JOURNAL OF BACTERIOLOGY, Oct. 1967, p. 1260-1261

Vol. 94, No. 4
Printed in U.S.A.

Defined Medium Allowing Maximal Growth of *Rhodomicrobium vannielii*

WILLIAM C. TRENTINI

Department of Biology, State University of New York, Buffalo, New York 14214

Received for publication 28 June 1967

E. Duchow and H. C. Douglas (J. Bacteriol. **58**:409, 1949) found *Rhodomicrobium vannielii* required no added growth factors. A basal salts solution, an organic hydrogen donor, carbon dioxide furnished as bicarbonate, and light were necessary and sufficient for slow, sustained growth. Yeast autolysate was found to stimulate growth. This stimulation was not replaced by mixtures of either or both B vitamins and amino acids.

Investigations reported since that time have used media containing yeast extract. Since complex growth media are unsuitable for many types of studies, it has become necessary to establish a defined growth environment that would permit the degree of enhanced growth obtained in yeast extract.

The isolation and cultivation of *R. vannielli* strain C-2859 have been described by W. C. Trentini and M. P. Starr (J. Bacteriol. **93**:1699, 1967). $MgSO_4 \cdot 7H_2O$ is now used at 0.01%. The light intensity used, ca. 700 ft-c, was previously shown to be saturating. Cells were grown in roll tubes with recessed, butyl rubber stoppers (R. E. Hungate et al., J. Bacteriol. **91**:908, 1966). The stoppered tubes were autoclaved containing 17 ml of basal salts medium. After being filter-sterilized, the lactate, bicarbonate, and sulfide, plus appropriate supplements, were each added in a volume of 0.2 ml. All tubes were inoculated with 1 ml of cells grown in minimal medium (YE^-). Rates were followed through transfer in supplemented media. Supplement additions and sample withdrawal were accomplished with syringe and needle through the recessed stopper. Pressure differentials were balanced with sterile N_2. Growth was measured as turbidity (optical density) at 1,000 mμ in yield studies and, subsequent to the findings below, at 755 mμ when doubling times were determined. Reliable growth rates were obtained when a wavelength of 755 mμ was used instead of 680 or 1,000 mμ, by masking the phototube of the spectrophotometer and by inverting the cuvette several times immediately prior to reading the sample.

The effect on growth when various supplements were added to YE^- was screened by estimating the yield of cultures after 48 hr of incubation. These yield studies showed that: (i) yeast extract strongly stimulated growth; (ii) a mixture of 10 vitamins (V_A), including those used by Duchow and Douglas, resulted in yields that approached the level achieved in 0.1% yeast extract (YE^+); (iii) several combinations of 20 L-amino acids did not stimulate growth when employed at the approximate concentration present in 0.1% acid-hydrolyzed, vitamin-free Casamino Acids (CA). The use of CA as a supplement gave erratic yields, which, when averaged, were slightly greater than YE^-; and (iv), when the 10 vitamins of V_A were subsequently divided into three solutions (V_B, V_C, and V_D), only V_C resulted in the response level of YE^+. When the components of V_C were tested separately, only riboflavine (RIB) at 1 μg/ml was shown to be the source of enhanced growth.

TABLE 1. *Effect of various supplements to YE^- on the doubling time of Rhodomicrobium vannielii*

Supplement[a]	Doubling time (hr)[b]
CA, V_A	6.1
V_A	5.7
V_C	5.2
RIB	5.2
ASP, V_A	5.1
ASP, V_C	5.2
ASP, RIB	5.3
CA	7.5
YE^+	5.8
YE^-	10.5

[a] ASP = aspartic acid, 0.1 mg/ml. For other abbreviations, see text.

[b] Estimated from the change in turbidity (optical density) read at 755 mμ. Each value is an average of two or more experiments.

The degrees of stimulation achieved by adding the various supplements to YE^- were more adequately measured when the growth rates of the

various cultures were considered (Table 1). If the doubling time is taken as an accurate criterion of stimulation, then addition of riboflavine alone allows an average doubling time (5.2 hr) that is within the range (5 to 6 hr) of any single, multiple, or complex supplement tested.

Thus, in strain C-2859 a single vitamin, riboflavine, when added to the minimal medium described above, fully substitutes for yeast extract as a growth stimulant.

This investigation was supported by a Public Health Service Fellowship 7-F1-GM-33, 336-01A1 from the National Institute of General Medical Sciences.