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SHORT COMMUNICATION

Comparison of agar media for counts of viable soil bacteria

J. K. MARTIN
CSIRO, Division of Soils, Glen Osmond, 5064 South Australia

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The numbers of bacteria present in leachates from pots containing different plant species at different stages of growth have been reported (Martin, 1971). Total bacterial numbers were estimated by dilution plate counts using yeast-extract/peptone/soil-extract agar (YPS) (Bunt and Rovira, 1955) as the medium. Major disadvantages of soil-extract agars are the time required for preparation and the difficulty of maintaining a uniform medium (Harris and Keeney, 1968). Contrary to earlier opinions, it is doubtful if soil extracts contain microbial growth factors which cannot be replaced by known vitamins, amino acids, etc. (Taylor, 1951). Commercial dehydrated media developed for exacting pathogenic bacteria should therefore provide the base for a medium which is quickly prepared, standardized and would be expected to support the growth of a wide range of soil bacteria, providing the nutrient concentration is sufficiently low (Clark, 1965).

Comparison of YPS with 1% and 0·3% Bacto-tryptic soy broth (Difco) solidified with 1·5% agar (1% TSA and 0·3% TSA) showed that 1% TSA gave colony counts some 25% lower than YPS but there was no difference in the values obtained with YPS and 0·3% TSA for 40 separate samples of Mt. Compass soil. The results of additional comparisons made between YPS and 0·3% TSA with eight soils providing a range of properties are reported in this paper.

Four of the soils (Waitpinga, Millicent, Mt. Crawford, Kalangadoo) were air-dry samples collected under pasture (Ladd and Butler, 1972) and the remaining soils were fresh, collected from adjoining plots with different vegetative cover. A preliminary experiment established the dilution of each soil which gave a count between 50–250 colonies/plate. Ten ml of the required dilution were prepared from each soil and 10 plates of the two media were inoculated in a random manner with 0·1 ml aliquots of each of the soil suspensions.

There were no significant differences in the counts obtained with the two media for six of the eight soils tested (Table 1).

Table 1. Comparisons of numbers of bacteria growing on soil extract agar (YPS) and 0·3% tryptic soy agar (TSA) and of pseudomonads growing on King B agar and TSA, both with added antibiotics

Soil	'Total' bacteria† (Numbers/g oven-dry soil × 10^{-6})			Pseudomonads† (Numbers/g oven-dry soil × 10^{-3})		
	YPS	TSA	Difference‡	King B + antibiotics	TSA + antibiotics	Difference‡
Waitpinga	4.3	4.3	n.s.	1.5	1.1	**
Millicent	47.5	62.2	n.s.	19.8	19.2	n.s.
Mt. Crawford	4.8	2.8	***	<0.02	<0.02	-
Kalangadoo	4.6	3.1	*	<0.02	<0.02	-
Urrbrae - pasture	20.3	20.8	n.s.	225	286	***
Urrbrae - pines	35.8	36.3	n.s.	358	375	n.s.
Urrbrae - fallow	20.7	22.5	n.s.	173	188	n.s.
Urrbrae - lucerne	38.6	41.4	n.s.	757	1164	***

† Values are the geometric mean of 10 replicates.
‡ Significance of difference—*P < 0·05; **P < 0·01; ***P < 0·001. Calculated from $\log_{10}$ (bacterial counts/g oven-dry soil) with 10 replicates.

There was also no qualitative difference observed between the two media with any of the soils. The colonies on 0·3% TSA were discrete, there was no limitation of pigment formation and actinomycetes were readily recognized. Similar results were obtained by E. L. Merek (unpublished) who found that the numbers of bacteria isolated from soil with soil extract agars were no higher than those obtained with 0·1% TSA but were consistently higher than the values obtained with 3% TSA.

The earlier study (Martin, 1971) also showed a marked effect of plant species on the number of fluorescent pseudomonads in the leachates. Pseudomonad numbers were measured using the selective NPC medium of Sands and Rovira (1970), and represented 0·05–0·3% of the total numbers of bacteria in the leachates. However, the large difference in nutrient status of YPS and the NPC medium, which is based on the addition of an antibiotic mixture to the rich peptone Medium B of King *et al.* (1954), cast doubt on the validity of comparing the two counts. When it was observed that a small number of the colonies growing on 0·3% TSA fluoresced under u.v. light, a comparison was made of the number of fluorescent colonies growing on the NPC medium and on 0·3% TSA to which was added the antibiotic mixture employed in the NPC medium.

The comparisons were made concurrently with the comparison of YPS and 0·3% TSA using the same soil suspensions but at lower dilutions to provide 50–250 colonies/plate. The counts of fluorescent organisms were almost identical for the NPC and 0·3% TSA + antibiotics media for all soils. However significantly higher numbers of total colonies were obtained with the modified TSA medium for Urrbrae soil collected under pasture and lucerne (Table 1). Subsequent sub-culture showed that the additional counts were predominantly antibiotic-resistant non-fluorescent organisms. For routine estimation of fluorescent pseudomonads the NPC medium is preferable to the modified 0·3% TSA since fluorescent colonies are more easily counted under u.v. light.

These results confirm that estimates of total viable organisms obtained with 0·3% TSA are comparable with those using YPS and show that it is possible to replace soil extract agar by a commercially available dehydrated medium which is more easily prepared and should be reproducible, thus increasing the value of comparisons between laboratories of estimates of microbial biomass derived from plate dilution studies.

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between both sectors is now establishing a distinct scientific discipline of agricultural microbiology.

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