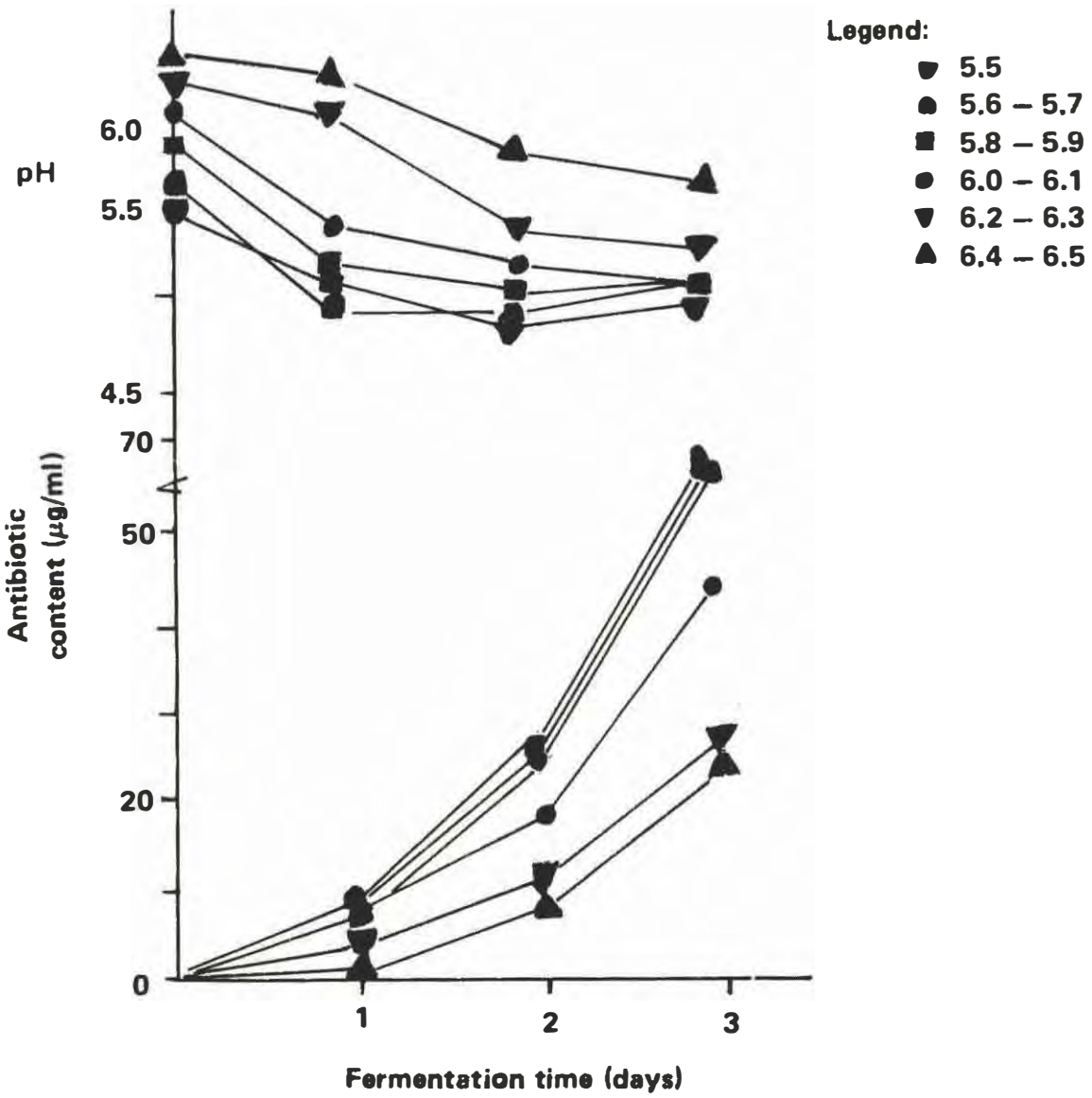

Fig. 4. Antibiotic content and pH of S-70-24B fermentation brew using different buffered coconut water.

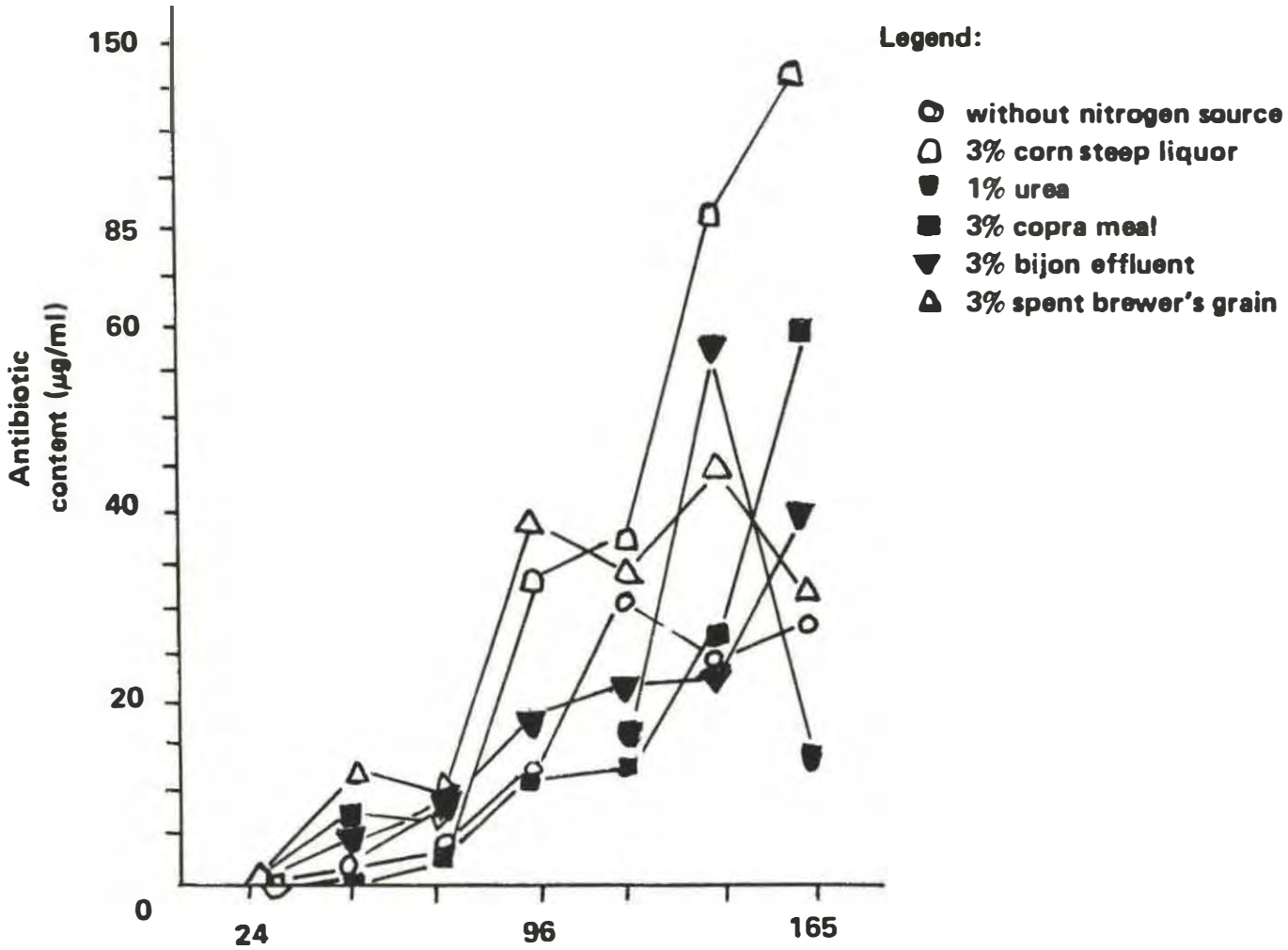

Fig. 5. Antibiotic content of S-70-24B brew using nitrogen supplemented buffered coconut water.

Table 6. Submerged fermentation of oxytetracycline production by S-70-24B utilizing nitrogen (inorganic) supplemented coconut water

Length of fermentation (hours)	*Inhibition zones* (mm)* Medium A	Medium B	Medium C	Medium D	Medium E
48	23.3 (20)	22.1 (16)	22.0 (16)	14.2 (3.5)	18.2 (75)
72	23.5 (22)	22.4 (18)	22.5 (19)	16.3 (5.3)	17.3 (64)
100	22.3 (17)	24.3 (27)	23.2 (20)	13.3 (2.6)	12.7 (23)
120	23.3 (20)	24.1 (24)	24.4 (26)	12.5 (23)	9.6 (1.8)

*Average of 3 trials

(Figures in parenthesis represents µg/ml)

Composition of fermentation media: Media A – coconut water (pH 5.1), Media B – coconut water (pH 7.2), Media C – coconut water + 0.1% $(NH_4)_2HPO_4$ (pH 5.7) Media D – coconut water + 0.1% $(NH_4)_2HPO_4$ (pH 7.2) and Media E – M-13 pH adj. to 7.2.

Composition of M-13 g/100 ml base-treated coconut water: corn steep liquor 4, sucrose 3, $CaCO_3$ 0.65, $(NH_4)_2SO_4$ 0.2, $MnCl_2$ 0.00033, $CuSO_4$ 0.00033 and $ZnSO_4$ 0.005.

Shake-flask fermentation of S-70-24B in buffered coconut water supplemented with organic nitrogen source

By supplementing the phosphate buffered coconut water with different nitrogenous substances, namely 3% corn steep liquor (45% solids), 1% urea, 3% copra meal, 2% spent brewers grain, 3% bijon effluent, it was observed (Fig. 5) that corn steep liquor at a concentration of 3% gave the highest antibiotic content of 145 μg/ml in 120 hours. At the start, the antibiotic production was nil. It was only on the 48th hour that the production began to rise and by 72nd, 96th and 120th hours, it increased rapidly. Antibiotic production was monitored by microbiological assay. Results are given in Table 7.

Table 7. Oxytetracycline production by S-70-24B in nitrogen (organic) supplemented buffered coconut water

Length of fermentation (Hours)	*Oxytetracycline content (μg/ml) Buffered Coconut Water Medium*					
	A	*B*	*C*	*D*	*E*	*F*
24	2.04	trace	5.85	trace	4.20	11.50
36	9.30	3.15	7.50	3.30	7.80	11.50
48	12.60	31.80	10.80	13.50	18.00	37.80
72	20.70	36.30	12.60	22.50	21.60	32.40
96	24.50	86.40	56.70	25.40	23.50	44.60
120	29.70	145.80	18.90	35.64	39.70	40.50

Medium
A – Buffered coconut water (BCW)
B – BCW with 3% cornsteep liquor (45% solids)
C – BCW with 1% urea
D – BCW with 3% copra meal
E – BCW with 3% bijon effluent
F – BCW with 2% Spent Brewer's grain

Isolation and identification of product

From 1,100 ml of pooled active brew, which was subjected to an isolation procedure for oxytetracycline see Fig. 4, 61.4 mg of antibiotic was isolated. The yield was small.

Chemical characterization

The isolated substance is a brownish yellow amorphous substance with a melting point of 213°C (microscope method). The compound is most soluble in water, ethanol, methanol and butanol; moderately soluble in acetone, ethyl acetate,

carbon tetrachloride and toluene; almost insoluble in chloroform, benzene, ether and petroleum ether.

By Rast method, the molecular weight was found to be 461.

Quantitative elementary analysis* gave the following composition: C–57.31%; H–5.28%; N–6.08% and O–30.85% and a molecular formula of $C_{22}H_{24}N_2O_9$ was obtained.

The physicochemical properties are summarized in Table 8.

Table 8. Physicochemical properties of S-70-24B antibiotic

M.W.	461
Molecular formula	$C_{22}H_{24}N_2O_9$
M. pt.	213°C
Solubility	
very soluble	water, methanol, ethanol, butanol
moderately soluble	acetone, toluene, ethyl acetate, carbon tetrachloride
poorly soluble	benzene, chloroform, ether, petroleum, ether
Specific rotation	−126° (MeOH)
Elementary analysis	C-57.31%, H-5.28%, N-6.08%, O-30.85%
Physical appearance	brownish yellow amorphous substance

Identification of antibiotic produced by S-70-24B as oxytetracycline was based on the comparison of its physical and chemical properties and its thin layer chromatographic results with the authentic samples of oxy-, chloro- and tetracycline.

The results of the test on the crude precipitate of S-70-24B and the three tetracyclines is given in Table 9 and shows that color reactions of S-70-24B are very similar to those of oxytetracycline. (Sevilla-Santos 1985) .

A comparison of the physical and chemical properties of the crude antibiotic with authentic samples of chlortetracycline, oxytetracycline and tetracycline is given in Table 10.

Table 9. Tests for identification of antibiotic produced by S-70-24B

Reagent	*Reaction*	*Antibiotic Powder Sample*			
		Chlortetra-cycline	*Tetra-cycline*	*Oxytetra-cycline*	*S-70-24B crude*
H_2SO_4 (conc.)	color	navy blue	dark violet	cherry red	old rose
2N NaOH	color	light yellow green	dark yellow green	dark yellow green	light yellow
not heated	UV	dark blue	none	green	light blue
heated 30 min.	color	pink orange	light yellow	yellow	light yellow
	UV	bright blue	light blue	light blue	bright blue
HCl (conc.)					
not heated	UV	none	none	yellow	yellow
heated	UV	none	none	yellow (greenish)	yellow

*UV fluorescence

As seen from the comparative data above, the antibiotic of S-70-24B is very similar, almost identical with oxytetracycline.

Thin layer chromatography

Table 11. shows the results of thin layer chromatography of the isolated compound S-70-24B and the authentic tetracyclines on silica gel G plates with a solvent system of organic solvent layer of Butanol-tartaric acid-water (100:6:100).

Antimicrobial spectrum

The antimicrobial spectrum of S-70-24B when tested against Gram positive and Gram negative bacteria, yeast and fungi by the agar dilution method is presented in Table 12. S-70-24B antibiotic exhibits good activity against the Gram positive bacteria with minimal inhibitory concentration of 0.1 μg/ml, and MIC against the Gram negative bacteria ranging from 1 to 10. In general, no activity was observed against *P. aeruginosa*, yeast and fungi.

Production of chlortetracycline by NRRL 2209

For the production of chlortetracycline by a known chlortetracycline producer, NRRL 2209, several fermentation experiments were conducted using as culture media or as diluent coconut water, or treated coconut water or buffered

Table 10. Physical and chemical properties of antibiotic S-70-24B and three tetracylines (Lat *et al., in press.*)

	Chlortetracycline	*Oxytetracycline*	*Tetracycline*	*Antibiotic S-70-24B*
a. Form	Golden yellow crystals	Yellow platelets	Yellow powder	Brownish yellow powder
b. Melting point	240°C (decomposed)	213-216°C	224-226°C	213°C
c. Specific rotation	−275.0 (MeOH)	−126.5 (MeOH)	−239 (MeOH)	−126 (MeOH)
d. Solubility	Soluble in water, slightly soluble in methanol, ethanol, butanol, acetone, ethyl acetate & benzene, insoluble in ether & petroleum ether.	Soluble in water, ethanol & methanol, slightly soluble in 2-propanol & toluene.	Soluble in water, slightly soluble methanol & ethanol, insoluble in ether.	Soluble in water, butanol, ethanol & methanol, slightly soluble in acetone, carbon tetracychloride, insoluble in benzene, chloroform, ether & petroleum ether.
e. Elementary analysis	C – 55.17% H – 4.84% N – 5.85% O – 26.73%	C – 57.39% H – 5.25% N – 6.08% O – 31.27%	C – 59.45% H – 5.44% N – 6.30% O – 28.8 %	C – 57.31% H – 5.28% N – 6.08% O – 30.85%
f. Molecular weight	478.88	460.44	444.43	461

Table 11. Mobility of tetracyclines and crude antibiotic S-70-24B

Antibiotic compound	*Spot*	*hRf values*
Chlortetracycline (CTC)	1	44
Oxytetracycline (OTC)	1	50
Tetracycline (TC)	1	32
Mixture of CTC, OTC, TC	3	44
		52
		31
Crude antibiotic S-70-24B		
Batch 1	2	49, 37
Batch 2	1	40
Batch 3	1	53
Batch 4	1	53
Batch 5	1	53

TLC plates: Silica gel G
Solvent system: Butanol-tartaric acid-H_2O (100:6:100)

Table 12. Antimicrobial spectrum of antibiotic S-70-24B

Test organisms	*Minimal inhibitory concentration (μg/ml)*
Micrococcus pyogenes var. *aureus*	0.1
Bacillus subtilis	0.1
B. cereus var. *mycoides*	0.1
Sarcina lutea	0.1
M. flavus	0.1
Alkaligenes faccalis	1.0
Escherichia coli	1.0
Salmonella gallinarum	10.0
Pseudomonas aeruginosa	> 100.0
Saccharomyces cerevisiae	> 100.0
Candida albicans	> 100.0
Fusarium moniliforme	> 100.0

coconut water. In some trials, protein sources locally available such as corn steep liquor, urea, fermented mother liquor, were added as protein supplement.

The use of coconut water as the sole fermentation media gave negative antibiotic activities. Buffered coconut water gave an antibiotic content of 0.09 μg/ml and 0.14 μg/ml of vitamin B_{12} activity for NRRL 2209 Isolate no. 1; and 0.02 μg/ml antibiotic with 0.08 μg/ml vitamin B_{12} activity for Isolate no. 2. The above results are very small and not practical.

With distilled water as diluent of a starch-corn steep liquor (CSL) medium, the antibiotic activity obtained was 0.56 μg/ml with a vitamin B_{12} activity of 0.14 μg/ml. Using buffered coconut water instead of distilled water as the diluent, no activity was observed.

Details of the study will be published later.

Discussion

It has been shown that S-70-27C, a local streptomyces isolate (both the parent and the mutant strain) could use 10% coconut water in the fermentation medium for tetracycline production (Joson *et al.* 1983). In the present study addition of 10% and 50% coconut water to the medium seem to inhibit the biosynthesis of the antibiotic. Certain substances present in the coconut water might have exerted some inhibitory action. To remove or inactivate the inhibitory factor, precipitation by addition of a base, mild and drastic oxidation, adsorption and ion-exchange treatment were tried. Only the base precipitation method gave favorable result.

To stabilize the pH during fermentation $CaCO_3$ was added. Phosphate buffer was also used to attain and maintain the desired pH values. For chlortetracycline production by NRRL 2209, coconut water as the sole culture medium gave negative results while for oxytetracycline production by S-70-24B, there was antibiotic production but with low activity. Buffering with phosphate (mono and di-basic) gave good results both for chlortetracycline and oxytetracycline production; the antibiotic produced by NRRL 2209 was very small in quantity while S-70-24B gave better results.

The results obtained in the supplementation of buffered coconut water with corn steep liquor confirmed previous findings (Evans, 1983). For S-70-24B, the antibiotic activity of the brew went up to about 145 μg/ml, a good and promising result.

For vitamin B_{12} production however, distilled water diluent seems to be better than buffered coconut water.

In the use of the seed or inoculum, both the spore suspension inoculum and the preformed inoculum could be used with the same end result. The only difference is that in the preformed inoculum using buffered coconut water the same amount of antibiotic was produced 24 hours earlier than the spore-suspension inoculum.

Summary and Conclusions

A local Streptomyces NIST S-70-24B (closely similar to *S. rimosus*) produced oxytetracycline 70 μg/ml in buffered coconut water with an initial pH of 5.5 to 5.9 on the third day of shake-flasks fermentation.

Addition of ammonium phosphate, or sodium phosphate and/or corn steep liquor increased the oxytetracycline titer to 145 μg/ml in a 5-day fermentation period.

Oxytetracycline was recovered from the fermentation brew and the yield was 61.4 mg of crude precipitate for every liter of the brew. It was identified as oxytetracycline by comparison of its properties with those of the three tetracyclines.

The antimicrobial spectrum of the product shows more activity against the Gram positive test bacteria than against the Gram negative ones.

Coconut water has been found to be a promising substrate for future development in a larger scale of oxytetracycline production but is not a very appropriate culture medium for chlortetracycline production.

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