JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1985, p. 303–305
0095-1137/85/080303-03$02.00/0

Vol. 22, No. 2

NOTES

Chemically Defined Medium for Oral Microorganisms

SIGMUND S. SOCRANSKY, JoANN L. DZINK, AND CLAIRE M. SMITH*

Forsyth Dental Center, Boston, Massachusetts 02115

Received 14 January 1985/Accepted 17 April 1985

We formulated a chemically defined medium consisting of 14 inorganic salts, 23 amino acids, 23 vitamins and other factors, seven purines and pyrimidines, and glucose which would successfully support the growth of a wide variety of oral microorganisms. Of 204 oral isolates representing 20 genera and 60 species, 197 maintained viability through six serial transfers.

The development of a synthetic medium which would permit the growth of a wide spectrum of bacterial species would be useful in studies of microbial physiology and antigenicity. Chemically defined media have been developed for the growth of strains of oral streptococci (1, 6) and oral *Actinomyces* spp. (2). However, these media were tested for the growth of strains within a single genus. The present investigation describes a "general-purpose" chemically defined medium which supports the growth of oral strains representing a wider variety of species and genera.

The composition of the chemically defined medium is presented in Table 1. The ingredients were divided into four blocks: inorganic salts, amino acids, purines and pyrimidines, and vitamins and other factors. The inorganic salts were weighed out and crushed in a mortar and pestle to evenly disperse the salts throughout the mixture. The amino acid block was prepared in the same fashion. Similarly, the vitamins and other factors formed a third mixture. The purines and pyrimidines were made up in 0.1 M KOH as a 20-fold-concentration solution. Each block was stored separately: inorganic salts and amino acids at room temperature, purines and pyrimidines at 4°C, and vitamins and other factors (dessicated) at 4°C. To make up the medium, 2.21 g of the inorganic salt block, 2 g of the amino acid block, 84 mg of the vitamins and other factors block, and 2 g of glucose were weighed out and dissolved, in that order, in 1 liter of distilled water. Five milliliters of the purine and pyrimidine stock solution per 100 ml of final medium was then added and mixed. Sodium bicarbonate and L-cysteine were dissolved in the medium immediately before use. The pH was adjusted to 7.2 with 1.0 M KOH, and the medium was filter sterilized by passage through a 0.20-μm Nalgene filter. A 100×-concentrated hemin stock solution (50 mg of hemin in 100 ml of 0.1 M K_2HPO_4) was sterilized by autoclaving, and 1 ml was aseptically added per 100 ml of final medium. The medium was aseptically dispensed into the wells of microtiter plates by using a MIC 2000 dispenser (Dynatech Laboratories, Alexandria, Va.).

A total of 31 reference strains were obtained from the American Type Culture Collection, and 29 strains were obtained from the Forsyth Dental Center, whereas 142 fresh isolates were derived from samples of subgingival plaque

TABLE 1. Composition of chemically defined medium

Block	Amt (mg/liter)
Inorganic salts	
$ZnSO_4$	0.4
KI	0.1
$CuSO_4$	0.04
Boric acid	0.5
$MgSO_4 \cdot 7H_2O$	700.0
$FeSO_4 \cdot 7H_2O$	5.0
$MnSO_4$	5.0
NaCl	100.0
K_2HPO_4	200.0
$CaCl_2$	100.0
KH_2PO_4	1,000.0
Sodium molybdate	0.5
KNO_3	100.0
Amino acid[a]	
L-glutamic acid	200.0
DL-alanine	100.0
L-Leucine	100.0
Glycine	100.0
L-Valine	100.0
L-Tryptophan	100.0
L-Threonine	100.0
L-Serine	100.0
L-Lysine hydrochloride	100.0
L-Arginine	100.0
L-Histidine	100.0
L-Glutamine	100.0
L-Asparagine	100.0
L-Methionine	100.0
L-Isoleucine	100.0
L-Proline	100.0
L-Aspartic acid	100.0
L-Phenylalanine	100.0
L-Tyrosine	20.0
L-Cystine	5.0
L-Ornithine hydrochloride	20.0
L-Hydroxyproline	20.0
Vitamins and other factors[a]	
Spermine tetrahydrochloride	1.0
Spermidine trihydrochloride	1.0
Putrescine dihydrochloride	1.0
Beta-alanine	10.0
Pimelic acid	0.1

Continued

* Corresponding author.

TABLE 1—*Continued*

Block	Amt (mg/liter)
Choline chloride	50.0
DL-Mevalonic acid lactone	0.1
Pyridoxal	1.0
Pyridoxine hydrochloride	1.0
Pyridoxamine dihydrochloride	1.0
Nicotinic acid	1.0
Nicotinamide	1.0
p-Aminobenzoic acid	0.1
Folic acid	1.0
Calcium pantothenate	1.0
Riboflavin	1.0
Thiamine hydrochloride	1.0
myo-Inositol	10.0
D-Biotin	0.1
DL-6,8-Thioctic acid	0.1
Nicotinamide adenine dinucleotide	1.0
Vitamin B_{12}	0.01
Purines and pyrimidines[a]	
Adenine	10.0
Guanine	10.0
Cytosine	10.0
Thymine	10.0
Xanthine	10.0
Hypoxanthine	10.0
Uracil	10.0
Other	
$NaHCO_3$	1,000.0
L-Cysteine	500.0
Hemin	5.0
Glucose	2,000.0

[a] From Sigma Chemical Co., St. Louis, Mo.

(Table 2). Fresh isolates were identified as described by Tanner et al. (5) and Dzink et al. (3, 4). All cultures were maintained by weekly transfer on Trypticase soy agar supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) in an anaerobic atmosphere of 80% N_2–10%CO_2–10%H_2 at 35°C. Pure cultures were transferred from the blood agar plates and placed into 2 ml of Mycoplasma broth (BBL) supplemented with 5 μg of hemin per ml and 0.1% glucose. After 24 h of anaerobic growth, 0.05 ml of each culture was inoculated into the wells of microtiter plates containing 0.1 ml of the chemically defined medium. The microtiter plates were anaerobically incubated at 35°C for 48 h. Growth in each well (1.5 μl) was then serially transferred every 48 h for five additional passages in the defined medium by using a MIC 2000 inoculator. At each passage, growth was determined by measuring the optical density at 580 nm with an Artek Vertical Beam reader (Artek Systems Corp., Farmington, N.Y.). After the last transfer, 1.5 μl of each culture was replicated onto the surface of a blood agar plate by using the MIC 2000 inoculator and incubated anaerobically for 48 h at 35°C. The blood plate was used to check the viability and purity of each culture.

Table 2 summarizes microbial growth in the chemically defined medium. Since reference cultures and fresh isolates of the same species gave similar levels of growth, their results were combined. Of 204 total strains, 7 (4%) failed to grow on the blood agar plate after the serial transfers. These included two strains of *Wolinella recta*, three strains of *Streptococcus intermedius*, and one strain each of *Fusobacterium nucleatum* and *Veillonella parvula*. Most micro-

TABLE 2. Growth of oral isolates in chemically defined medium

Organism	No. of isolates with final optical density[a] at 580 nm of:		
	<0.45	0.45–0.75	>0.75
Actinobacillus actinomycetemcomitans	0	8	0
Actinomyces israelii	0	2	2
Actinomyces naeslundii	0	1	3
Actinomyces odontolyticus	0	1	6
Actinomyces viscosus	0	0	6
Actinomyces meyerii	0	0	1
Actinomyces sp. (cluster groups 3 and 8)	0	0	5
Arachnia propionica	0	0	4
Bacterionema matruchotii	0	0	2
Bacteroides buccae	0	0	1
Bacteroides corporis	1	0	0
Bacteroides denticola	0	0	2
Bacteroides gingivalis	0	4	0
Bacteroides intermedius	0	0	2
Bacteroides oralis	0	0	1
Bifidobacterium dentium	0	0	2
Bifidobacterium infantis	0	0	2
Bifidobacterium sp.	0	2	3
Capnocytophaga gingivalis	0	2	2
Capnocytophaga ochracea	0	6	0
Capnocytophaga sputigena	0	3	0
Eubacterium aerofaciens	0	2	1
Eubacterium combesii	1	0	0
Eubacterium contortum	0	1	0
Eubacterium cylindroides	0	1	0
Eubacterium lentum	0	2	0
Eubacterium moniliforme	0	1	0
Eubacterium necrogenes	0	1	0
Eubacterium timidum	0	4	0
Eubacterium sp. (cluster group 33)	0	0	1
Fusobacterium nucleatum	1	0	2
Haemophilus aphrophilus	0	2	0
Lactobacillus acidophilus	0	0	1
Leptotrichia buccalis	0	0	1
Peptococcus prevotii	0	2	0
Peptostreptococcus anaerobius	0	0	3
Peptostreptococcus micros	0	2	0
Peptostreptococcus productus	0	1	0
Propionibacterium acnes	0	0	12
Propionibacterium avidum	0	0	1
Propionibacterium jensenii	0	0	1
Propionibacterium lymphophilum	0	0	1
Propionibacterium sp. (cluster groups 26 and 41)	0	3	2
Selenomonas aputigena	0	1	0
Staphylococcus capitus	0	1	0
Staphylococcus hominis	0	3	0
Staphylococcus xylosus	0	3	0
Streptococcus acidominimus	0	3	0
Streptococcus intermedius	0	0	11
Streptococcus milleri	0	0	4
Streptococcus mitis	0	1	0
Streptococcus morbillorum	0	15	0
Streptococcus mutans	0	0	16
Streptococcus salivarius	0	0	2
Streptococcus sanguis I	0	0	6
Streptococcus sanguis II	0	0	3
Streptococcus uberis	0	0	3
Veillonella parvula	0	1	0
Wolinella recta	0	0	0

[a] Standardized to a 1-cm light path.

organisms exhibited at least moderate growth through the six serial transfers and retained their viability, in spite of the purposefully small inoculum-to-medium ratio. The small-inoculum was used to minimize the carryover of nutrients from the initial Mycoplasma broth. The dilution factor through six serial transfers approximated 10^{-10}.

The medium has certain deficiencies for routine use. It must be freshly made and used immediately after preparation because it precipitates when stored in the cold or when frozen. The medium also cannot be autoclaved but must be sterilized by filtration. The medium supported the growth of strains of a wide variety of microorganisms tested in this investigation. Caution must be employed in extrapolating these results to species where limited numbers of strains were tested. The medium, on the other hand, can be readily altered to fit the needs of specific species or the investigation. Finally, the medium has been used successfully to grow cultures in multiliter amounts. For example, strains of *Streptococcus mutans* provided cell yields of 3 to 4 g (wet weight) per liter in this medium when higher levels of glucose were provided.

This work was supported by Public Health Service grants DE-04881 and DE-02847 from the National Institute of Dental Research.

LITERATURE CITED

1. **Carlsson, J.** 1970. Chemically defined medium for growth of *Streptococcus sanguis*. Caries Res. **4**:297–304.
2. **Christie, A. O., and J. W. Porteous.** 1962. The cultivation of a single strain of *Actinomyces israelii* in a simplified and chemically defined medium. J. Gen. Microbiol. **28**:443–454.
3. **Dzink, J. L., C. M. Smith, and S. S. Socransky.** 1984. Semiautomated technique for identification of subgingival isolates. J. Clin. Microbiol. **19**:599–605.
4. **Dzink, J. L., S. S. Socransky, J. L. Ebersole, and D. E. Frey.** 1983. ELISA and conventional techniques for identification of black-pigmented *Bacteroides* isolated from periodontal pockets. J. Periodontal Res. **18**:369–374.
5. **Tanner, A. C. R., C. Haffer, G. T. Bratthall, R. A. Visconti, and S. S. Socransky.** 1979. A study of the bacteria associated with advancing periodonitis in man. J. Clin. Periodontol. **6**:278–307.
6. **Terleckyj, B., N. P. Willet, and G. D. Shockman.** 1975. Growth of several cariogenic strains of oral streptococci in a chemically defined medium. Infect. Immun. **11**:649–655.