SHORT ARTICLES

CONTRASTING GROWTH ON DIFFERENT PEPTONES

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SOME bacteria isolated at the hospital laboratory in Hexham grew on a medium containing Oxoid Peptone L37 (OP), but not on blood agar containing home-made tryptic digest of meat. This was confirmed at the Regional Public Health Laboratory in Newcastle, where other organisms behaving similarly were later isolated. It was noted that the strains showed satellitism with other bacterial species. Seven of these recently isolated strains were then examined at the Public Health Laboratory in Sunderland. All grew badly on blood agar made with home-made extract of heart and Evans Peptone, batch no. N89900 (HME), and better or much better on a medium containing Oxoid Blood Agar Base (OBA).

Of the seven strains subcultured at Sunderland five were *Escherichia coli* and two were streptococci. The colonies of the former in early subcultures were less than 0.2 mm in diameter after 24 hr at 37°C on HME but 2–3 mm on OBA; in smears stained by Gram's method the bacteria were pleomorphic, fusiform, irregularly stained, and up to 15 µm long from HME but alike, parallel sided, uniformly stained, and only 5 µm long from OBA. The two strains of streptococci grew slightly better on OBA, their action on the blood in the two media was different, but the Gram-stained smears were not distinguishable.

The cause of these phenomena could have been a toxic substance in some of the media. But the original observation at Hexham had been that the demanding strains grew on MacConkey agar containing OP, and not on blood agar with tryptic digest of meat. Toxicity, which one might expect to be reduced by blood, therefore seemed unlikely to account for the failure of the tryptic digest. Nor did charcoal improve the growth on a medium made with Evans Peptone (EP), but this medium if layered on OBA allowed growth as luxuriant as on OBA itself.

It seemed likely that OP contained some nutrient that the other media lacked and this was sought in eight other nitrogen-containing nutrients {Yeastrel; Oxoid peptone (old batch); Lab-Lemco (Oxoid); tryptone (Oxoid); enzymic hydrolysate of lactalbumin; acid hydrolysate of casein (Oxoid); Oxoid peptone (batch no. 460); Difco Proteose Peptone no. 3 (control 513512)}. Of these, only OP (batch no. 460) and Difco Proteose Peptone no. 3 enhanced the growth of the demanding strains. Both peptones contained aerobic spore-bearing bacilli and it could be shown that these bacilli alone, without the peptone, produced satellitism of the demanding strains. Failure of the Yeastrel to enhance growth showed that the essential nutrient was not V-factor. It remained only to show that these organisms modify EP to make it suitable for the growth of the demanding strains. The numerical yield of bacterial cells, more easily measured than the diameter of colonies and more constant after repeated subculture, was used to contrast the behaviour of the peptones and their modifications.

MATERIALS AND METHODS

Media. Peptone water was made by dissolving 1 g of peptone in 100 ml demineralised water. The pH was adjusted to 7.0–7.2 and the medium sterilised at 115.5°C for 15 min. Evans Peptone (batch no. N89900) and Oxoid Peptone L37 (batch no. 460) were used. Different strengths of the latter in the former were made by mixing the prepared peptone

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waters. For determining the yield of bacterial cells the medium was distributed in amounts of 2 ml in 120×12 mm tubes plugged with cotton-wool. To test for the effect of pre-conditioning by the growth of the spore-bearing bacilli isolated from Oxoid and Difco peptones, 50-ml amounts of peptone water in 112-ml bottles were sown with the bacilli and incubated at 37°C for 48 hr. The cultures were heated at 50°C for 1 hr, and autoclaved; the cells were removed by centrifugation and the pH of the supernatant fluid was adjusted to 7·7·2.

TABLE I
Yields of demanding Escherichia coli contrasted with those of E. coli O111 in mixtures of peptone waters

Organism	The 1 g peptone per 100 ml medium contained		Number of bacteria per 0·02 ml of the following log ₁₀ dilution of medium						
	Oxoid peptone (per cent.)	Evans peptone (per cent.)	1	2	3	4	5	6	7
<i>E. coli</i> O111 (control)	0	100	...	...	...	...	IC	25	0
	30	70	...	...	...	...	IC	7	2
	60	40	...	...	...	...	IC	14	1
	100	0	...	...	...	...	IC	8	0
<i>E. coli</i> no. 32319 (demanding)	0	100	20-100	2	2	0	0	0	...
	30	70	CG	CG	IC	20-100	8	1	...
	60	40	CG	CG	CG	IC	20-100	5	...
	100	0	CG	CG	CG	IC	27	3	...
<i>E. coli</i> no. 19616 (demanding)	0	100	20-100	11	1	0	0	0	...
	30	70	CG	IC	20-100	28	2	0	...
	60	40	CG	CG	IC	20-100	12	1	...
	100	0	CG	CG	IC	20-100	18	1	...
<i>E. coli</i> no. 26284 (demanding)	0	100	11	0	0	0	0	0	...
	30	70	CG	IC	60	10	2	0	...
	60	40	CG	CG	IC	IC	16	1	...
	100	0	CG	CG	IC	IC	20-100	2	...

CG = Confluent growth; IC = innumerable discrete colonies; ... = not done.

Organisms. *E. coli* O111 (NCTC9111) and *Salmonella senftenberg* (NCTC10384), which had been maintained on Dorset's egg medium, but were cultured on nutrient agar just before use and grew equally well in the two peptone waters, were used as controls. *E. coli* no. 32319, 26284, 19616, and 34686, which were maintained on nutrient agar containing OP, were chosen for study from the seven demanding strains. The viable cells, after incubation for 24 hr at 37°C, were counted by the method of Miles and Misra (Miles, Misra and Irwin, 1938) with 0·01M phosphate buffer as the diluent (Report, 1966) and nutrient agar with OP as the plating medium.

RESULTS

Comparison of peptones

The control strain *E. coli* O111 yielded as many cells in EP as in OP (table I). Three of the demanding strains (no. 32319, 19616, and 26284) yielded in EP between 10⁻³ and 10⁻⁵

TABLE II

Yields of demanding Escherichia coli contrasted with those of Salmonella senftenberg in media made with Evans peptone, Oxoid peptone, and Evans peptone modified by the growth of aerobic spore-bearing bacilli obtained from Oxoid peptone (EOASB)

Organism	Peptone	Number of bacteria per 0.02 ml of the following log ₁₀ dilution of medium						
		1	2	3	4	5	6	7
<i>S. senftenberg</i> (control)	Evans	...	...	...	...	CG	47	2
	Oxoid	...	...	...	...	IC	23	3
	EOASB	...	...	...	...	IC	24	2
<i>E. coli</i> no. 32319 (demanding)	Evans	IC	52	11	2	0	...	...
	Oxoid	CG	CG	CG	IC	67	9	...
	EOASB	CG	CG	CG	IC	22	0	...
<i>E. coli</i> no. 19616 (demanding)	Evans	8	1	0	0	0	...	...
	Oxoid	CG	CG	CG	IC	48	4	...
	EOASB	CG	CG	CG	IC	50	2	...
<i>E. coli</i> no. 26284 (demanding)	Evans	8	1	0	0	0	...	...
	Oxoid	CG	CG	CG	IC	35	...	...
	EOASB	CG	CG	CG	IC	27	4	...

CG = Confluent growth; IC = innumerable discrete colonies; ... = not done.

TABLE III

Yields of demanding Escherichia coli compared with those of E. coli O111 in peptone unmodified and modified by the growth of aerobic spore-bearing organisms isolated from Oxoid (EOASB) and Difco (EDASB) peptones

Organism	Peptone	Number of bacteria per 0.02 ml of the following log ₁₀ dilution of medium				
		2	3	4	5	6
<i>E. coli</i> O111 (control)	Evans	CG	CG	IC	20-100	3
	EOASB	CG	CG	IC	20-100	4
	EDASB	CG	CG	IC	20-100	6
<i>E. coli</i> no. 34686 (demanding)	Evans	20-100	20-100	4	0	0
	EOASB	CG	CG	IC	20-100	3
	EDASB	CG	IC	20-100	20-100	6
<i>E. coli</i> no. 19616 (demanding)	Evans	20-100	11	3	0	0
	EOASB	CG	IC	IC	20-100	1
	EDASB	CG	IC	20-100	20-100	7
<i>E. coli</i> no. 26284 (demanding)	Evans	3	0	0	0	0
	EOASB	CG	IC	20-100	9	1
	EDASB	CG	IC	20-100	20-100	3

CG = Confluent growth; IC = innumerable discrete colonies; ... = not done.

of the numbers that they gave in OP, and the yield in 30 per cent. OP was probably less than in 100 per cent. OP.

Effect of spore-bearing bacilli

Two experiments were done with modified EP. In the first the yield in EP was contrasted with that in OP and with that in EP modified by the growth of spore-bearing bacilli obtained from OP (table II). In the second experiment, growth in EP was contrasted with that in EP modified either by the spore-bearing bacilli from OP or from Difco peptone (table III).

The control organisms grew equally well in all these media. In EP, growth of the demanding strains was much improved by previous growth of spore-bearing bacilli in the medium.

SUMMARY

Seven strains of bacteria were isolated in two laboratories in North East England on a medium containing Oxoid Peptone L37, but not on other media. Four of them produced relatively few viable cells in Evans Peptone water. However, this peptone could be modified by growing in it aerobic spore-bearing bacilli isolated from either Oxoid Peptone or Difco Proteose Peptone no. 3. After preconditioning Evans Peptone yielded as many cells as Oxoid Peptone.

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