

Variations in the Metal Content of Some Commercial Media and Their Effect on Microbial Growth

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The cation content in commercial media obtained from two manufacturers showed considerable variation. Even different batches of the same make were found to be inconsistent in the content of metal ions. With cultures of *Cytophaga* sp. and *Yersinia pseudotuberculosis* in base media, growth stimulation was dependent on additions of certain commercial media. It could be demonstrated that this stimulation was derived solely from increased Mg^{2+} concentration in the media.

Existing knowledge concerning the cation composition of bacteria rests mainly upon measurements derived from analyses of a limited number of different species (e.g., 2, 4-7). An understanding of bacterial cation composition is of importance in devising nutrient media which are optimal not only for growth but also for the formation of endo- and exocellular products. In many instances, a certain complex medium gives satisfactory results while others fail. Similarly, a medium from one commercial firm is satisfactory, whereas purportedly identical media from other firms are less favorable or are completely unsuitable. Only a few descriptions of common commercial media include the quantitative contents of cations. Kempner (3) has published a thorough investigation of the ion content of nutrient broth from Difco Laboratories, Detroit, Mich.

Our interest in the cation contents of commercial media originated from an investigation in which it was found that different complex media resulted in the production of different quantities of a cholinesterase-liberating factor from a *Cytophaga* sp. It could be demonstrated that the production of this factor was dependent on the magnesium content of the medium (1).

MATERIALS AND METHODS

Procedures for chemical analysis. For analysis of cation content, the different media (Oxoid Ltd., London, and Difco, Detroit, Mich.) were dissolved in deionized water (10%, w/w) and analyzed with the aid of an atomic absorption spectrophotometer (EEL model 140). Mean values were calculated from the determinations of a minimum of three and a maximum

of six different solutions of the same batch of medium; the determinations were performed by three different people. The media were taken from fresh unopened bottles and dissolved without drying.

Submerged culture procedures. The organisms *Cytophaga* sp. (NCMB 1314) and *Yersinia pseudotuberculosis* were maintained on agar slants. The basal medium for *Cytophaga* contained tryptone T (Oxoid Ltd. London), 5 g/liter; KCl, 0.1 g/liter; deionized water, 1,000 ml.

The basal medium for *Y. pseudotuberculosis* contained $Na_2HPO_4 \cdot 12H_2O$, 9 g/liter; KH_2PO_4 , 4 g/liter; $(NH_4)_2SO_4$, 1 g/liter; sodium citrate $\cdot 2H_2O$, 0.5 g/liter; glucose, 4 g/liter; $Na_2S_2O_5$, 0.1 g/liter; thiamine, 0.28 mg/liter; calcium pantothenate, 0.5 mg/liter; L-cystine, 50 mg/liter; glutamic acid, 25 mg/liter; nicotinamide, 0.6 mg/liter; deionized water, 1,000 ml.

When used for growth experiments, these media were supplemented with either 0.1 mg of magnesium ion per liter or an amount of the selected commercial media which contained the same amount of magnesium and was adjusted to pH 7.2 with 0.1 M NaOH. Subcultures were grown overnight on a rotary shaker in the appropriate basal medium. The experimental cultures (50 ml in 250-ml Erlenmeyer flask) were inoculated with 2 ml of subculture and incubated for 3 days on a rotary shaker at 24 C. Growth was measured daily in terms of extinction at 650 nm in a Hitachi spectrophotometer (model 101).

RESULTS AND DISCUSSION

Although microbial media from different manufacturers often carry the same name and thus give the impression of being identical, they may differ in important details; e.g., they do not contain the same amount of measured cations (Table 1). Thus one of the more commonly used media, yeast extract, contains about four times more

TABLE 1. Contents of six cations in one batch each of different commercial media^a

Medium	Micrograms per g of medium					
	Mg ²⁺	Fe ²⁺	Ca ²⁺	Zn ²⁺	Cu ²⁺	Mn ²⁺
Tryptone (Difco B 123)	347	45	19	20	4	3
Tryptone (Oxoid L 42)	297	47	546	43	2	3
Tryptone T (Oxoid L 43)	10	29	22	18	2	2
Casitone (Difco B 259)	407	21	102	16	4	3
Casamino Acids (Difco B 230)	18	4	17	5	6	<1
Casein hydrolysate (Oxoid L 41)	32	58	14	18	2	<1
Proteose peptone (Difco B 120)	380	8	83	8	5	2
Proteose peptone (Oxoid L 46)	805	26	134	47	4	1
Tryptose broth (Difco B 62)	285	23	238	33	3	4
Tryptose (Oxoid L 47)	925	69	52	48	9	2
Peptone (Difco B 118)	448	16	25	9	2	<1
Bacteriological peptone (Oxoid L 37)	816	38	249	21	7	11
Nutrient broth (Difco B 3)	296	16	18	6	3	2
Nutrient broth (Oxoid CM 1)	217	21	44	14	8	2
Yeast extract (Difco B 127)	2,150	57	22	126	11	3
Yeast extract (Oxoid L 21)	513	158	4	197	18	1

Values are means of three to six determinations.

TABLE 2. Contents of three cations in three different batches of some commercial media^a

Medium	Batch no.	Micrograms per g of medium		
		Mg ²⁺	Zn ²⁺	Ca ²⁺
Yeast extract (Difco B 127)	8034	2,150	126	22
	1261	2,100	120	75
	2302	1,850	135	40
Yeast extract (Oxoid L 21)	4773	513	197	4
	4746	443	143	2
	2880	315	190	1
Nutrient broth (Oxoid CM 1)	7005	217	14	44
	5874	100	11	16
	7860	160	14	47
Tryptone T (Oxoid L 43)	3233	10	18	22
	2366	23	27	50
	366	23	21	50

^a Values are means of three to six determinations.

magnesium in the Difco product as compared to the Oxoid preparation. On the other hand, the Oxoid product contains three times more iron than the Difco medium.

When cation limitation is important, Casamino Acids (Difco) or tryptone T (Oxoid) is generally accepted as a suitable complex medium to be used with or without additions of vitamins or other growth stimulants.

Another interesting aspect, from the consumer's viewpoint, is whether one batch of a certain commercial product is comparable to another batch of the same product. From the limited number of batches tested by us, it appears that one batch of a certain make is not necessarily comparable to other batches of the same make (Table 2).

The effect of cation concentration on growth is shown in experiments with *Cytophaga* sp. (NCMB 1314) and *Y. pseudotuberculosis* grown in their respective basal media. When these media were supplemented with either 0.1 mg of Mg²⁺/liter or with an amount of certain selected commercial media containing a corresponding quantity of Mg²⁺, growth resulted in about the same comparatively low optical densities (Fig. 1). After the experiments were completed, the amount of magnesium was measured in the filtered, spent culture fluid and found to be undetectable. It appears, therefore, that Mg²⁺ is the limiting nutrient in the basal media.

In a further experiment, a supplement of 3 mg of Mg²⁺/liter to the basal media gave a greater increase in growth than that obtained with only the basal media, which suggests that nutrients in the basal media other than Mg²⁺ had not been exhausted in the previous experiments. The contribution to growth provided by additions of commercial media consists, in our experiments, of nothing more than an enrichment of Mg²⁺.

The underlying purpose in publishing these data is to emphasize the necessity of determining and adjusting the cation content of commercial media used in cultivation. Thus, one factor which might possibly contribute to undesirable product formation would be eliminated.

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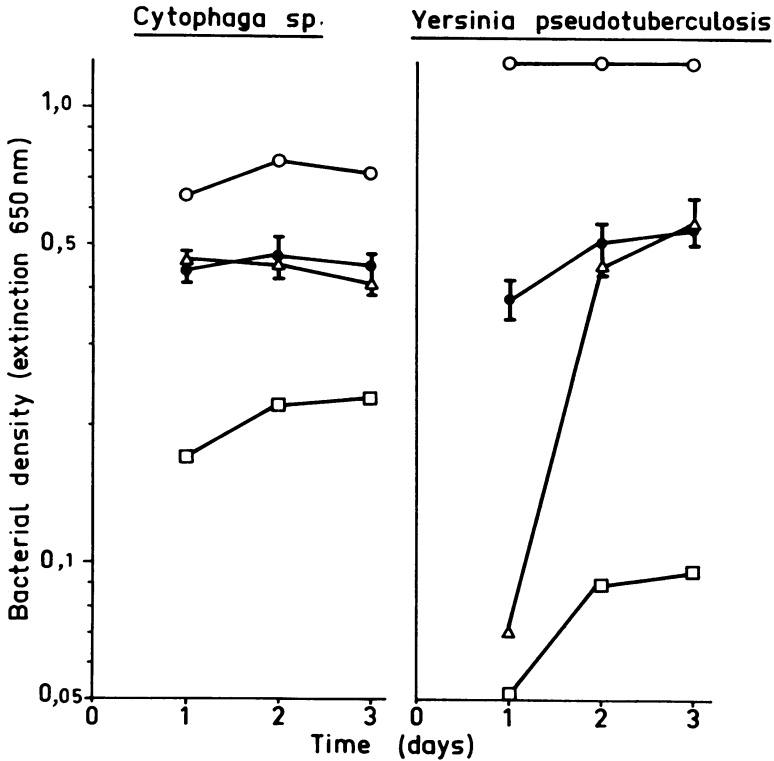


FIG. 1. Growth of *Cytophaga* sp. NCMB 1314 and *Yersinia pseudotuberculosis* in different media. Symbols: □, growth in the respective basal media; △, growth in the basal media supplemented with 0.1 mg of Mg²⁺ per liter; I, growth in the basal media supplemented with either 10 g of Tryptone T (Oxoid) per liter, 0.4 g of nutrient broth (Oxoid) per liter, 0.17 g of yeast extract per liter (Oxoid), 0.33 g of tryptone (Oxoid) per liter, or 3.3 g of casein hydrolysate per liter (Oxoid); the magnesium content of the added commercial media corresponds roughly to 0.1 mg of Mg²⁺ per liter; ○, growth in the basal media supplemented with 3 mg of Mg²⁺ per liter.

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