UTILIZATION OF VARIOUS CARBOHYDRATES BY *STREPTOMYCES GRISEUS* FOR PRODUCTION OF STREPTOMYCIN AND GROWTH

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The development of a chemically defined medium (4, 5) for growth and production of streptomycin by *Streptomyces griseus*, (Krainsky) Waksman and Henrici, 1943, provides accurate methods for nutritional studies with this microorganism. This synthetic medium, consisting of simple organic and inorganic chemicals furnishes critical tests for the utilization of other substance when substituted for various constituents because the nutritional factors are controlled and also because in the absence of any constituent growth is greatly diminished and streptomycin production is reduced or lacking. With these exact methods available, the utilization of various carbohydrates by *S. griseus* was studied by substituting these for glucose of the original medium. The results of this study and a description of materials, methods, and experiments are reported herein.

METHODS* AND MATERIALS

The culture of *S. griseus* used throughout these studies was secured from Waksman (6) and is his strain number 9 from No. 18-16 originally obtained from heavily manured field soil (3, 7).

Streptomycin was estimated by the paper disc-plate assay method (2) against *Bacillus subtilis* in Bacto-Streptothricin Assay Agar and reported as units per ml. (2).

H-ion concentrations were determined electrometrically with a Beckman pH meter.

Growth of the microorganism was determined by weighing the vacuum dried mycelial mat that developed on the surface of the liquid medium.

Growth conditions were provided by an air-conditioned culture room in which the air was exchanged and the temperature and humidity were automatically controlled at 26°C. and 40 percent relative humidity (dry bulb 78-79°F. and wet bulb 60-65°F.). The cultures were grown for ten days in 125 mls. Erlenmeyer Pyrex flasks containing 50 mls. of medium which was 2 cm. deep with an air surface of 0.66 cm^2 per ml. of liquid.

Inoculum for seeding the media was obtained by growing the fungus for 7-10 days on 200 ml. of beef-extract liquid medium in rectangular, two-quart milk bottles laid horizontally (air or mat surface of 192 cm^2 per bottle). Inoculum on this medium was utilized to prevent any selective adaptation of the fungus to the special medium through repeated transfers. The mat, consisting of spores and mycelium, was added to sterile distilled water (one mat per 100 ml.) and thoroughly broken up by means of a Waring blender operated at high speed for 10 seconds. A small amount (4 to 5 drops) of this inoculum was introduced aseptically into each flask of medium. To avoid submergence of spores the inoculum was applied to the glass surface of tilted flasks just above the medium. This permitted the spores

* The methods are similar to those used in developing the synthetic medium (4, 5).

to remain on the surface of the liquid, a condition which is apparently necessary for growth. Thereafter, the inoculated flasks were stationary until the medium was assayed for streptomycin.

The basal synthetic medium (4, 5) was as follows: KH_2PO_4 2.38g; K_2HPO_4 . $3H_2O$ 5.65g; mg. SO_4. $7H_2O$ 1.0g; $ZnSO_4$. $7H_2O$ 0.0115g; $FeSO_4$. $7H_2O$ 0.0111g; $CuSO_4$ 0.0064g; MnCl . $4H_2O$ 0.0070g; and amonium lactate 5.4g. per 1000 mls. of distilled water. The H-ion concentration was pH 6.95.

The carbohydrates used were: l-arabinose, l-xylose, rhamnose, d-mannose, d-galactose, maltose, lactose, callibiose, melibiose, trehalose, raffinose, melezitose, mannitol, dulcitol, dextrin, inulin, glycogen, salicin and gum acacia.

EXPERIMENTS AND RESULTS

To determine what carbohydrates were utilized by *Streptomyces griscus,* experiments were designed to substitute each of the carbohydrates for glucose in the synthetic medium prior to sterilization at 15 lbs. pressure for 20 minutes. With the experiments in six replications a total of 300 ml. of medium was used with each carbohydrate and this was divided accurately into 50 ml. portions. (Approximately 0.1 percent of powdered calcium carbonate was added to three of each series. As there was but little effect upon growth and streptomycin production, the results of the two series were combined). Monosaccharides were added in 0.05 molar concentrations and polysaccharides were used in 0.025 molar concentrations. Those

TABLE 1.—THE EFFECT OF CARBOHYDRATES IN A SYNTHETIC MEDIUM ON GROWTH, PRODUCTION OF STREPTOMYCIN AND CHANGES IN H-ION CONCENTRATION BY *Streptomyces Griseus* IN SURFACE CULTURE

Carbohydrate	Concentration	Units of Streptomycin per ml.	Weight of Mat. per 300 ml.	pH
1-Arabinose	0.05 M.	0	188.9	7.86
1-Xylose	0.05 M.	15.1	701.5	7.04
Rhamnose	0.05 M.	11.1	448.5	7.52
d-Mannose	0.05 M.	42.3	624.6	7.50
d-Galactose	0.05 M.	34.6	724.0	7.32
Maltose	0.025M.	62.3	645.1	7.28
Lactose	0.025M.	11.1	363.5	8.14
Cellibiose	0.025M.	41.5	512.1	7.41
Melibiose	0.025M.	10.5	269.6	8.34
Trehalose	0.025M.	11.8	443.8	7.96
Raffinose	0.025M.	10.7	337.8	8.27
Melezitose	0.025M.	9.7	330.4	8.38
Mannitol	0.05 M.	42.2	452.6	7.83
Dulcitol	0.020M.	9.1	192.0	8.35
Dextrin	0.5%	26.1	423.6	7.76
Dextrin	1.0%	40.5	641.3	7.04
Inulin	0.5%	13.8	130.4	8.27
Inulin	1.0%	18.7	91.9	8.31
Glycogen	0.5%	18.0	129.3	7.66
Glycogen	1.0%	25.35	145.4	7.23
Salicin	0.5%	10.3	.....	8.08
Salicin	1.0%	8.0	.....	8.11
Gum acacia	0.5%	13.8	285.9	8.19
Gum acacia	1.0%	12.8	230.4	8.19
None	.....	10.3	241.6	8.19

compounds of which the molecular weights were not known were added in both 0.5 percent and 1.0 percent concentrations. At the end of the 10-day growth period the streptomycin was assayed and H-ion concentration was determined in the medium of individual flasks. The amount of growth was determined by the dry weight of the six combined mycelial mats. The results of this preliminary survey given in Table 1 (average of six flasks) show that d-mannose, d-galactose, maltose, cellibiose, mannitol and dextrin supported good growth of the organism as well as good production of streptomycin. l-Arabinose, l-xylose, rhamnose, lactose, melibiose, trehalose, raffinose, melezitose, dulcitol, inulin, glycogen, salicin and gum accacia gave little if any increase in growth or steptomycin production.

Since our interest was chiefly in streptomycin production, only those compounds giving relatively good production were selected for further study. These were: d-mannose, d-

TABLE 2.—THE RELATIONSHIP OF CARBOHYDRATE CONCENTRATION IN A SYNTHETIC MEDIUM TO GROWTH, PRODUCTION OF STREPTOMYCIN, AND CHANGES IN CONCENTRATION BY *Streptomyces Griseus* IN SURFACE CULTURE

Carbohydrate	Concentration	Units of Streptomycin per ml.	Weight of Mat. per 300 ml.	pH
d-Mannose	0.01 M.	23	390.2	8.10
	0.03 M.	68	464.2	7.95
	0.05 M.	101	699.4	7.74
	0.08 M.	99	780.2	7.45
	0.10 M.	82	924.2	7.35
d-Galactose	0.01 M.	22	323.2	8.10
	0.03 M.	42	503.2	7.93
	0.05 M.	49	653.5	7.85
	0.08 M.	59	788.1	7.60
	0.10 M.	63	927.4	7.40
Maltose	0.01 M.	18	407.3	8.06
	0.03 M.	62	781.9	7.83
	0.05 M.	106	1,083.5	7.37
	0.08 M.	86	1,476.8	6.84
	0.10 M.	68	1,615.0	6.54
Cellibiose	0.01 M.	59	603.6	7.67
	0.03 M.	112	800.8	7.60
	0.05 M.	79	1,100.9	6.92
Mannitol	0.01 M.	22	332.4	8.06
	0.03 M.	57	631.9	7.78
	0.05 M.	101	802.5	7.49
	0.08 M.	50	895.0	6.52
	0.10 M.	69	1,319.6	6.72
Dextrin	0.5%	31	593.7	7.72
	1.0%	73	952.4	7.19
	2.0%	66	1,356.6	6.39
	3.0%	24	1,406.1	5.82
	4.0%	17	1,280.7	5.97
None	.....	18	160.5	8.12

galactose, maltose, cellibiose, mannitol and dextrin. To determine the optimal concentrations for production of streptomycin and growth dextrin was employed at 0.5, 1.0, 2.0, 3.0 and 4.0 percent concentrations, and the others were all used at 0.01, 0.03, 0.05, 0.08 and 0.10 molar concentrations. Three hundred mls. of medium equally divided into six flasks were used at each concentration. At the end of the 10-day growth period streptomycin was assayed and the weight of mycelial mat and the H-ion concentrations were determined. These results are shown in Table 2.

DISCUSSION

It is evident from the results of the first part of the studies shown in Table 1 that some carbohydrates may be substituted for glucose in this medium and that they give good production of streptomycin and growth, but that others are not utilized by the organism.

The relationship of streptomycin production to concentration of certain carbohydrates is illustrated graphically: figure 1, d-mannose; figure 2, d-galactose; figure 3, maltose; figure 4, cellibiose; figure 5, mannitol; figure 6, dextrin. With mannose the peak of streptomycin production as shown by the curve occurred at a concentration somewhere between 0.05M and 0.08M. Using d-galactose as the carbohydrate, streptomycin production steadily increased with the concentration of galactose up to the highest concentration used (0.1M). Whether production would have continued to increase to even higher levels before it started to decrease with increasing concentrations of sugar was not indicated. Maltose gave a peak production at a concentration near 0.05M, and at higher concentrations the amount of streptomycin decreased. With cellibiose maximum production of streptomycin occurred at 0.03M concentration, which is about

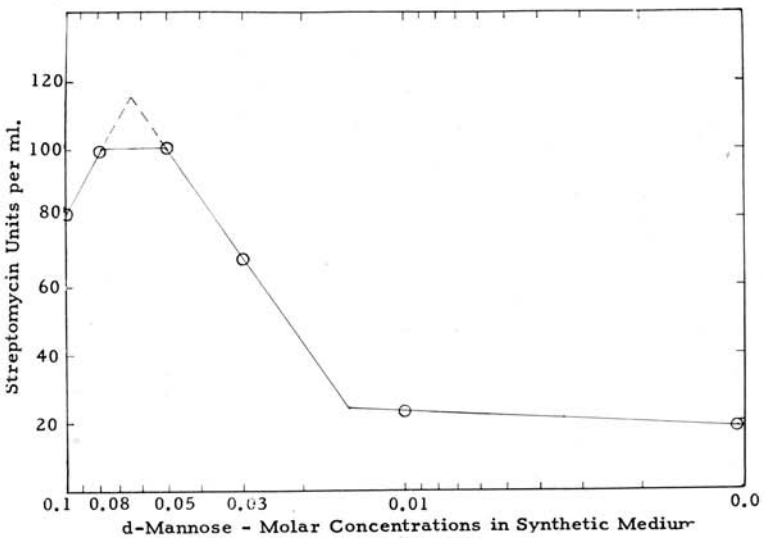

FIG. 1.—Relation between Streptomycin production in units per ml. and molar concentrations of d-mannose.

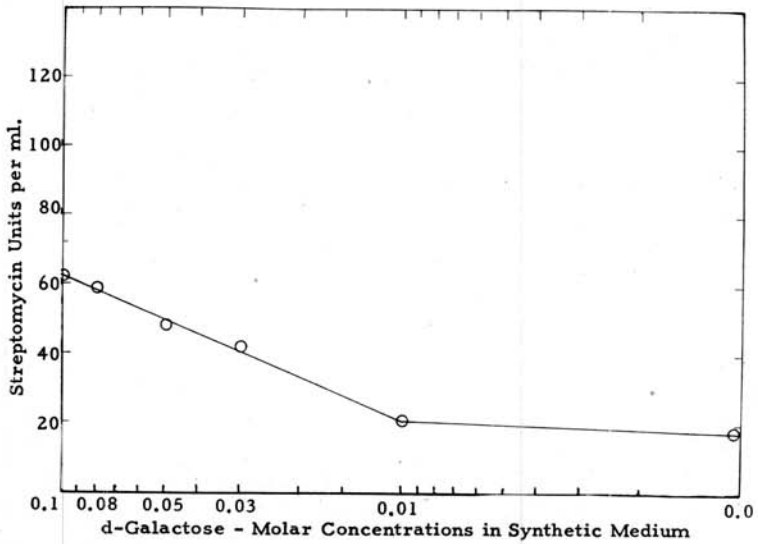

FIG. 2.—Relation between Streptomycin production in units per ml. and molar concentrations of d-galactose.

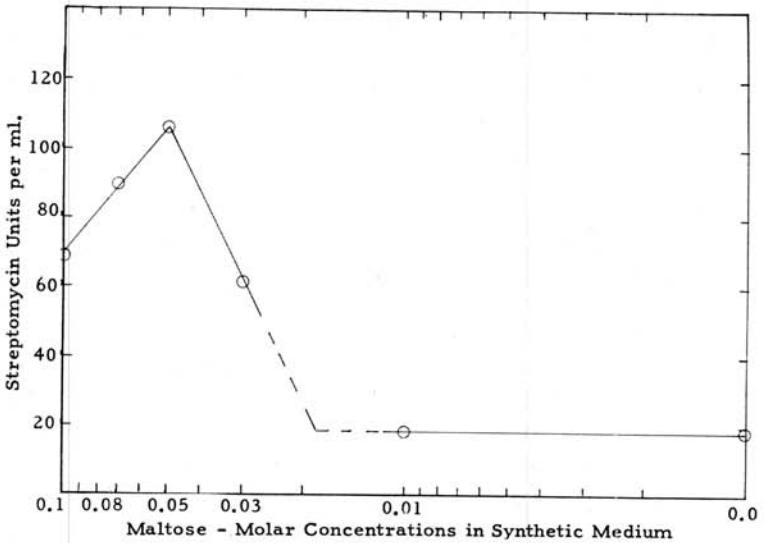

FIG. 3.—Relation between Streptomycin production in units per ml. and molar concentrations of maltose.

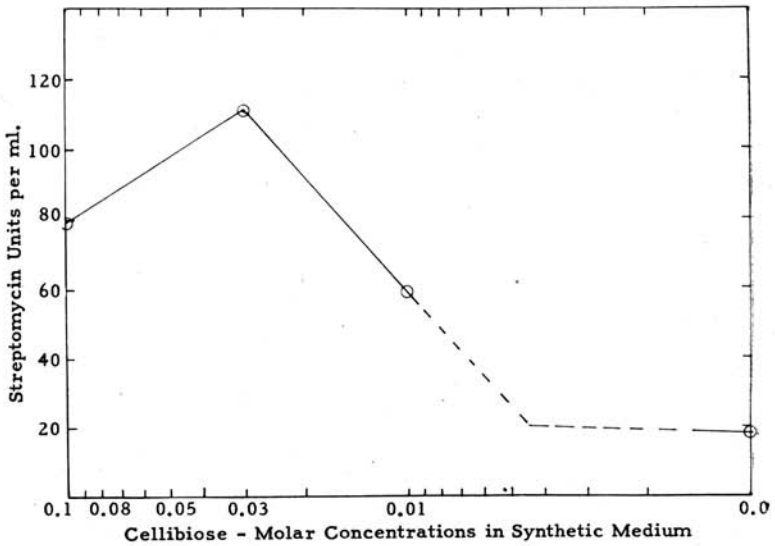

FIG. 4.—Relation between Streptomycin production in units per ml. and molar concentrations of cellibiose.

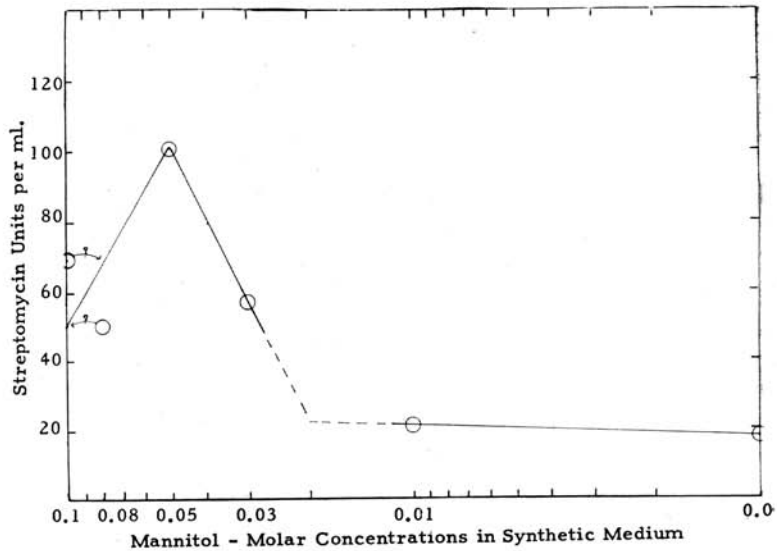

FIG. 5.—Relation between Streptomycin production in units per ml. and molar concentrations of mannitol. (.08 and .1 molar values were probably reversed by error in numbering).

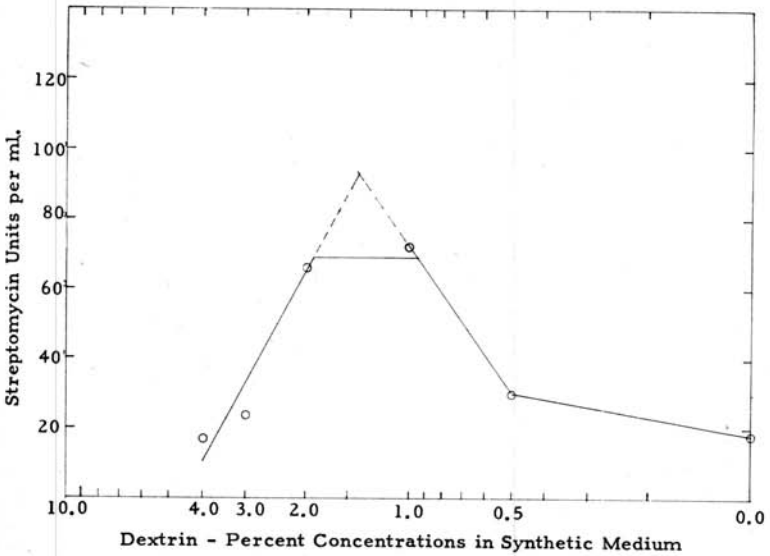

FIG. 6.—Relation between Streptomycin production in units per ml. and percentage concentrations of dextrin.

one-half the concentration (0.05M) of the more favorable monosaccharides necessary for the peak. It is interesting that the peak in production for maltose and the monosaccharides occurred at the same concentrations, but about twice the concentration of cellibiose necessary for the peak production of streptomycin. Since maltose and cellibiose are similar except for the alpha and beta linkage respectively between the two glucose units, the results suggest that the organism does not break the alpha linkage, but utilizes the maltose through direct fermentation as mentioned by Leibowitz and Hestrin (1). With cellibiose the organism apparently is able to break the beta linkage to form two molecules of glucose, and thus would need a concentration only half that of maltose for maximum production. Mannitol gave a peak production of streptomycin at a concentration between one and two percent.

The relationship between growth and production of streptomycin at various concentrations is similar with all the carbohydrates studied. The peak for streptomycin was reached at a concentration from 0.03M to 0.05M, but the weight of the mycelial mat continued to increase up to the highest concentrations used in the experiments. Dextrin is an exception to this in that maximum growth was obtained at concentration of 3.0 percent and decreased at a higher concentration. Figure 7 illustrates graphically the relationship between streptomycin units per ml., weight of mycelial mat and pH at the concentrations of maltose used. The streptomycin units per ml. rise rather sharply to a maximum of 106 at 0.05M concentration and gradually decrease to a value of 68 at a concentration of 0.1M. The weight of mycelial mat increases steadily with increased concentrations of sugar. The weight of mycelial mat was greatest (1,615 mg.) at 0.1M maltose and probably would have continued to rise at higher concentrations.

In all cases with good production of streptomycin there was a white,

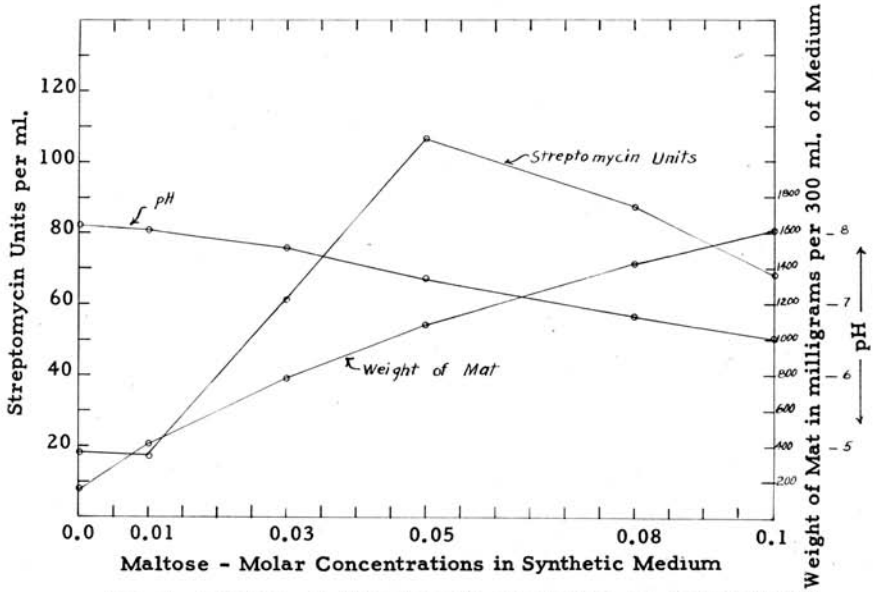

FIG. 7.—Relation of Streptomycin production in units per ml., weight of mycelial mat, and pH, to molar concentration of maltose.

cottony aerial growth. At higher concentrations of dextrin the organism produced a waxy growth that had a tendency to submerge.

SUMMARY

1. *Streptomyces griseus* in a chemically defined basal medium utilized d-mannose, d-galactose, maltose, cellibiose, mannitol, and dextrin for production of streptomycin and growth.

2. It does not utilize l-arabinose, l-xylose, rhamnose, lactose, melibiose, trehalose, raffinose, melezitose, dulcitol, inulin, glycogen, salicin or gum acacia.

3. The optimum concentrations for streptomycin production from the monosaccharides utilized and maltose appear to be between 0.05M and 0.08M. With cellibiose this optimum concentration is near 0.03M.

4. Maximum growth occurs at a higher concentration of carbohydrate than the optimum concentration for maximum production of streptomycin.

REFERENCES

1. LEIBOWITZ, J. AND S. HESTRIN. Alcoholic fermentation of the aligosaccharides. Advances in Enzymology *5*:87-127, 1945.
2. LOO, Y. H., P. S. SKELL, H. H. THORNBERRY, JOHN EHRLICH, J. M. MCGUIRE, G. M. SAVAGE AND J. C. SYLVESTER. Assay of streptomycin by the paper-disc plate method. Jour. Bact. *50*: 701-709, 1946.
3. SCHANTZ, ALBERT, ELIZABETH BUGIE, AND S. A. WAKSMAN. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proc. Soc. Exp. Biol. Med. *55*: 66-69, 1944.
4. THORNBERRY, H. H. Nutrient requirements of an antibiotic soil fungus, *Streptomyces griseus* (Krainsky) Waksman and Henrici. Phytopathology *36*: 412, 1946.
5. THORNBERRY, H. H. AND H. W. ANDERSON. Synthetic medium for *Streptomyces griseus*, and the production of streptomycin. (In press).
6. WAKSMAN, SELMAN A. Letter Sept. 1. 1944 to H. W. Anderson, University of Illinois.
7. WAKSMAN, SELMAN A. AND ALBERT SCHANTZ. Streptomycin—a review. Jour. Amer. Pharm. Assoc., Practical Pharm. Edition *6*: 308-321, 1945.