STUDIES ON THE NUTRITIONAL REQUIREMENTS OF STREPTOMYCES GRISEUS FOR THE FORMATION OF STREPTOMYCIN

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In view of the marked activity of streptomycin *in vitro* against a number of species of organisms relatively resistant to penicillin, such as *Escherichia coli* (Schatz, Bugie, and Waksman, 1944), certain of the *Salmonella* (Robinson, Smith, and Graessle, 1944), *Klebsiella pneumoniae* (Donovick, Hamre, Kavanagh, and Rake, 1945), and *Mycobacterium tuberculosis* (Schatz and Waksman, 1944), as well as its *in vivo* therapeutic behavior against such infecting agents (Jones, Metzger, Schatz, and Waksman, 1944; Robinson, Smith, and Graessle, 1944), investigations of this antibiotic as well as of the characteristics and growth requirements of the causative organism, *Streptomyces griseus*, are now being vigorously investigated in many laboratories. Chemical studies on streptomycin have already resulted in the preparation of crystalline derivatives (Fried and Wintersteiner, 1945; Kuehl, Peck, Walti, and Folkers, 1945).

Schatz, Bugie, and Waksman (1944) state that the production of streptomycin by *Streptomyces griseus* requires in the culture medium the presence of a specific growth-promoting substance supplied by beef extract or corn steep liquor. The medium recommended contained peptone, beef extract, glucose, and sodium chloride. We have studied various other materials as sources of nutrition for *Streptomyces griseus* and have found that it is possible to devise media including neither beef extract nor corn steep liquor that yield as much as 250 units[1] of streptomycin per ml, and from which streptomycin is recovered more readily in a purified state. The latter advantage arises because certain of the basic constituents of beef extract are concentrated in a manner similar to that of streptomycin and are found as impurities in the end product. The present paper deals chiefly with media employing soybean meal as the source of nitrogen.

The experiments described below were all conducted in a uniform fashion. A dilute suspension of *S. griseus* spores was prepared by suspending in distilled water the surface growth of this organism grown on Krainsky's asparagine glucose agar. Since the spores wet with great difficulty, the suspension was shaken with glass beads for half an hour. The resultant even suspension was stored at 4 C and was used for some months to inoculate the various media tested.

The media to be tested were dispensed in 200-ml amounts in the earlier experiments, and later in 100-ml amounts in 500-ml Erlenmeyer flasks. After being autoclaved, each flask was inoculated with 0.5 ml of spore suspension. The

[1] This is an average peak figure for medium no. 8. Individual shake flasks have on occasion been found to contain more than 350 units per ml of broth.

flasks were then incubated at 24 C on a shaking apparatus oscillating approximately 100 strokes per minute.

From the third through the sixth or seventh days of incubation, samples were taken daily for pH determination and streptomycin assay. The samples were clarified by centrifugation; the supernatant fluids were removed and heated in a boiling water bath for two minutes and assayed by the 2-ml broth dilution method (Donovick, Hamre, Kavanagh, and Rake, 1945). The constituents of the media employed and the streptomycin concentrations obtained in the various broths

TABLE 1

Streptomycin production in various media

MEDIUM NO.	CONSTITUENTS*				NO. OF REPLICATE FLASKS	VOL. OF MEDIUM PER FLASK		DAYS OF INCUBATION				
	Soybean meal†	Glucose	Beef extract	Sodium chloride				3	4	5	6	7
		%	%	%		ml						
1	1.5	1.0			2	100	pH	6.8	7.0	6.9	7.5	7.8
							u/ml‡	1.6		5.7	5.7	9.8
2§	1.5	1.0	0.5	0.5	4	200	pH	7.0	7.1	7.4	7.2	7.4
							u/ml	6.1	10.7	27.2	37.4	73.2
3	1.5	1.0	0.1		3	100	pH	7.3	7.8	8.2	8.3	8.5
							u/ml	37.1	44.1	96.2	121.0	114.0
4	1.5	1.0	0.1	0.5	6	100	pH	7.1	7.7	8.2	8.4	
							u/ml	123.5	147.0	146.0	158.0	
5	1.5	1.0	0.2	0.5	5	100	pH	6.8	7.7	8.2		
							u/ml	69.2	156.0	160.0		
6§	1.5	1.0	0.5	0.5	7	100	pH	7.2	7.9	8.3	8.6	
							u/ml	41.5	100.0	168.0	181.0	
7	1.5	1.0		0.5	16	100	pH	7.1	7.6	8.1	8.3	..
							u/ml	120.5	170.0	187.5	212.0	
8	1.0	1.0		0.5	6	100	pH	7.0	7.1	7.4	7.9	8.3
							u/ml	129.0	148.0	201.0	236.0	237.0

* Made up in distilled water.

† The soybean meal employed contained from 41 to 44 per cent protein.

‡ Units streptomycin per ml broth.

§ Media "2" and "6" were the same except that "2" was dispensed in 200 ml per flask while "6" was in 100-ml amounts.

are shown in table 1 and figure 1. These data represent a summary of the results of many replicate experiments.

Under the conditions employed in these studies the volume of medium per flask was extremely important. For example, in medium no. 2 the average flask yielded in the broth only 73.2 units of streptomycin per ml in 7 days, rising to 140 units per ml at a later date. When only 100 ml of the same medium was employed per flask (see no. 6), the average flask contained 181 units per ml of broth on the seventh day. Consequently, in most of the experiments only 100 ml of medium per 500-ml Erlenmeyer flasks was employed.

If the media are considered in the ascending order of activity obtained in the

broth, it will be noted that a medium consisting of only soybean meal and glucose (no. 1) is distinctly deficient, yielding less than 10 u per ml in 7 days. The addition of 0.1 per cent beef extract but no sodium chloride (no. 3) improves the medium considerably but is only approximately half as effective as adding sodium chloride and no beef extract to a soybean glucose preparation (nos. 7 and 8). It would appear from these results that beef extract may supply certain necessary salts but is otherwise not required when soybean meal is employed. In fact, when media 4, 5, and 6 are compared with 7 and 8 it appears that the addition of beef

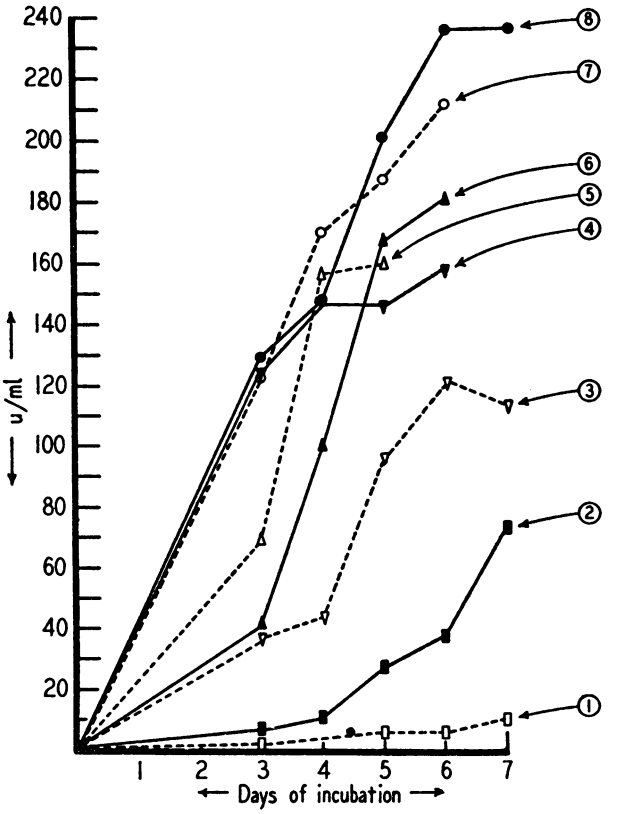

FIG. 1. STREPTOMYCIN PRODUCTION IN VARIOUS MEDIA

extract to a soybean meal, glucose, sodium chloride mixture is somewhat detrimental to the rise in streptomycin content in the broth. We have routinely observed that the rise in activity in a culture of *S. griseus* is greatest during or shortly after sporulation has begun. Thus, whereas mycelial growth is excellent in the presence of beef extract, sporulation is delayed. This may account for the lower streptomycin yields obtained in broths nos. 4, 5, and 6.

Preliminary studies have shown that substitution of the sulfate ion for the chloride ion in the salt added to soybean medium causes no appreciable change in the streptomycin concentration obtained in the broth. On the other hand, sub-

stitution of magnesium for the sodium ion gave lower streptomycin concentrations.

SUMMARY

The volume of medium in shake-flask cultures of *Streptomyces griseus* plays an important role in the concentration of streptomycin obtained in the broth.

Beef extract is not required for streptomycin production in a medium containing soybean meal, glucose, and sodium chloride. In fact, the addition of beef extract to such a medium in shake flasks delays somewhat the production of this antibiotic.

It is necessary to add an inorganic salt, e.g., sodium chloride, to soybean meal media for streptomycin production.

Preliminary studies indicate that sodium sulfate may be substituted for sodium chloride, but the substitution of magnesium chloride for either of these two electrolytes gives lower streptomycin yields.

Beef extract may supply a certain amount of the salt required in a soybean meal medium.

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