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Short Communication

Utilization of carbon and nitrogen sources by *Streptomyces kanamyceticus* M 27 for the production of an Anti bacterial antibiotic

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We tested a number of carbon and nitrogen compounds for their effect on the production of an antibacterial antibiotic by *Streptomyces kananmyceticus* M27. Dextrose was found to be the most suitable carbon source, though maltose, sucrose, and soluble starch gave moderate yields. $(NH_4)H_2PO_4$ and yeast extract were adequate nitrogen sources for antibiotic production. There was, however, no direct relation between the growth of the organism and antibiotic formation. The pH of the medium might be an important factor for antibiotic formation, as media giving high antibiotic yields showed an alkaline pH.

Key words: Nitrogen compounds, *Streptomyces kananmyceticus* M27, dextrose, antibacterial antibiotic

INTRODUCTION

The study of the formation of antibiotics usually involves a search for optimal media for their production. This is achieved by a systematic study of the suitability of large number of carbon sources and nitrogen sources. Early reports showed the *streptomyces* species could utilize sugars, alcohols and some organic acids. On the basis of the utilization of different carbon sources, Pridham and Gottieb (1948) characterized different actinomycetes. Grove et al. (1955) studied the carbon utilization of *Streptomyces kananmyceticus* for kanamycin production in complex media and reported that glucose, maltose, dextrin, starch, lactose, and sucrose are better carbon sources than glycerol. Other authors have also studied the effects of different sugars and nitrogen sources on antibiotic production (Majumdar and Majumdar, 1967). The present paper describes the utilization of different carbon and nitrogen sources for growth of and antibiotic production by *S. kanamyceticus*.

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MATERIALS AND METHODS

The culture of *S. Kanamyceticus* M27 was maintained on maltose-sodium nitrate-mineral agar slants (5,6) at 28°C and was subcultured at monthly intervals. The effects of different carbon and nitrogen sources was studied in the basal medium consisting of: K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; $CaCl_2.2H_2O$, 0.04 g; $FeSO_4.7H_2O$, 0.005 g; $ZnSO_4.7H_2O$, 0.0005 g and water, 1,000 ml. The pH was adjusted to 7.5. The carbon sources and phosphate were sterilized separately and added just prior to inoculation. For studying the effect of carbon sources on antibiotic production, sodium nitrate in 0.51% concentration was included in the basal medium; the medium was adjusted to pH 7.2 and sterilized.

For the development of inoculums, 30 ml of Lepage broth was placed in a 100 ml Erlenmeyer flask, pH adjusted to 7.5 and the medium was sterilized. It was inoculated with a well-sporulated slant culture of *S. Kanamyceticus* (10 days old) and kept on a rotary shaker (220 rpm) for 18 h at 28°C. A 5 ml portion of this broth was used to inoculate 30 ml of the fermentation medium contained in a 100 ml Erlenmeyer flask. Usually, triplicate flasks were used for each test. The flasks were kept on a rotary shaker (220 rpm). Occasional checking of the flasks to drop adhering cells

into the medium was necessary during the first 48 h of shaking. Incubation temperature was 28°C.

To determine kanamycin potency, the filtered broth was diluted with potassium phosphate buffer (pH 8) according the Grove and Randall (1955), and the estimation of kanamycin was made by a modified cup-plate method, with *Bacillus subtilis* as test organism. Kanamycin sulfate was used as the standard, and the results are expressed in terms of µg of antibiotic per ml.

Table 1. Effect of carbohydrate on production of the antibiotic by *S. kanamyceticus* in a synthetic medium.

Carbohydrate concentration (1.5%)	Antibiotic activity (units/ml)	
	5th day	7th day
Sucrose	1.66	1.66
Maltose	0.93	6.66
Dextrose	1.66	6.93
Soluble Starch	0.53	0.00

Table 2. Effect of dextrose concentration on production of the antibiotic by *S. kanamyceticus* in a synthetic medium.

Dextrose concentration (%)	Antibiotic activity (units/ml)	
	5th day	7th day
0.5	0.93	0.93
1.0	1.00	1.00
1.5	1.66	5.60
2.0	1.66	6.66

Table 3. Effect of Nitrogen Sources on Production of the Antibiotic by *S. kanamyceticus*.

Nitrogen sources (84 mg of nitrogen per 100 ml)	Antibiotic activity (units/ml)	
	5th day	7th day
$NaNo_3$	1.66	4.33
Yeast Extract	6.66	6.93
Peptone	4.33	4.93
$(NH_4)H_2PO_4$	6.93	8.33

RESULTS AND DISCUSSION

A number of carbohydrates were investigated for their effect on growth of *S. kanamyceticus* M27 and on its antibiotic production. Dextrose proved to be an excellent carbon source for antibiotic formation, although maltose is also a good carbon source for antibiotic production.

Table 4. Effect of (NH_4) H_2PO_4 concentration of Production of the antibacterial Antibiotic by *S.Kanamyceticus* M_{27}.

$(NH_4)H_2PO_4$ (%)	Antibiotic activity (units/ml)	
	5th day	7th day
0.5	6.33	8.26
0.68	8.10	9.00
1.0	7.00	8.00

Sucrose and soluble starch are poor carbon source for antibiotic production (Table 1). It is possible that these carbon sources are utilized rapidly for the synthesis of cellular material so that little would be available as carbon and energy source for antibiotic synthesis. Dextrose may be utilized less rapidly, and thus it is available during the phase of antibiotic production. It is, however, interesting to note that antibiotic formation is not solely dependent on cellular growth. As dextrose was an excellent carbon source for antibiotic production by *S. kanamyceticus*, different levels of dextrose were tested to determine the optimal concentration for antibiotic production. Dextrose at a concentration of 2 g/100 ml gave maximal antibiotic titers (Table 2) a higher dose decreased the yield (not shown).

The medium for testing different nitrogen sources contained the basal mineral salts plus 2% dextrose. The amino acids and inorganic nitrogen compounds were employed at a concentration equivalent to 84 mg of nitrogen per 100 ml. Highest antibiotic yield was obtained in a synthetic medium containing $(NH_4)H_2PO_4$ as the nitrogen sources (Table 3). The optimal concentration for antibiotic production was found to be at a concentration 0.68% (Table 4).

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REFERENCES

Grove DC, Randall WA (1955). Assay methods of antibiotics. pp.91-93. Medical Encyclopedia, Inc. New York.

Majumdar MK, Majumdar SK (1967). Utilization of carbon and nitrogen-containing compounds for neomycin production by *S. fradiae.* Appl. Microbiol. 15:744-749.

Pridham TG, D Gottlieb (1948). The Utilization of carbon compound by some actionmycetes as an aid for species determination. J. Bacteriol. 56:107-114.