EFFECT OF CULTURE MEDIUM ON THE INHIBITORY ACTIVITY OF STREPTOMYCIN

by W. F. K. CHRISTIAN

Food Research Institute, Council for Scientific and Industrial Research, Accra

EFFECT OF CULTURE MEDIUM ON THE INHIBITORY ACTIVITY OF STREPTOMYCIN

By W. F. K. CHRISTIAN

Food Research Institute, Council for Scientific and Industrial Research, Accra.

Summary

The inhibitory effect of streptomycin on the growth of germinated spores of *Bacillus subtilis* strain SF 40 is investigated in a number of culture media. The minimum inhibitory concentration of the antibiotic is found to depend on the nature of the medium. Streptomycin is most inhibitory in media least favourable for growth, whereas in those media in which growth is heavy the antibiotic shows reduced inhibitory effect. It is suggested that the differences in the antibacterial activity of streptomycin in the different media may be due to differences in the amounts of glucose, sodium chloride and organic nitrogenous substances in the media.

Introduction

The nature of the culture medium has been found by a number of workers to be an important factor influencing the antibacterial activity of streptomycin. Wallace, Rymer, Gibson and Shattuck (1945) have reported that suspensions of *Eberthella typhosa* showed marked drops in viable cell counts when exposed to streptomycin in nutrient broth and nutrient broth diluted with an equal volume of water, whereas in brain heart infusion broth the same concentration of streptomycin had no effect. They also found that *Staphylococcus aureus* suspensions under similar conditions showed great decreases in viable cell counts in all three media, but grew out heavily in brain heart infusion broth.

Donovick and Rake (1946) have also shown that if they raised the concentration of tryptone from 0.5 to 1.0% in a tryptone-water medium, the minimum inhibitory concentration of streptomycin against *Klebsiella pneumoniae* was raised from 0.036 to 0.084 units/ml.

Green and Waksman (1948) also found that the antibacterial potency of streptomycin decreased with an increase of glucose, sodium chloride and organic nitrogenous substances in the medium.

In the present study, the effect of streptomycin on the growth of germinated spores of a strain of *Bacillus subtilis* in different media was investigated. As streptomycin does not inhibit germination of spores, the concentration of the antibiotic at which germinated spores failed to grow into cells was taken as the inhibitory concentration of the antibiotic in the particular growth medium.

Materials and methods

Five different culture media were used. These were, nutrient broth, nutrient broth diluted 10-fold (referred to as dilute nutrient broth in the text), yeastrel dextrose broth, yeastrel dextrose broth diluted 10-fold (referred to as dilute yeastrel dextrose broth in the text), Knight and Proom inorganic ammonium basal medium to which 0.25% (W/V) glucose and 2.5 mM alanine had been added.

The nutrient broth was an Oxoid preparation and contained Lab-Lemco, 1.0g; yeast extract (Oxoid L 20), 2.08g; peptone (Oxoid L 37), 5.0g; sodium chloride, 5.0g; distilled water, 1 litre.

The yeastrel dextrose broth was made up of Lab-Lemco, 10.0g; Oxoid bacteriological peptone, 10.0g; yeastrel, 3.0g; sodium chloride, 5.0g; glucose, 5.0g; distilled water, 1 litre.

The composition of the Knight and Proom medium was as follows: potassium hydrogen phosphate, 1.5g; diammonium hydrogen phosphate, 7.0g; calcium chloride dihydrate, 0.3g; magnesium sulphate ($MgSO_4.7H_2O$), 0.5g; manganous sulphate ($MnSO_4.4H_2O$), 40.0mg; ammonium molybdate, 2.0 mg; distilled water, 1 litre. To every 10 ml of this

basal medium was added 0.5 ml of 5% glucose, and 0.5 ml of 50mM alanine before use. The glucose and alanine were sterilized separately before being added to the medium; alanine was added to assist germination in the medium.

The test culture used was *Bacillus subtilis* strain SF 40 which was obtained from the culture collection of the Department of Agricultural Sciences, Leeds University. A washed spore suspension of the test culture, free from vegetative cells, was prepared, pasteurised at 70°C for 10 min and stored in the refrigerator at 4°C.

A stock solution of streptomycin of concentration 100 μg/ml was kept at 4°C. From this, dilutions were prepared when required.

The experimental method consisted of exposing spores of the test organism in each of the 5 culture media to different concentrations of streptomycin and observing, under the phase contrast microscope, the concentration of streptomycin at which germinated spores failed to grow into vegetative cells. Each experiment was repeated once.

Three concentrations of streptomycin (0.5, 1.0 and 5.0 μg/ml) were used in each experiment. To obtain the required concentration of streptomycin, 0.2 ml of a streptomycin solution of a concentration 10 times higher than the final concentration required was added to 1.6 ml of the medium in a test tube. To this was added 0.2 ml of a heat-shocked spore suspension to bring the total volume to 2 ml. Before inoculation, the spore suspension was diluted and adjusted to give a final concentration in the growth medium of about 100 spores per field under the phase contrast microscope.

Two controls were set up in each experiment. In one control, which was to check on the stability of the spore suspension used, 0.2 ml of the adjusted spore suspension was added to 1.8 ml of Ringer's solution in a test tube. The spores in the Ringer's solution were expected to remain ungerminated and, therefore, phase bright throughout the experiment. Before the test tubes were placed in the water bath and at each reading, the spore control was examined for the percentage of spores which were phase bright. The spore controls remained 100% phase bright in all control tests indicating that the spore material was in a suitable condition for the experiments.

The other control consisted of 1.8 ml of the medium under test plus 0.2 ml of spore suspension and no antibiotic.

All test tubes including those of the controls were incubated at 37°C in a water bath. For all media, excluding yeastrel dextrose broth, readings were taken at 4, 8 and 22h. In the case of yeastrel dextrose broth, the richest of the 5 media used, readings were taken at 60 min, 90 min, 2h then at hourly intervals up to 4 h, and subsequently at 8 and 22h.

To take a reading, a loopful of the inoculated medium was withdrawn from a test tube and placed on a water agar block protected by means of a No. 1 cover slip, and examined under the phase contrast microscope. The percentage of ungerminated phase bright and germinated phase dark spores and spores which had developed into vegetative cells were assessed by examining a number of fields and doing a number of spot counts. The extent of vegetative growth in the controls without antibiotic was also noted.

Results and discussion

Results obtained indicate that the inhibitory effect of streptomycin is affected to a marked degree by the type of medium. The results were identical for both experiments for each test except in dilute yeastrel dextrose broth.

In yeastrel dextrose broth (Table 2) which, in composition, was by far the richest of the culture media used, inhibition of the test organism occurred only at the highest streptomycin concentration of 5.0 μg/ml. This was noticeable 90 min after the experiment had started.

When the yeastrel dextrose broth was diluted, the minimum inhibitory concentration of streptomycin dropped to 0.5 μg/ml. (Table 1). This effect was most noticeable at the 4th hour of incubation, after which the organism showed a tendency to overcome the inhibitory effect of the antibiotic at this concentration. In the first experiment there was only 5% vegetative growth at a concentration of 0.5 μg/ml after 4h incubation in this

TABLE 1

Percentage occurrence of different forms of B. subtilis *strain SF* 40 *in various culture media at specified times and concentrations of streptomycin*

Culture medium	Form of the test organism	4 h				8 h				22 h			
		Streptomycin concentration in μg/ml.				Streptomycin concentration in μg/ml.				Streptomycin concentration in μg/ml.			
		0.0	0.5	1.0	5.0	0.0	0.5	1.0	5.0	0.0	0.5	1.0	5.0
Nutrient broth	Phase bright spores	0	0	0	0	0	0	0	0	0	0	0	0
	Phase dark spores	15	20	100	100	10	20	100	100	10	10	100	100
	Vegetative cells	85	80	0	0	90	80	0	0	90	90	0	0
Dilute nutrient broth	Phase bright spores	0	0	0	0	0	0	0	0	0	0	0	0
	Phase dark spores	100	100	100	100	50	100	100	100	20	100	100	100
	Vegetative cells	0	0	0	0	50	0	0	0	80	0	0	0
Dilute yeastrel dextrose broth	Phase bright spores	0	0	0	0	0	0	0	0	0	0	0	0
	Phase dark spores	25	100	100	100	15	95	100	100	100	95	100	100
	Vegetative cells	75	0	0	0	85	5	0	0	0	5	0	0
Knight and Proom medium	Phase bright spores	0	0	0	0	0	0	0	0	0	0	0	0
	Phase dark spores	90	100	100	100	20	100	100	100	20	100	100	100
	Vegetative cells	10	0	0	0	80	0	0	0	80	0	0	0

TABLE 2

Percentage occurrence of different forms of B. subtilis *strain SF* 40 *in yeastrel dextrose broth at specified times and concentrations of streptomycin*

Form of the test organism	1 h				1.5 h				2 h				3 h				4 h				8 h				22 h			
	Streptomycin conc. in μg/ml				Streptomycin conc. in μg/ml				Streptomycin conc. in μg/ml				Streptomycin conc. in μg/ml				Streptomycin conc. in μg/ml				Streptomycin conc. in μg/ml				Streptomycin conc. in μg/ml)			
	0.0	0.5	1.0	5.0	0.0	0.5	1.0	5.0	0.0	0.5	1.0	5.0	0.0	0.5	1.0	5.0	0.0	0.5	1.0	5.0	0.0	0.5	1.0	5.0	0.0	0.5	1.0	5.0
Phase bright spores	0	0	2	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0
Phase dark spores	90	90	93	98	20	20	35	98	20	20	30	100	15	20	20	100	10	20	20	100	5	5	15	100	0	0	5	100
Vegetative cells	10	10	5	0	80	80	65	0	80	80	70	0	85	80	80	0	90	80	80	0	95	95	85		100	100	95	0

medium (Table 1), while in the repeat experiment there was 25% and 10% growth into vegetative cells at 0.5 μg/ml respectively between the 4th and 8th hours of incubation. At 22h incubation growth had increased to 20% at a streptomycin concentration of 1.0μg/ml in the second experiment, but there was still total inhibition at 5.0 μg/ml.

The minimum inhibitory concentration of streptomycin in nutrient broth was 1.0 μg/ml in both experiments (Table 1). This was observed in 4h and remained the same throughout the experiment. On diluting the medium, the antibiotic became more inhibitory in its effect and prevented growth at 0.5 μg/ml. The effect was, however, noticeable only after 8h incubation.

In the medium of Knight and Proom to which alanine and glucose had been added, streptomycin was observed to inhibit growth at the lowest concentration of 0.5 μg/ml. This took place between the 4th and 8th hours of incubation (Table 1).

The minimum concentration at which streptomycin is inhibitory to *Bacillus subtilis* SF 40 would appear to be related to the nutrient level of the medium. Streptomycin was least inhibitory in yeastrel dextrose broth in whose controls the test organism showed the most rapid and luxuriant growth producing in 22h masses of vegetative cells in long chains (Table 2).

When yeastrel dextrose broth was diluted, growth in the controls was not as heavy as it was in the undiluted medium. A decrease in vegetative growth was, however, accompanied by an increase in the inhibitory activity of the antibiotic (Table 1).

A parallel phenomenon was observed with nutrient broth. Diluting this medium also resulted in less vegetative growth, and an increase in the antibacterial activity of streptomycin (Table 1).

Vegetative growth in the Knight and Proom medium was the poorest. In the control of this medium, the growth rate was slow and the vegetative cells produced were very short and were mostly simple out-growths from germinated spores. In this medium, which was clearly not adequate for the growth of the test organism, the antibiotic was very active and was inhibitory at the minimum concentration of 0.5 μg/ml (Table 1).

Thus it may be inferred that in a rich medium able to support rapid and heavy growth of the test organism, streptomycin is least inhibitory, while in a medium which is not very favourable for growth, and in which the organism, therefore, grows more slowly, the antibiotic exerts its greatest bacteriostatic effect. When any medium is diluted, it presumably becomes less able to support growth and the inhibitory activity of the antibiotic is consequently increased.

The marked reduction in the bacteriostatic activity of streptomycin in yeastrel dextrose broth may be attributed to its comparatively high content of glucose, sodium chloride and organic nitrogenous substances. According to Green and Waksman (1948), the antibacterial potency of streptomycin decreases with an increase in glucose, sodium chloride and organic nitrogenous matter in the medium. These substances in adequate amounts would appear to make the medium more favourable for growth thereby minimising the antibacterial effect of streptomycin.

Nutrient broth with less nitrogenous matter, no glucose, but the same amount of sodium chloride would be less effective than yeastrel dextrose broth in counteracting the action of streptomycin. When diluted, both nutrient and yeastrel dextrose broths would contain less amounts of these substances per unit volume and would, therefore, allow the antibiotic to be more inhibitory.

The medium of Knight and Proom, lacking in organic nitrogenous matter and sodium chloride would therefore be expected to allow streptomycin to show increased inhibition of the growth of vegetative cells.

Acknowledgement

The author wishes to thank Mr J. Wolf of the Food Bacteriology Section of the Department of Bacteriology, Leeds University, for his assistance and advice in carrying out this work.

References

DONOVICK, R. and RAKE, G. (1946) Influence

of certain substances on the activity of streptomycin: I. Modifications in test culture. *Proc. Soc. expt. Biol. Med.* **61,** 224.

GREEN, S. R., and WAKSMAN, S. A. (1948) Effect of glucose, peptone and salts on streptomycin activity. *Proc. Soc. expt. Biol. Med.* **67,** 281.

WALLACE, G. I., RYMER I., GIBSON, O., and SHATTUCK, M. (1945) Studies on mode of action of streptomycin. I. Effect of culture media. *Proc. Soc. expt. Biol. Med.* **60,** 127.

Received 23 Jul 69.