THE USE OF SILICA GEL FOR THE ENUMERATION AND ISOLATION OF *AZOTOBACTER* IN SOIL

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While Winogradsky (1925 and 1926) used silica gel for the isolation of *Azotobacter*, Ashby (1907), Martin (1940) and Jensen (1940) used agar as the solidifying agent either for the plate count of *Azotobacter* or for the isolation of the same. In view of the cumbersome procedure involved in the preparation and sterilization of silica gel the use of agar was more popular. However, the agar that is usually available in bacteriological laboratories contains small amounts of nitrogen and as such even organisms, that do not fix nitrogen, may grow and form colonies. In attempts to enumerate *Azotobacter* from soil at I.A.R.I. different agar media Jensen (1940), Ashby (1907) and Martin (1940) were tried and it was observed that besides *Azotobacter*, colonies of several other organisms particularly of actinomycetes appeared on the agar plates.

Actinomycetes most frequently occurring on the agar plates was plated on plain agar (containing 0·3% N*) and it was found to be able to grow well on these plates. It did not fix any nitrogen in Jensen's liquid medium for *Azotobacter*.

Trials were therefore made to simplify the silica gel method so that it may be used for the enumeration of *Azotobacter* in the soil.

Method of preparation of silica gel.—Recently there has been a trend towards improving the method of preparation of silica gel. Taylor (1950) described a greatly improved method of the preparation of silica gel and Pramer (1957) has described a modified method. These methods require the use of an activated resin column and repeated adjustments of pH and concentrations, and are too cumbersome to be widely adopted. An attempt was made in this laboratory to simplify the procedure. Sterilisation by

* According to the specification laid by the Society of American Bacteriologists (*Manual of Microbiological Methods*,1957) the nitrogen in Bacteriological Grade Agar should not be more than 0·32%.

autoclaving was replaced by sterilisation with ultra-violet rays to avoid the formation of blow holes. The technique followed is as follows:—

A 20% solution of water-soluble crystalline sodium silicate is prepared by slow heating. This alkaline solution is cooled and filtered. A known volume of sodium silicate solution is then poured into a conical flask containing an equal volume of 33% HCl and the mixture thus prepared in small lots is then poured into sterilised Petri-dishes (12–15 c.c. for 9 cm. diameter dishes) and left undisturbed overnight for gelation to take place. The gel formed is transparent. These plates are then washed free from acid in running tap-water for about 48 hours. The pH of the draining water is tested by methyl red. The gel is freed from chlorides by keeping the plates immersed in distilled water which is changed after 5–6 hours till the washing is free from chlorides as tested by $AgNO_3$. This can be done usually in two to three washings. The pH of the gel is neutral at this stage. The plates are kept inverted on filter-paper sheets to allow the water to drain off. These plates are sterilized by exposure to ultra-violet light of 2437 Å wavelength at a distance of 70 cm. for $\frac{1}{2}$ hour in the plating room.

For use of these plates in enumeration of *Azotobacter*, 2 c.c. of four times concentrated sterilised nutrient mixture (including $CaCO_3$) as contained in Jensen's agar medium are poured on the sterilised and chloride-free silica gel plates and water is allowed to evaporate by exposure of the plates to dust-free air in the plating room. Thereafter an exposure of an hour's duration to ultra-violet light in the closed plating room suffices to sterilise the plates. Scrapings from the plate were smeared on slides and stained with Rose Bengal and examined under the microscope. No organisms could be detected. Nutrient broth inoculated with these scrapings also failed to show any growth.

These plates are now ready to receive soil suspension inoculum of convenient dilutions. After pouring the soil suspension on the plates they are dried by exposure to dust-free air in a plating room sterilised with ultra-violet rays. Control plates (not inoculated with soil suspension) similarly exposed failed to show any growth in peptone broth or in Jensen's agar slants used for maintaining nitrogen fixing organisms.

The comparative development of *Azotobacter* and actinomycete colonies on Jensen's agar medium and silica gel medium (prepared as described here) from mixed cultures of these was ascertained in repeated experiments and it was seen that the silica gel media largely favoured the appearance of *Azotobacter* almost to the exclusion of others; development of other organisms was more in the agar medium in which *Azotobacter* was suppressed to a great extent (*see* Table I).

TABLE I

Comparative development of Azotobacter *and actinomycete colonies from mixed inocula on silica gel and Jensen's agar*

	Number of colonies (Average of four plates)	
	Azotobacter	Actinomycetes
Silica gel ..	11·5	None
Jensen's agar ..	0·5	30

The relative usefulness of the agar and silica gel plates for the enumeration of *Azotobacter* in soil was further tested by plating soil suspension from the same set of samples from a bacterization experiment with *Azotobacter* and phosphobacterin with wheat crop. As in other instances, no colonies, other than those of *Azotobacter*, appeared on the silica gel plates whereas the Jensen's agar plates showed colonies of other organisms as well as those of *Azotobacter*. The counts obtained are tabulated in Table II.

TABLE II

Count of Azotobacter *on silica gel and Jensen's agar medium from soil samples from pot culture experiment on wheat*

(Dilution 1 : 100)

Treatment	Silica gel *Azotobacter* (average of four plates)	Jensen's agar (Average of four plates)	
		Azotobacter	Other organisms
1. Control ..	82·25	11·25	190·25
2. Phosphobacterin ..	129·25	10·25	211·75
3. *Azotobacter* ..	125·75	6·5	292·75
4. *Azotobacter* + Phosphobacterin	92·00	10·5	224·00
5. F.Y.M. ..	87·00	8·0	150·25
6. F.Y.M. + Phosphobacterin	116·00	10·25	211·75
7. F.Y.M. + *Azotobacter* ..	107·50	4·25	235·50
8. F.Y.M. + Superphosphate + *Azotobacter* + Phosphobacterin	161·50	15·75	214·00

The counts obtained on agar plates were too low to be relied upon as the actinomycetes seriously hampered the correct enumeration of *Azotobacter*. The counts of *Azotobacter* obtained on silica gel plates were higher.

Pure cultures of *Azotobacter* were obtained by three successive platings on silica gel. The difficulty that is usually experienced in eliminating a closely associated rod form contaminant when plated on Jensen's agar or other medium has been easily overcome by employing silica gel.

SUMMARY

A simplified method for the preparation of silica gel is described. The gel has been found to be useful in enumerating *Azotobacter* population in the soil as only *Azotobacter* colonies appeared in the plates containing this gel. It is also possible to obtain pure cultures of *Azotobacter* by employing this technique.

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