less than 2. An added factor is the uniformity with which these animals respond to low dilutions of MEF1 virus as compared with the irregularity of reactors to those of the Lansing strain. This offers an advantage for certain experiments with rodent-adapted poliomyelitis viruses.

VITAMIN REQUIREMENTS OF BACILLUS COAGULANS

ROBERT C. CLEVERDON, MICHAEL J. PELCZAR, JR., AND RAYMOND N. DOETSCH

Department of Bacteriology, University of Maryland, College Park, Maryland

Received for publication April 15, 1949

During studies on some thermophilic members of the genus *Bacillus*, it was found that *Bacillus coagulans* NRS 27, a eurithermophile, could be serially cultivated at 37 C and at 55 C in a medium of the following composition: vitamin- and salt-free casein hydrolyzate, 1 per cent; L-cystine, 0.001 per cent; DL-tryptophan, 0.01 per cent; NaCl, 0.1 per cent; D-glucose, 0.5 per cent; K_2HPO_4, 0.5 per cent; thiamine, 1 μg per ml; niacin, 1 μg per ml; biotin, 0.04 μg per ml.

TABLE 1

Growth of B. coagulans NRS 27, at 37 C and 55 C, in casein hydrolyzate medium, with combinations of niacin, thiamine, and biotin

VITAMIN CONCENTRATION, μg/ML			GROWTH RESPONSE	
Niacin	Thiamine	Biotin	37 C	55 C
1	1	0.04	66*	53*
1	1	0.004	62	46
1	1	0.00004	41	25
1	1	0.0000004	10	0
1	1	0	0	0
1	0.01	0.04	0	0
1	0	0.04	0	0
0.1	1	0.04	66	53
0.01	1	0.04	62	51
0	1	0.04	0	0
0	0	0	0	0

* Average of replicate serial 48-hour transfers. Figures represent turbidity as measured with Fisher electrophotometer, 100 minus reading of light transmittance, using 425 B filter.

Table 1 shows the growth response of this organism to the three vitamins in the medium described above.

The results indicate that this organism requires a relatively high concentration of the three vitamins reported and each of these vitamins is essential

for growth. Growth in the casein hydrolyzate medium with niacin, thiamine, and biotin is less prompt and less abundant than in trypticase soy broth; but the spore yield, which is consistently low for this organism, is equal in both media. At the lower temperature, more spores are formed; the cells are wider and shorter, and stain more evenly with Giemsa stain.

FACTORS AFFECTING THE ELABORATION OF PIGMENT AND POLYSACCHARIDE BY SERRATIA MARCESCENS

MARY I. BUNTING, CARL F. ROBINOW, AND HENRY BUNTING

Osborn Botanical Laboratory, Yale University, and Department of Pathology, Yale School of Medicine, New Haven, Connecticut

Received for publication April 18, 1949

The purpose of this note is to confirm and extend the interesting observations recently reported by J. P. Duguid (J. Path. Bact., **60**, 265, 1948). He has demonstrated very clearly that capsule formation in *Aerobacter aerogenes* is encouraged whenever growth is checked by limiting quantities of a nitrogen source or phosphate, provided an excess of carbohydrate is available. Under such conditions capsules are large and the cells, depleted of their cytoplasmic ribonucleotide, reveal their nuclei very conspicuously when stained with basic dyes.

We have made essentially the same observations with *Serratia marcescens*, using the Hy strain, which has typical coccoid cells. The results were particularly clear when the cultures were abundantly aerated on a shaker. In a simple medium with 0.5 per cent peptone, 1.0 per cent glycerol, and 0.1 per cent K_2HPO_4, or Na_2HPO_4, there was little evidence of capsule formation, the cells stained uniformly with basic dyes, and pigmentation was scarcely evident. However, when the peptone was reduced to 0.1 per cent, or when the added phosphate was omitted from the medium, the results after 48 hours at 30 C were very different. A great deal of capsular material was elaborated; simple basic dyes such as methylene blue revealed deeply staining nuclear bodies in cytoplasm that stained very faintly indeed; pigmentation was intense. The identity of the nuclei was established with Giemsa and Feulgen preparations.

Moreover, when smears of cells grown for 2 or more days on the shaker in peptone glycerol medium lacking added phosphate were treated with periodic acid and exposed to Schiff's reagent (McManus: Nature, **158**, 202, 1946), the cytoplasmic material surrounding the nuclei was stained an intense pink. The nuclei appeared as unstained zones in the cells. The cells were not stained by Schiff's reagent when the periodate treatment was omitted. This recolorization of Schiff's reagent following treatment with periodic acid has been ascribed to the presence of polysaccharide material (Hotchkiss: Arch. Biochem., **16**, 131, 1948). Young cells from the same cultures did not give a positive periodate-Schiff's reaction; these cells were not highly pigmented and gave little evidence of