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Studies on the inhibitory properties of sodium thioglycollate on the germination of wet spores of clostridia

By D. A. A. MOSSEL
Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands
and H. BEERENS
Laboratory of Anaerobic Bacteriology, Pasteur Institute, Lille, France

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The use of thioglycollate broth for the testing of sterility of surgical apparatus remains the current medical laboratory practice (Meeks, Pembleton & Hench, 1967), despite repeated warnings of bacteriologists (Hirsch & Grinsted, 1954; Galesloot, 1961) that sodium thioglycollate may inhibit the germination of the spores of certain clostridia. In order to avoid obtaining dangerous, false negative results in the future due to this phenomenon, we report the results of an investigation of this subject.

Spores contained in broth solutions were used as these could be expected not to be more sensitive to toxicity of a culture medium than dry spores occurring on contaminated instruments, catgut, gauze, gloves, etc.

MATERIALS AND METHODS

The clostridia studied were from the culture collection of the Laboratory of Anaerobic Bacteriology, Pasteur Institute, Lille, France. Three groups of organisms were chosen: predominantly proteolytic types (*Cl. bifermentans*, *Cl. histolyticum* and *Cl. sporogenes*), mixed glycolytic-proteolytic strains (*Cl. perfringens* and *Cl. septicum*) and predominantly glycolytic types (*Cl. acetobutylicum*, *Cl. butyricum* and *Cl. tertium*).

Spore-bearing cultures of these organisms were obtained in the following way. The strains were cultivated in an anaerobic jar, using a broth containing meat extract, yeast extract, glucose and meat particles (Mossel *et al.* 1965), until fully grown cultures had been obtained. The tubes were then evacuated, sealed and left at room temperature for about 2 months. These cultures were then heated for 10 min. at 80° C. before use in our experiments. Such a heat treatment will certainly kill all vegetative cells; slight losses of spores may also occur (Roberts, Gilbert & Ingram, 1966), but these are not important in these experiments.

The following four media were used for the spore counts, for comparison with the reference medium.

Medium 1. Thioglycollate agar U.S.P. (Pittman, 1946; *U.S. Pharmacopoeia*, 1965). Tryptone, 15 g.; yeast extract, 5 g.; glucose, 5·5 g.; sodium thioglycollate,

A.R., 0·5 g.; sodium chloride, 2·5 g.; cystine hydrochloride, 0·5 g.; agar, 15 g.; glass-distilled water, 1 l.; pH = 7·2 ± 0·1.

Medium 2. As medium 1, but without thioglycollate; indicated in Table 1 as U.S.P. – TH.

Medium 3. Thioglycollate agar, Difco (*Difco Manual*, 1953). Casitone, 15 g.; yeast extract, 5 g.; sodium thioglycollate, A.R., 0·3 g.; cystine hydrochloride, 0·25 g.; agar, 15 g.; glass-distilled water, 1 l.; pH = 7·2 ± 0·1.

Medium 4. As medium 3, with glucose 5 g./l. added.

As the reference medium agars containing cysteine (Quastel & Stephenson, 1926) were used. These, in all instances, contained 0·5 g./l. cysteine-HCl, 5 g./l. yeast extract and 2·5 g./l. disodium phosphate. For the proteolytic clostridia 2·5 g./l. tryptone and 3 g./l. meat extract paste were used as further sources of nitrogen, whereas for the glycolytic types 15 g./l. soya peptone was preferred instead; these had been established to be the optimal media in the respective instances in an earlier investigation (Mossel *et al.* 1965).

Counts were made in oval cross-section tubes in triplicate. Only those dilutions were taken into account that contained between 30 and 200 colonies. Incubation was for up to 3 days at 37° C. in all instances.

RESULTS AND DISCUSSION

The results obtained are shown in Table 1. These data have been evaluated there by the introduction of the ratio R = count in reference cysteine agar, divided by the count in a given other medium.

Comparing U.S.P.-thioglycollate agar (medium 1) with the reference medium shows that it is inadequate for the spores of eight strains of clostridia, including all the proteolytic types, poor for three strains, suboptimal for two strains and satisfactory for one. Difco thioglycollate agar (medium 3) was inadequate for the spores of five strains, suboptimal for another five, but acceptable for four others. Hence the Difco formula appeared to be considerably better than the U.S.P. This may be due to the richer type of peptone used in the former medium, because omitting the thioglycollate from the U.S.P. medium resulted in a decisive improvement in only four cases, leaving the counts of the spores of most proteolytic clostridia at a level 10^{-3}–10^{-4} times the reference counts.

It is a striking fact that the addition of glucose to the Difco formula led to a considerable improvement in count with four strains. In fact, the Difco formula plus glucose was only quite inadequate for *Cl. histolyticum* and definitely suboptimal for one strain of *Cl. acetobutylicum*; the recoveries of the other clostridia were all virtually within the region $R < 3$. It seems, finally, worth while to stress that only in two intances was R less than 1·0. Both the two occasions were with strain *Cl. butyricum* 1375 and R remained over 0·5, which is still a quite acceptable recovery.

The results of these experiments confirm and extend earlier observations of the great superiority of media containing cysteine (Hirsch & Grinsted, 1954; Galesloot, 1961). The conclusions for medical laboratory practice must be that: (i) the use of sodium thioglycollate in sterility test media should be discontinued; (ii) it should be replaced by cysteine hydrochloride.

Table 1. *Comparative spore counts of fourteen strains of various metabolic types of* Clostridium, *made in media with and without thioglycollate*

(The figures in parentheses indicate the ratio R = [count in reference medium]/[count in TH type medium]).

		Spore count per ml. × 100				
		Thioglycollate type media				Reference cysteine agar
Strain		USP	USP – TH	Difco	Difco + gluc.	
Cl. acetobutylicum	A 1	< 0·01 (+ +)*	< 0·01 (+ +)	0·05 (+ +)	27·6 (2·4)	67·4
	A 23	0·09 (51)	2·0 (2·3)	< 0·01 (+ +)	0·5 (9·2)	4·6
	A 462	< 0·01 (+ +)	< 0·01 (+ +)	0·2 (+ +)	22·6 (1·2)	27·5
Cl. butyricum	T	5·6 (1·1)	1·9 (3·4)	2·6 (2·5)	2·6 (2·5)	6·4
	1375	1·6 (7·8)	18 (0·7)	8·8 (1·4)	14·2 (0·9)	12·4
Cl. tertium	Ut	0·3 (33)	4·2 (2·3)	1·1 (9)	8·5 (1·2)	9·9
Cl. perfringens	8238	1·7 (2·6)	2·4 (1·9)	3·7 (1·2)	3·2 (1·4)	4·5
	166/63	0·6 (13)	4·9 (1·6)	3·9 (2)	7·2 (1·1)	7·9
Cl. septicum	SE 05	< 0·01 (+ +)	< 0·01 (+ +)	1·6 (1·9)	2·1 (1·4)	3·0
Cl. bifermentans	701	< 0·01 (+ +)	< 0·01 (+ +)	4·7 (2·2)	3·0 (3·4)	10·3
Cl. histolyticum	Co	< 0·01 (+ +)	< 0·01 (+ +)	< 0·01 (+ +)	< 0·01 (+ +)	28·0
Cl. sporogenes	1039	< 0·01 (+ +)	< 0·01 (+ +)	3·6 (7·5)	22·4 (1·2)	27·2
	13499	< 0·01 (+ +)	< 0·01 (+ +)	< 0·01 (+ +)	21·2 (2·4)	51·8
	72840	< 0·01 (+ +)	0·8 (14)	3·2 (3·5)	8·2 (1·4)	11·2
No. of strains for which R is:						
satisfactory, i.e. ⩽ 2		1	3	4	8	—
suboptimal, i.e. 2·1–10		2	3	5	5	—
poor, i.e. 10·1–100		3	1	0	0	—
inadequate, i.e. > 100		8	7	5	1	—

* (+ +) indicates > 100.

SUMMARY

Wet spores of fourteen strains of *Clostridium*, representing eight species, were enumerated in four different types of thioglycollate agar and in a similar medium in which thioglycollate was replaced by cysteine-HCl. Thioglycollate appeared to be toxic, in principle, to almost all strains tested, although the degree of toxicity was influenced by other components of the medium. These experiments entirely confirm earlier observations and must lead to the conclusion that the use of thioglycollate in sterility test media should be discontinued and that cysteine-HCl should be used as the redox potential reducing compound instead.

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