SHANNON INDEX FOR ESTIMATING THE DIVERSITY OF NITROGEN-FIXING LEGUME NODULE BACTERIA USING RANDOMLY AMPLIFIED POLYMORPHIC DNA

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The process of biological nitrogen fixation is perhaps the most important in the global cycling of this nutrient. Soil bacteria belonging to the genera *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* (generally referred to as rhizobia) are capable of nodulating legume species developing a symbiotic partnership. Intensive applications of nitrogen fertilizers in agricultural soils may lead to adverse effects in environment. Therefore, it should be the priority of national agricultural policies to maximize the benefits of the biological nitrogen fixation process. This requires the use of rhizobial strains as inoculants. In order to achieve this, the first step is to investigate their diversity in the soils, followed by screening strategies for selection of highly efficient nitrogen fixing strains and, finally, field tests to evaluate their agronomical performance. The emergence of molecular biology techniques enabled the assessment of the diversity of microorganisms using the technique RAPD (Randomly Amplified Polymorphic DNA). The end-products of this technique are banding patterns of the nodule isolates, called DNA fingerprints (3). According to the respective banding patterns, the nodule isolates were classified in different groups. We propose the use of the Shannon index, described in (1), as a rhizobial diversity measure. Generally, this index is used to measure the diversity of taxonomic groups, but could also be applied to evaluate the genetic diversity of microorganisms. This index is based on richness, in this case the number of different groups, and the relative abundance of the isolates in each group.

So, the index (H) was calculated by the following equation:

$$H = -\sum_{i=1}^{k} p_i \ln p_i$$

where, k is the number of groups and p_i , the relative abundance of isolates in each group ($i = 1, 2, \ldots, k$).

Generally, diversity estimates depend on the sample size (**n**). Hence, it is necessary to determine the minimum number of nodules which corresponds to a value H no longer dependent on **n**. In order to study this, we adapted the Pielou´s pooled quadrat method (2) to the specific case where the samples are nodule isolates. So, the method consists of random ordination of the nodule isolates and then calculation of the diversity index in a cumulative manner. A graph was constructed plotting the number of pooled samples against the diversity estimate in order to access the sample size ($\mathbf{n_o}$) at which the curve flattens off. Additionally, it is interesting to evaluate the precision of the diversity index estimate (H) when the sample size $\mathbf{n_o}$ was used. The standard error of the estimated H was evaluated using the Bootstrap method. If the standard error is too high, the sample size (**n**) must be increased so as to increase the precision of the diversity index estimate. The validation of this method for evaluating rhizobial diversity will assist in the study of the impacts different types of agricultural practices may impose to the environment. The quantitative approach to diversity studies, using mathematical indices estimates and associated standard error, will allow statistically sound analyses of microbial diversity, which is impossible by the use of molecular techniques on its own.

References:

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