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Simplified Method for the Preparation of Silica Gel Media

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A simplified and reliable method for the preparation of a variety of silica gel media is described. The growth of common microorganisms on these media was examined.

Due to the recent shortage of agar the use of silica gel plates for isolation and propagation of microorganisms has become a more attractive prospect. This shortage has caused the price of agar, if available, to increase from about 0.5 cents/plate to 1.1 cents/plate.

Although the use of silicic acid as a solidifying agent has been recognized since reported by Winogradsky (4), the preparation of silica gel plates has been enshrouded with problems and uncertainties. Silica gel plates prepared by the method of Funk and Krulwich (1) contain water on the surface due to syneresis and must be incubated in a high humidity atmosphere to prevent drying of the gels. Additionally, the plates contain a high concentration of phosphates (33.4 g/liter). The procedure of Sommers and Harris (3), although using ion exchange resin to lower the pH of the silicate solution (thereby avoiding high phosphate concentration), leads to difficulties in that gelation occurs at pH values above 7.0. The technique of Roslycky (2), although leading to high quality gels, necessitates the sterilization and subsequent mixing of two separate components of the medium each time plates are prepared. Additionally, the final pH of plates prepared by this method (7.2 ± 0.2) cannot be controlled to a specific value. Finally, the above techniques of silica gel preparation have not been examined as to their use in preparation of specific defined and complex media for use with a variety of common microorganisms.

The method outlined below has been used for the routine preparation of silica gel plates of various media, both defined and complex.

(i) Dissolve 13.4 g of $Na_2SiO_3 \cdot 9H_2O$ in 100 ml of distilled water. Mineral salts may be added at this time if not adversely affected by high pH.

(ii) Adjust the sodium silicate solution to pH 10 by the addition, with constant stirring, of cation exchange resin (Bio-Rad Laboratories, AG 50 WX-8).

(iii) Mix for 30 min and re-adjust to pH 10 by the addition of further resin.

(iv) Remove the resin by filtration with Whatman no. 1 filter paper.

(v) Dissolve nutrients (e.g., glucose, yeast extract, or mineral salts) in the silicate solution.

(vi) Sterilize the solution by passage through a 0.45-μm membrane filter preceded by a prefilter.

(vii) Adjust the solution to pH 7.0 with sterile 5 N phosphoric acid. This adjustment usually requires the addition of a maximum of 1.6 g of phosphate per liter.

(viii) Pour the medium into sterile plates and allow to gel.

The process of gelation takes 35 to 40 min at room temperature, but at 55 C this time is reduced to 15 to 20 min. One and one-half to 2 h after plating, the plates obtained via this procedure have a hardness approximating that of 2.0% agar and can be streaked with an inoculating needle. Using this procedure no water droplets form on the plate surface due to syneresis during either the initial gelation of the plates or during a subsequent 2- to 3-week incubation period. The mechanisms involved in gelation and syneresis are still not fully understood. Therefore, the explanation of why this procedure is relatively trouble free is not known, although presumably the pH and the low temperatures maintained by avoiding autoclaving are involved. Whereas the plates exhibit some shrinkage with prolonged incubation, drying or cracking is not a problem and incubation at high humidity is not necessary. The degree of clarity of the plates obtained with this technique is greater than that obtained with purified agar. This is advantageous in the detection of those microorganisms forming small and/or nonpigmented colonies. The cost of silica gel plates prepared by this procedure is about 1.0 cent/plate using technical grade sodium silicate and 2.3 cents/plate using certified grade sodium silicate, which makes it economically attrac-

TABLE 1. *Growth of common microorganisms on silica gel media*

Organisms	Colony diam after 24 h (mm)		
	Glucose-salts silica gel	Plate count silica gel	Nutrient silica gel
Rhizopus oligospora	25	30	35
Aspergillus niger	20	25	25
Saccharomyces cerevisiae	5	5	4
Pseudomonas fluorescens	15	7	6
Pseudomonas aeruginosa	15	20	10
Serratia marcescens	5	4	10
Bacillus subtilis	4	6	5
Escherichia coli	6	5	5
Staphylococcus aureus	5	5	8
Streptococcus fecalis	2	5	6
Proteus mirabilis	6	7	6

tive. Furthermore, silica gel provides a plate of known composition, whereas agar contains undefined components.

Variation of the initial pH was attempted in an effort to remove additional sodium from the silicate solution, but any reaction below pH 10 led to an increase in gelation time, reduced hardness, or syneresis.

The versatility of this technique was examined by testing a variety of common microorganisms for growth on the following silica gel plates containing various nutrients: (i) glucose-salts silica gel: glucose, 1.0 g; KH_2PO_4, 0.185 g; Na_2HPO_4, 0.125 g; $(NH_4)_2SO_4$, 0.20 g; $MgSO_4 \cdot 7H_2O$, 0.04 g; distilled water, 100 ml; (ii) plate count silica gel: tryptone, 0.5 g; yeast extract, 0.25 g; glucose, 0.1 g; distilled water, 100 ml; (iii) nutrient silica gel: beef extract, 0.3 g; peptone, 0.5 g; distilled water, 100 ml.

All microorganisms tested exhibited growth within 24 h on the media utilized (Table 1). These results indicate that silica gel plates containing appropriate nutrients could be used for the routine isolation and/or propagation of a wide variety of microorganisms.

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