Influence of Culture Media and Incubation Conditions on the Population and Diversity of Microorganisms Cultivated from Soils and Termite Guts

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Abstract

In the last several years information on the gut ecosystem of termites has continued to be gathered. Most studies on termite gut microbial communities have been focused on wood feeding termites but studies on soil feeders remain sparse owing to their typically remote habitat, delicate nature and the difficulty of establishing permanent laboratory cultures. Studies have shown soil feeding termites' house important antibiotic producing bacteria in their gut, nest and surrounding soil. An alternative approach was used in this study to isolate such bacteria from the soil, gut and mound of soil feeding termites from Kakamega Forest. The samples were collected from two sites Kalunya Glade and Lirhanda Hill. The study was also extended to the soils found in Juja. The cultivation procedure included: use of media with minimal nutrients, incubation at three different temperatures (25 ^{o}C, 30 ^{o}C and 37 ^{o}C) and observing the trend and counting of colony forming units (cfu) for an extended cultivation period. Dilution and heat shock method was also used during cultivation to target isolation of Actinobacteria. Through statistical analysis, Kakamega Forest soil samples had higher counts of cultivable microorganisms' (cfu/g) with a mean of 1.65×10^{8} cfu/g than Juja soils samples that had a mean of 8.5×10^{7} cfu/g. However clay soils from Juja and kakamega Forest had higher cfu/g counts than all the other soils samples with means of 1×10^{8}cfu/g and 2.02×10^{8} cfu/g respectively. The termite gut had high cfu/g counts with a mean of 3.10×10^{9}cfu/g with an optimum cultivation temperature of 37 ^{o}C while the mound samples had the least number of cfu/g counts with a mean of 6.25×10^{7} cfu/g at optimum cultivation temperatures of 25 ^{o}C and 30 ^{o}C. The results showed that the effect of incubation temperature on a number of cultivable microorganisms was significant when the dilution and heat shock method was used where the optimum cultivation temperature for termite guts, soil and termite mound samples was 30^{o}C. Extending the incubation period was significant as cfu/g

count increased with time on most samples. Hundred and thirty seven (137) isolates were screened for their antagonistic effects on various test organisms. Fifty one percent of the isolates were antagonist to *Escherichia coli*. Fifty seven percent of the isolates were antagonists to *Bacillus subtilis* while 55% of the isolates were antagonist to *Candida albicans*. Enzymatic activities of the isolates showed that 65% of the total isolates were starch degraders, 54% were casein degraders and 68% of the isolates were able to liquefy gelatin. Lastly, 11% of the isolates were cellulose degraders the majority of which were obtained from termite gut and mound. Isolates from Juja soil had the highest number of non degraders as compared to Kakamega Forest soils. The isolates were characterized using morphological, biochemical and molecular methods. Phylogenetic analysis of amplified 16S rRNA gene sequence revealed eight isolates from gut, mound and soil were closely related to *Bacillus thuringiensis.* An isolate from surrounding soil was closely related to *Bacillus pumilus* while two isolates from the mound were closely related to *Bacillus subtilis*. An isolate from surrounding soil of termites was closely related to *Brachybacterium paraconglomeratum* and showed very strong *in vitro* antagonistic effects. Two Gram negative bacterial isolates obtained from surrounding soil were closely related to *Pseudomonas aeruginosa* and *Serratia marcescens.* In conclusion, the study was able to cultivate microorganisms in low nutrient media from the gut, mound and surrounding soil of *Cubitermes severus.* These isolates were antibiotic producers and had the ability to degrade gelatin, casein, starch and cellulose an indication of the role they play in their habitat.