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EFFECTS OF MOST-PROBABLE-NUMBER METHOD AND PLATE COUNT METHOD ON FUNGAL POPULATION DENSITY AT DIFFERENT pH OF ASSAY MEDIUM

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Abstract

The most-probable-number method was adopted to evaluate population density of fungus using two fungal models in potato-dextrose media of pH 5.6 and 3.5. The results were compared with plate count methods. Concentration of propagules ranged of 6.11 - 6.55 log cfu/ml in *Rhizopus*), 6.01 - 6.09 log cfu/ml in *Trichoderma* at pH 5.6 and 6.68 - 6.75 log cfu/ml (*Rhizopus*), 6.10 - 6.32 log cfu/ml (*Trichoderma*) at pH 3.5. The order of results was - most-probable-number > spread plate> pour plate. Population density was relatively higher at pH 3.5 and no significant differences ($p \leq 0.05$) among the methods recorded.

Fungi are known to play valuable role in agriculture, food processing and waste recycling from ancient times. Concurrent estimation of fungal concentration as a factor of product formation for monitoring mass fermentation is vital. Conventionally plate count techniques are by and large reliable for estimating fungal population density (Schnurer 1993, Santiago and Motta 2008).The most-probable-number (MPN) method thus restricted to bacteria finds scope for fungal enumeration as well presently. This technique allows accurate counting because the liquid medium can be incubated for a longer period of time relatively to plate count techniques and offers miniaturisation possibility (Obispo and Dehority 1992, Hunsinger *et al.* 2005, Bhowmik *et al.* 2013). However, reports on applicability of MPN technique in fungal enumeration are scanty. Hence for the first time performance of MPN technique at variable pH of assay medium was tested. The results were compared with plate count techniques with an aim to confirm suitability of MPN in fungal research. The results were validated through two fungal species.

Commercially available fungal selective media, namely potato-dextrose agar (@ 39 g/l, pH 5.6) and potato-dextrose broth (@ 24 g/l, pH 5.6) and their respective acidified formulations (pH 3.5) (HiMedia Laboratories Pvt. Ltd., India) were used in the investigation.

The models, namely *Rhizopus oryzae* (ITCC 7382.09) and *Trichoderma reesei* (local isolate) were individually grown on 10 ml PDA in 100 ml Erlenmeyer flasks at 30 ± 2°C for 2 and 3 days, respectively. The fungal propagules comprising of spore-cum-mycelial fragments suspension was prepared by washing the growth surface for three times with approximately 30 ml of sterile 0.9% (w/v) NaCl and was stored at 4°C. Decimal dilutions of suspensions of harvested propagules were tested up to $1:10^6$ were prepared using a sterile 0.9% (w/v) NaCl.

From each dilution, 1 ml aliquot was inoculated in 5 dilution replicate tubes of 12 × 75 mm size (Fisherbrand disposable culture tubes) containing 2 ml potato-dextrose at pH 5.6 and acidified potato-dextrose at pH 3.5 liquid media individually thereby minimizing the size and volume of glass vessels and culture media of the normal MPN technique. The positive tubes with mycelial

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growth were recorded at 96 hrs (30 ± 2°C) incubation and population density was expressed as colony forming units (cfu)/g on dry weight basis (Cochran 1950). Spread plate (SP) and pour plate (PP) assay were conducted concomitantly in Petri dishes (90 mm diameter) by 15 ml potato-dextrose agar media of pH 3.5 and 5.6 individually.

All the experiments were repeated for five times independently. The mean values were transformed to logarithmic form and data were subjected to ANOVA and DMRT ($p \leq 0.05$).

Fig. 1 depicts higher number of fungal colonies in acidified potato-dextrose medium (pH 3.5). Correspondingly MPN method on acidified potato-dextrose medium at pH 3.5 resulted highest propagule count for *R. oryzae* (6.75 log cfu/ml), followed by spread plate method (6.71 log cfu/ml), and pour plate method (6.68 log cfu/ml) (Table 1). Potato-dextrose medium at pH 5.6 recorded lower number of propagules relative to acidified medium but with a similar trend i.e. MPN (6.55 log cfu/ml) > SP (6.50 log cfu/ml) > PP (6.11 log cfu/ml). The trend of result was parallel to *T. reesei.* MPN method in general resulted insignificantly ($p \leq 0.05$) highest propagule count as compared to plate count techniques for both the models under study. The order of results between MPN and plate count methods is in conformity to Hunsinger *et al.* (2005). A thin but conspicuous submerged growth was observed at 42 hrs (*R. oryzae*) and 24 hrs (*T. reesei*) in MPN tubes, while above 48 hrs in agar-plates normally. At extended incubation, the matured cultures bore signature dark hue that varied with pH for *Trichoderma* (Fig. 2).

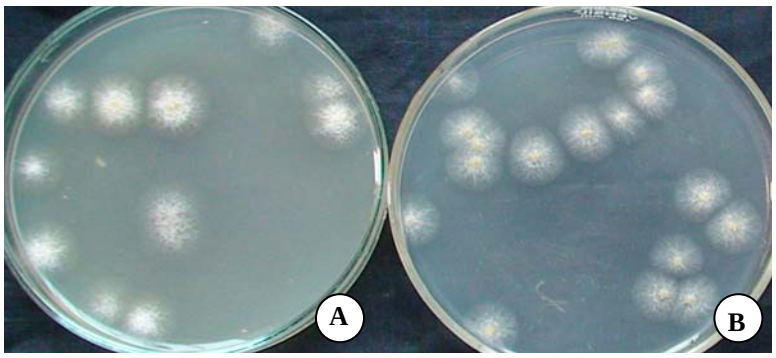

Fig. 1. Effect of (A) potato-dextrose agar (pH 5.6) and (B) acidified potato-dextrose agar (pH 3.5) on the colony count of *Rhizopus oryzae.*

Table 1. Effect of pH of potato-dextrose media and enumeration methods on fungal population density.

pH	Method	Population density* (log cfu /ml)	
		Rhizopus oryzae	*Trichoderma reesei*
5.6	MPN**	6.55 ± 0.02^{c}	6.09 ± 0.02^{bc}
	SP	6.50 ± 0.01^{c}	6.06 ± 0.01^{c}
	PP	6.11 ± 0.02^{d}	6.01 ± 0.01^{d}
3.5	MPN	6.75 ± 0.00^{a}	6.32 ± 0.01^{a}
	SP	6.71 ± 0.01^{ab}	6.12 ± 0.02^{b}
	PP	6.68 ± 0.02^{b}	6.10 ± 0.02^{b}

*Mean values ± SD of five replicates are given. Means sharing a letter in the column are not significantly different at $p \leq 0.05$ by DMRT. **MPN most-probable-number, SP spread plate, PP pour plate.

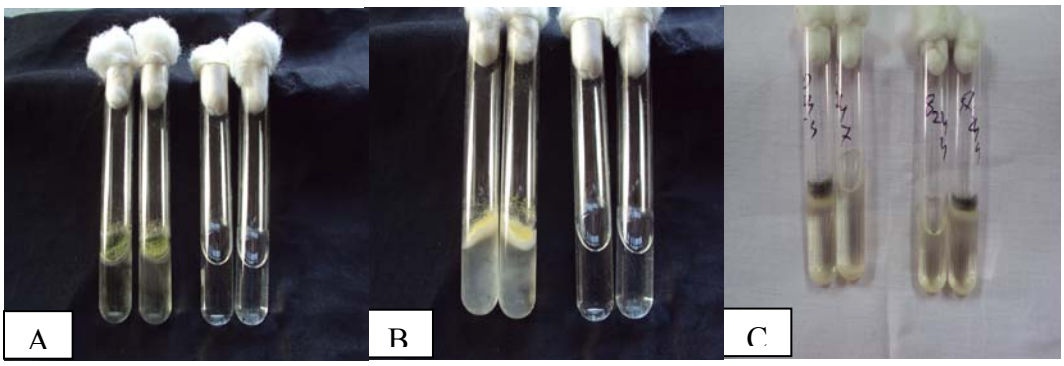

Fig. 2A-C. Growth characteristic of *Trichoderma reesei* on (A) potato-dextrose liquid medium, (B) acidified potato-dextrose liquid medium; and (C) *Rhizopus orzyzae* on potato-dextrose (left) and acidified potato-dextrose (right) liquid medium; along with their corresponding uninoculated control tubes.

It is evident from this work that MPN method can duly replace the plate count methods for enumeration of fungal population density with miniaturisation possibility.

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