Short Communication

Membrane Filter Method for Isolation and Characterization of Thermophilic Bacteria

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We have reported the isolation of various thermophilic bacteria from extreme environments such as hot springs¹⁾ and hot compost samples.²⁾ During growth experiments for the isolated thermophiles we find difficulties in growing them on agar plates at high temperature about 70°C. It was often encountered that even dense cell suspension samples from their log phase growth cultures formed no colonies on agar plates, or if any, much fewer colonies than expected from total cell number. The troubles are likely due to unusual physical conditions on agar plates at high temperature that is, for example, quick evaporation of moisture which is unfavorable for thermophiles to grow and form colonies. Colony

forming efficiency can be improved by several methods such as water vapor saturation,³⁾ wrapping agar plate with Salan Wrap,⁴⁾ adding soluble starch or other substrate to medium⁵⁾ or spreading 0.5 ml, instead of 0.1 ml cell suspension on agar plates. These procedures, however, are not fully satisfactory.

In order to characterize biochemical activities of thermophilic microorganisms and to develop their genetics, it is essential to establish a procedure for good colony forming efficiency to get reliable viable count and get them purified. In this paper we report that the above troubles can easily be overcome by using agar plate on which membrane filter is placed.

The medium composed of 0.8% pepton, 0.4% yeast extract, 0.2% NaCl (pH is adjusted to 7.2 before autoclaving)⁶⁾ is used for growing thermophiles, *Thermus flavus* AT62, *Thermus thermophilus* HB8 and the medium described by Brock⁴⁾ is used for growing *Thermus aquaticus* YT1, respectively. For solid medium 3% agar was added. One tenth ml of cell suspension appropriately diluted by 0.85% NaCl solution was spread on agar plates with or without membrane filters (0.45 $\mu\text{m}\phi$ from Toyo Roshi Co. Ltd.) at 70°C.

Over night liquid culture of *T. flavus* AT62 at 76°C gave approximately 10^9 CFU (colony

a

b

FIG. 1. Colonies on Membrane Filter-Agar Plates of *Thermus flavus* AT62 (a) and *Thermus thermophilus* HB8 (b).

TABLE I. THREE STRAINS OBTAINED FROM A *Thermus flavus* AT62 STOCK CULTURE

Strain	Contents (%)	Color	Max. temp. ^a	Gram stain.	Spore	Shape of cell
Y	95	Deep yellow	> 75°C	—	—	Rod
W	4.5	Light yellow	> 75°C	—	—	Rod
SP	0.5	Pale yellow	74°C	+	+	Rod

^a Maximum temperature for growth.

forming unit) per ml of culture broth on agar plates with membrane filters (Fig. 1a) but no colonies without membrane filters. Similar results were obtained with *T. thermophilus* HB8 (Fig. 1b), *T. flavus* AT61, BS1, BS2 and BK1 but some different result with *T. aquaticus* TY1. Liquid culture of *T. aquaticus* YT1 at 72°C gave colonies on agar plates either with or without membrane filters and approximately the same numbers of CFU were obtained on both plates. But colonies on membrane filter are very compact as compared with large thin colonies without membrane filter. Generally, thermophilic bacteria tested gave more compact colonies on membrane filter-agar plates than on agar plates without membrane filters.

Using this procedure we checked the purity of a *T. flavus* AT62 stock culture and found that the culture was about 95% pure with two minor contaminants. We named them strain Y (95% dominant), W and SP respectively. Physiological and morphological properties tested with strain Y coincided with the description of original AT62 strain.¹⁾ Strain W has no yellow pigment, resembling *Thermus* X1,⁷⁾ while most *Thermus* species are yellow colored. Strain SP is spore-forming rod and does not grow above 75°C (Table I).

Another trouble of growing thermophilic bacteria on agar plates at high temperature

(65~70°C) is that motile bacteria often grow on all over agar plates instead forming countable colonies. The membrane filter-agar plate method turned out useful in that motile thermophilic bacteria can form compact colonies without failure.

The reason why extreme thermophile can grow much better on agar plate-membrane filter system is not clear. However it is likely that membrane filter can keep favorable physical conditions like high moisture content of agar plate surface. This procedure seems to be very useful for establishing and examining pure cultures of extremely thermophilic bacteria.

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