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University of Calcutta, Kolkata 700 019, India

Studies on the optimisation of cultural conditions of a strain of *Streptomyces setonii* (19 NRA1) for antibiotic production

S. SHIBURAJ AND T. K. ABRAHAM
Division of Microbiology, Tropical Botanic Garden & Research Institute, Palode, Thiruvananthapuram 695562, Kerala

The antibiotic biosynthesis is a specific property of some species or even some strains of microorganisms and it depends greatly on the cultural conditions. It is necessary to develop the optimum conditions to make the production of an antibiotic feasible. The present study deals with the optimisation of the cultural conditions such as temperature, time, suitable media, pH, aeration, concentration of nutrients etc. of a *Streptomyces* strain, producing a broad-spectrum antibiotic. This strain was isolated from the forest soils of Neyyar wild life sanctuary of Southern Western Ghats of Kerala State. Cultural, physiological, morphological and chemotaxonomical characteristics of the strain were evaluated following standard methods. The strain is identified as *Streptomyces setonii.*

Key words : Antibiotic production, cultural condition, *Streptomyces setonii* (19 NRA1)

INTRODUCTION

There are thousands of antibiotics discovered so far and many of them are clinically important and have revolutionised the medical science to a great extent. However, an intensive search for novel antibiotics and potential antibiotic producing strains is going on world-wide as many pathogens acquire resistance to known antibiotics. The Actinomycetes are regarded as a major potential source of new bioactive substances. The members of the genus *Streptomyces* have been widely exploited as producers of antibiotics and are considered as the most important taxa of industrial microorganisms. The antibiotics produced by the *Streptomyces* strains account for more than half of the known antibiotics of microbial origin.

The antiobiotic biosynthesis is a specific property of some species or even some strains of microorganisms and it depends greatly on the cultural conditions. It is necessary to develop the optimum conditions to make the production of an antibiotic feasible. The present study deals with the optimisation of the cultural conditions of a *Streptomyces* strain 19NRA1, producing a board-spectrum antibiotic, which was isolated from the forest soils of Neyyar wild life santuary of southern Western Ghats of Kerala State.

MATERIALS AND METHODS

Characterisation of the strain

The methods recommended by International Streptomyces Project (ISP) for characterisation of the *Streptomyces* spp. has been followed (Shirling and Gottlieb, 1966). Substrate and aerial mass colour was determined using the colour codes of Methuen Handbook of colour (Kornerup and Wanscher, 1967). The morphology and spore surface morphology were determined by light and Scanning Electron Microscope (Hitachi) following standard methods (Laboratory manual Actinomycetes, 1998). The cell was analysis of the strain wall also done following standard methods (Staneck and Roberts, 1974).

Maintenance media

The *Streptomyces* strain was maintained in Sabaraud's agar slants at 28°C. The bacterial

cultures were maintained on nutrient broth and fungal strains on Sabaraud's broth and kept at 4°C.

Assay method

The antibiotic was assayed following agar-cup method (U. S. Pharmacopoeia, 1980) against standard test organisms. The antibiotic titre was determined by measuring the diameter of inhibition zone around the cups. The test organisms used were *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 739), *Pseudomonas aeurugenosa* (MTCC 741), *Staphylococus aureus* (MTCC 740), *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 36).]

Selection of suitable media

Ten different media combinations (Atlus and Perks, 1993) were tested for maximum production of the antibiotic. Culture filtrates were collected and assayed after five days of incubation at room temperature on a shaker kept at 120 rpm.

Standardisation of physiological parameters

The effect of incubation period, temperature, pH of the media and aeration on the maximum antibiotic production were determined and standardised using the selected suitable medium. The results are presented in Tables 1-3 and Fig 1.

RESULTS AND DISCUSSION

The microscopic observasions of the strain 19NRAI showed separate aerial and substrate mycelia with Rectus-flexus type conidial chains on the aerial mycelia. The cell wall was chemo type I and have L-DAP as cell wall peptidoglycan. The chemo-taxonomic and general cultural characteristics were consistent with assignment of the strain to the genus *Streptomyces* Waksman & Henrici (1943). The spore surface morphology as revealed by SEM was smooth. The growth on different ISP media showed that the aerial mass colour was white and the reverse colony colour was yellow brown (Table 1). The strain was melanin positive and was capable of reducing nitrate. Using the keys of Nonomura (1974), Szabo *et al.* (1975), and by comparison to published description (Shirling and Gottlieb, 1966; Waksman and Henrici, 1974), the culture 19NRA1 was classified as a strain of *Streptomyces setonii* (Millard and Burr, 1926; Waksman, 1953).

Production of an antibiotic in laboratory condition is associated with excessive nutrient supplies and the *Streptomyces* strains need suitable medium and growth conditions for the maximum accumulation of antimicrobial metabolites. In the present study ten different broths had been used (Table 2). Broth containing 1.5% glucose, 0.1% $CaCO_3$, 1.5% soyameal, 0.25% glycerol, 0.5% NaCl and 0.1% Yeast extract gave good antibiotic yield compared to others.

The production of antibiotics is sometimes evident during the growth of organisms but it usually depends upon the production of primary metabolites. In the present study growth showed gradual increase by the 4th day and attained maximum by the 8th day. Antibiotic accumulation was maximum on the 6th day and then gradually it decreased (Fig. 1). It is assumed that precursors and other factors required for antibiotic production are produced during the early growth phase where primary metabolism occurs vigorously (Iwai and Omura, 1992).

It is evident from Fig. 1 that the effect of pH on growth and antibiotic accumulation by *Streptomyces* strain 19NRA1 increased with the increase in pH. It attained maximum at neutral pH (6.5 - 7) and there after gradually decreased.

Normally *Streptomyces* strains prefer temperature near 27°C for antibiotic production. In the present experiment room temperature (30±2°C) proved ideal for antibiotic production (Table 3). An aerated and submerged condition in a rotary shaker at 120 rpm was ideal for the antibiotic accumulation.

The effect of different carbon sources on antibiotic accumulation is shown in Fig. 2. It also revealed that 2% starch and 1% glycerol in the broth had been found suitable for antibiotic accumulation and 1% Tryptone as nitrogen source instead of soyameal gave good antibiotic yield (Fig 3). A modified broth wih 2% starch, 1% glycerol and 1% tryptone was found ideal for the maximum accumulation of antibiotic using the *Streptomyces setonii* strain 19NRA1.

The Actinomycete isolate 19NRA1 is a *Streptomyces* strain with Rectus-flexus conidial chains and belongs to the white series. The physiological conditions like, temperature of about 30 ± 2°C, shaking at 120 rpm in a rotary shaker and a pH range of 6.8-7 are ideal for antibiotic production. The modified broth without glucose and soyameal and substituting with 2% starch, 1% glycerol and 1% tryptone proves ideal for antibiotic production using *Streptomyces setonii* strain 19NRA1.

Table 1 : Cultural characteristics of *Streptomyces* strain 19NRA1.

Medium	Cultural characteristics			
	Growth	Reverse colony colour	Aerial mycelium (Growth & Colour)	Soluble pigments
ISP 2	Good	Yellow Brown	Good, White	None
ISP 3	Good	Yellow Brown	Good, White/light grey	None
ISP 4	Good	Yellow Brown	Good, White	None
ISP 5	Good	Yellow Brown	Good, White	None
Glucose-Aspargine agar	Good	Yellow Brown	Good, White	None
Czapek's agar	Moderate	Yellow Brown	Good, White	None
Nutrient agar	Moderate	Yellow Brown	None	None
Tap water agar	None	Yellow Brown	Trace, White	None

Effect of carbon and nitrogen sources

The carbon and nitrogen sources of the selected medium were replaced by ten different carbohydrates and nitrogen compounds separately to determine the suitable ones for the maximum production of antibiotic. The percentage of the selected carbon and nitrogen source was also standardised. The results are presented in Figs. 2 and 3.

Table 2 : Effect of different media on antibiotic accumulation of *Streptomyces* strain 19NRA1.

Culture broths	Cell mass (g/ 100 ml)	Antibiotic activity against test organisms used (inhibition zone in diameter)					
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Saccharomyces cervisiae*
Glucose-aspargine	0.287	18	15	16	16	14	12
Glycerol-aspargine	0.352	17	19	19	17	14	13
Antibiotic assay broth	0.962	26	37	34	23	16	14
Sabaraud's broth	0.76	20	24	26	21	15	—
Nutrient broth	0.043	—	—	—	—	—	—
Starch casein broth	0.878	22	26	20	20	14	10
Potato-glucose broth	0.306	20	27	20	17	15	12
AGS broth	0.226	—	18	—	—	—	—
Glucose-peptone	0.276	—	16	16	—	—	—
S. S. broth	0.32	25	30	—	22	—	—

Table 3 : Effect of aeration and temperature on antibiotic accumulation.

Aeration conditions (rpm)	Average antibiotic activity at different temperatures (mm diameter)				
	20°C	25°C	30°C	35°C	40°C
Stationary	0	0	0	0	0
50	0	0	0	0	0
70	0	0	16	0	0
120	12	12	21.5	16	0
150	0	0	19.5	0	0

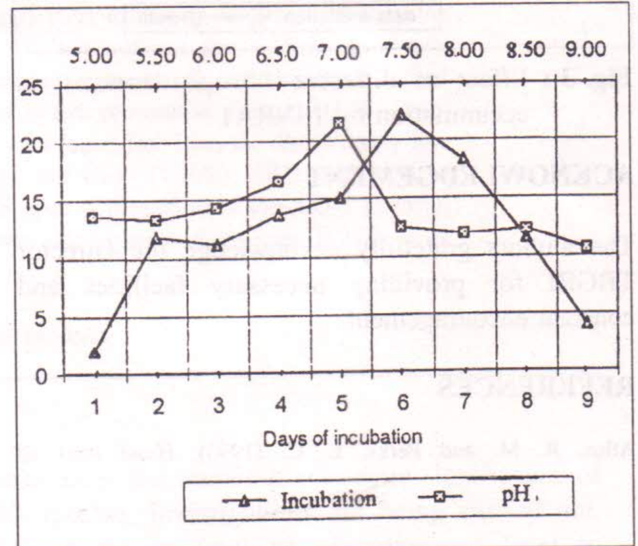

Fig. 1 : Effect of incubation period and pH of the medium on antibiotic accumilation by 19NRA1 pH of the medium.

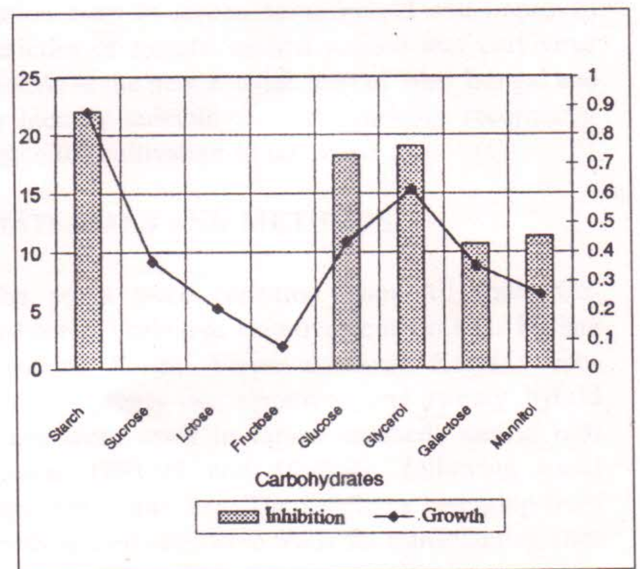

Fig. 2 : Effect of carbohydrates on antibiotic accumilation by 19NRA1

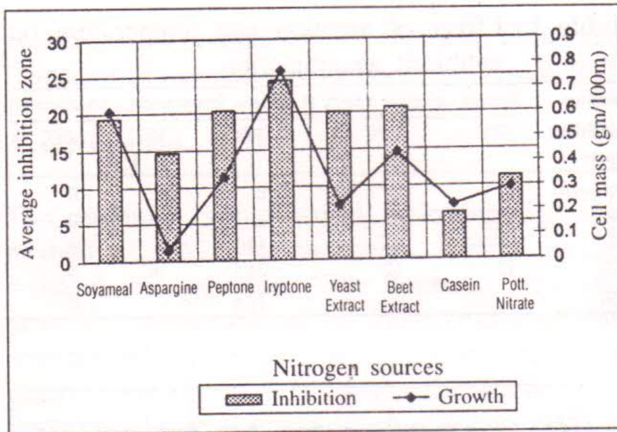

Fig. 3 : Effect of different nitrogen sources on accumilation by 19NRA1

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REFERENCES

Atlus, R. M. and Perks, L. C. (1993). *Hand book of Microbiological Media.* CRC Press London.

Buchanan, R. R. and Gibbons, N. E. (Ed) (1974). *Bergey's Manual of Determinative Bacteriology,* 8Th Ed. Williams & Wilkins & Baltimore.

Iwai Y. and Omura, S. (1982). Culture conditions for screening of new antibiotics. *J. Antobiotics.* **35**(2) 123-141.

Laboratory manual–Actinomycetes, (1998) Microbial Type Culture Collection and Gene Bank, IMTECH, Chandigarh.

Korncrup. A. and Wanscher, J. H. (1967). *Methuen Hand Book of Colour,* Methuen & Company Ltd., London.

Nonomura, H. (1974). Key for classification and identification of 458 species of the *Streptomyces* included in ISP *J. Ferment. Technol.* **52,** 78-92.

Shirling, E. B. and Gottlieb, D. (1966). Methods for characterisation of *Streptomces* species. *Intl. J. Bacteriology,* **16** : 313-340.

Staneck, J. L. and Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. *Appl. Microbiol.* **28**(3) : 226-231.

Szabo, I. M., Marton, M., Buti, I. and Fernandez, C. (1975). A diagnostic key for the identification of "species" of *Streptomyces* and *Streptoverticillium* included in the International Streptomyces Project. *Ata Botanica Academiae Hungarieae* **21** : 387-418.

The United States Pharmacopoeia, (1980) 20th revision.

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