Pakistan J. Sci. Ind. Res., 12, 236–238 (1969)

NUTRITIONAL REQUIREMENTS OF STREPTOMYCES ROSEOCHROMOGENES FOR CYCLOSERINE PRODUCTION

Part I.—Effect of Carbon and Nitrogen Sources

M.A. Qadeer, Bushra A. Mateen and M. Afzal Baig

Biological Evaluation and Fermentation Division, P.C.S.I.R. Laboratories, Lahore 16

(Received July 15, 1968; revised November 20, 1968)

The biosynthesis of cycloserine by different species of *Streptomyces* was studied. *Streptomyces roseochromogenes* produced maximum amount of cycloserine. Organic nitrogen sources such as urea or peptone gave better results of cycloserine production than inorganic nitrogen sources. Of the six carbon sources, starch was most suitable for optimum yield of antibiotic.

Introduction

Cycloserine is a broad spectrum antibiotic active against both Gram positive and Gram negative bacteria including mycobacteria such as *Mycobacterium ranae*. It has been found particularly effective in the treatment of tuberculosis and urinary tract infections in man.[1,2] It is also used for certain other bacterial infections and leprosy.[3] The antibiotic is produced by the microorganisms of the genus *Streptomyces* such as *S. orchidaceous*,[4–6] *S. garyphalus*,[7] *S. lavendulae*,[8–10] *S. roseochromogenes*[11,12] and *S. nagasakinesis*[13] and the work has mostly been reported in patents. The present paper describes the selection of Streptomyces strains and their nutritional requirements for the production of cycloserine in shake flasks as a preliminary to pilot plant studies.

Material and Methods

Organism.—The following strains of the genus *Streptomyces* were used: *S. lavendulae* IMI39045, IMI39046 and UGOC (University of Guelph, Ontario, Canada); *S. roseochromogenes* IFO3363, IFO3411, NRRL-B2036 and NRRL-B1230.

Media.—The composition of the media used in the present work are given in the Table 1. All reagents were of analytical grade. Glass-distilled water was used for the preparation of solutions. All media unless otherwise stated were autoclaved at 121°C for 15 min. The pH value of the medium was 6.8.

Inoculum Preparation.—All cultures were maintained on sporulation medium incubating at 30°C for 10 days. Vegetative inoculum was used in the present study. 25 ml inoculum medium in 300 ml shake flasks was inoculated with a loop of mycelium from the agar slant. It was incubated at 30°C for 48 hr.

Shake Flask Cultures.—For shake flask cultures 25 ml fermentation medium including 1 ml vegetative inoculum was held in a 300 ml conical flask; shaken on a rotary shaker (throw 1½ in) and rotated at 125 rev/min.

Analytical Method.—Cycloserine was estimated colorimetrically by the method of Jones.[14] The growth of *Mycobacterium tuberculosis* was inhibited by the cycloserine produced by all the strains.

Results

Selection of Strain.—Three strains of *S. lavendulae* and four of *S. roseochromogenes* were examined for cycloserine production. The amount of cycloserine produced 86 hr (peak yield; see Fig. 1) after inoculation, are shown in Table 2. The comparison of cycloserine production was carried out in the presence of different sources of nitrogen such as peptone and urea. *Streptomyces lavendulae* UGOC and *S. roseochromogenes* NRRL-B2036 produced cycloserine in both media. The latter gave better yield of cycloserine in the medium containing urea and peptone. For further studies, therefore, *S. roseochromogenes* NRRL-B2036 was used.

Effect of Nitrogen Source on Cycloserine Production.—Twelve sources of nitrogen were examined for their effects on the production of cycloserine by *S. roseochromogenes* NRRL-B2036. The results obtained 86 hr after inoculation are given in Table 3. The concentration of each nitrogen source corresponds to the nitrogen level of 0.22%. Nitrogen sources of organic nature, in general, gave better yields of cycloserine than those of inorganic sources. The production of cycloserine was maximum in the presence of urea (2.4 g/l) and peptone (1.95 g/l), at 86 hr.

Effect of Nitrogen Concentration.—Fig. 2 shows the effect of nitrogen concentration on the biosynthesis

TABLE 1.—COMPOSITION OF MEDIA EMPLOYED.

Constituents	Sporulation medium (g/l)	Inoculum medium (g/l)	Fermentation medium (gl/)
Glucose	10.0	10.0	—
Beef extract	1.0	1.0	—
Yeast extract	1.0	1.0	—
Casein (hydrolysed)	2.0	2.0	—
Agar	20.0	—	—
Starch	—	—	50.0
Urea	—	—	4.8(N2.25g)
$MgSO_4.7H_2O$	—	—	5.0
K_2HPO_4	—	—	5.0
$FeSO_4.7H_2O$	—	—	0.02
$ZnSO_4$	—	—	0.02
$MnSO_4$	—	—	0.01

TABLE 2.—ABILITIES OF THE *Streptomyces* SP. TO SYNTHESISE CYCLOSERINE.

	Cycloserine (g/l)	
	Urea as N source	Peptone as N source
Streptomyces lavendulae IMI39045 ..	Nil	Nil
S. lavendulae IMI 39046 ..	,,	,,
S. lavendulae UGOC	0.60	0.65
S. roseochromogenes NRRL-B2036 ..	2.4	1.95
S. roseochromogenes IFO 3363 ..	Nil	Nil
S. roseochromogenes IFO 3411 ..	,,	,,
S. roseochromogenes NRRL-B1230 ..	,,	,,

TABLE 3.—EFFECT OF DIFFERENT NITROGEN SOURCES ON THE PRODUCTION OF CYCLOSERINE BY *S. Roseochromogenes* NRRL-B2036.

Nitrogen source	Cycloserine produced(g/l)	Cell dry weight (g/l)
Urea	2.4	4.93
Peptone	1.95	7.60
Caseitone	1.0	5.18
Cornsteep liq. ..	0.60	2.15
Soyabean meal ..	1.1	6.80
Asparagin	0.12	2.52
$(NH_4)_2HPO_4$ ..	0.46	4.80
NH_4NO_3	0.35	3.10
NH_4Cl	0.32	3.88
$(NH_4)_2SO_4$	0.35	3.49
$(NH_4)_2CO_3$ ..	0.18	3.27
$NaNO_3$	0.18	1.95

TABLE 4.—EFFECT OF DIFFERENT CARBON SOURCES ON THE PRODUCTION OF CYCLOSERINE BY *S. Roseochromogenes* NRRL-B2036.

Carbon source	Percentage of carbon source	Cycloserine (g/l)
Starch (Sol.) ..	5%	2.40
Starch Maize* ..	2%	1.40
Corn Flour ..		1.00
Cane Molasses ..		0.86
Beet Molasses ..	5%	0.80
Maize* ..		0.66
Liqued Glucose* ..		0.28
Glucose ..		0.21

*Rafhan Maize Products Co. Ltd., Lyallpur. affiliated with Corn Products International, New York, U.S.A.

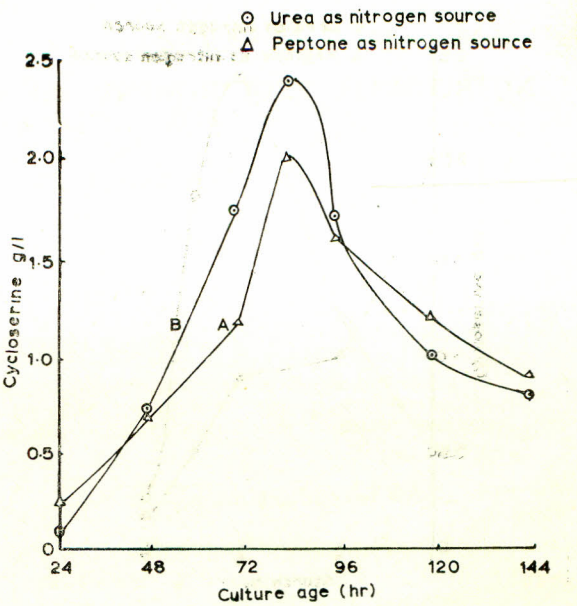

Fig. 1.

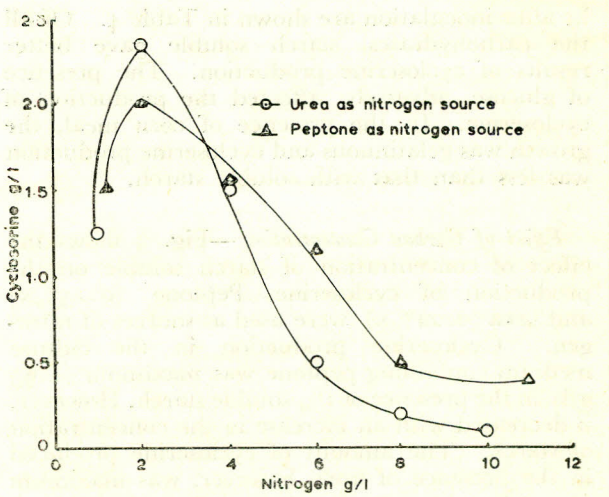

Fig. 2.

of cycloserine by *S. roseochromogenes*. The sources of nitrogen were peptone (1.0–6.0 g/l) and urea (1.0–8.0 g/l). The optimum concentration of nitrogen was 1.5 and 2.0 g/l in the presence of peptone and urea, respectively. The production of cycloserine was better in the presence of urea (2.4 g/l). Further increase in the concentration of nitrogen resulted in lowering the production of cycloserine.

Effect of Carbon Sources on Cycloserine Production.— Six sources of carbon were examined for their effects on cycloserine production by *S. roseochromogenes* NRRL-B2036. The results obtained 86

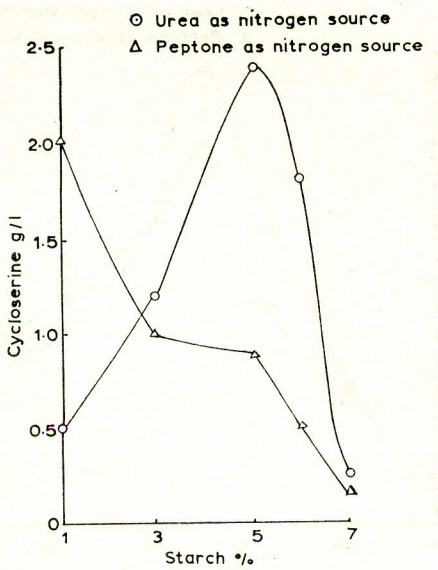

Fig. 3.

hr after inoculation are shown in Table 4. Of all the carbohydrates, starch soluble gave better results of cycloserine production. The presence of glucose adversely affected the production of cycloserine. In the presence of corn meal, the growth was gelatinuous and cycloserine production was less than that with soluble starch.

Effect of Carbon Concentration.—Fig. 3 shows the effect of concentration of starch soluble on the production of cycloserine. Peptone (0.15%N) and urea (0.22%N) were used as sources of nitrogen. Cycloserine production in the culture medium containing peptone was maximum (1.95 g/l), in the presence of 1% soluble starch. However, it decreased with an increase in the concentration of starch. The amount of cycloserine produced in the presence of urea, however, was maximum (2.4 g/l), when starch concentration was 5%. A further increase in the concentration of carbon affected the production of cycloserine.

Time required for the maximum biosynthesis of cycloserine in shake flasks was 86 hr, after which the yield markedly decreased (Fig. 1).

Discussion

The purpose of the present investigation was to select a strain of *Streptomyces* and to study optimum cultural conditions for the biosynthesis of cycloserine in agitated aerobic culture. Out of 6 strains of *Streptomyces* species, *S. roseochromogenes* NRRL-B2036 produced maximum amount of cycloserine. Effect of 12 kinds of nitrogen sources of organic nature, e.g. urea and peptone gave better results of cycloserine production (about 2.0 g/l), while in the presence of inorganic nitrogen sources it was greatly affected (0.10–0.5 g/l). The amount of nitrogen (1.5 g/l), as peptone produced less amount of cycloserine (1.95 g/l), than that of urea (2.4 g/l). The use of urea as a source of nitrogen in the production of cycloserine seemed most economical. Among the carbon sources studied, starch was a better source of carbon at a concentration of 5% when urea was used as a source of nitrogen. In the presence of peptone, however, 1% starch in the medium gave optimum yield. The mycelial dry weight was comparatively more when peptone was used as a source of nitrogen. It could be due to the availability of certain amino acids which stimulated the growth process.

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