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ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE IDENTIFICATION AND ENUMERATION OF AZOSPIRILLUM BRASILENSE cd.

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Currently employed methods for identification and enumeration of a specific bacterial strain, particularly when applied to plant roots under field conditions, have certain limitations: e.g., counting on solid media or by the Most Probable Number technique cannot exclude other bacteria with similar features. Qualitative methods such as direct and indirect immunofluorescence, tube agglutination, immunodiffusion, immunoelectrophoresis, capillary precipitation techniques and radioactive techniques are impractical for large scale field studies.

In recent years, Azospirillum brasilense has been used world-wide as a cereal inoculant that enhanced plant growth. However, the identification and enumeration of it present unique problems: it is known to have highly variable colonies and to form aggregates on roots, which prevent counting by dilution methods.

We suggest the Enzyme-Linked-Immunosorbent Assay (ELISA)-mostly used for the detection of viruses, as a reliable and sensitive method for the specific detection and enumeration of A. brasilense cd. The method can be applied to pure cultures, bacterial mixtures or plant roots.

Microtiter plates were coated overnight at 4°C with dilutions of bacterial suspensions or with a centrifuged extraction of roots from previously inoculated plants. After blocking with egg albumin, rabbit antibodies specific to A. brasilense cd. were applied to the plates. After washing, alkaline phosphatase conjugated to goat anti-rabbit IgG was added and subsequently plates were incubated with freshly prepared substrate (p-nitrophenyl phosphate) at 37°C. The resulting color was recorded at 405 nm. Between each step the plates were rinsed x 3 in PBS containing Tween 20. Scanning-electron microscopy of coated wells revealed a relatively small number of bacteria attached to the plastic, indicating the importance of

sufficient blocking. Additionally, inhibitions of the antibody binding by various bacterial suspensions or by root extracts were also performed.

The ELISA method exhibited a very high specificity towards A. brasilense cd. showing no cross-reaction with other wheat rhizosphere bacteria, including several strains of Azospirillum. The regular minimum practical sensitivity of the method was 10^4 Colony-Forming-Units CFU/sample. At higher bacterial numbers, sensitivity increased linearly up to $5 \cdot 10^8$ CFU/sample, yielding a useful standard curve.

The Elisa identified A. brasilense specifically even if the bacterial colony morphology was variable, in pure cultures and in mixtures of various other bacterial species. The system enabled the identification of A. brasilense cd. in roots of several cereals, like wheat, maize, sorghum, panicum, setaria, rye, barley and oats. Current studies are aimed to measure the colonization level of field grown inoculated wheat. In principle, the Elisa technique can be adopted for the detection and enumeration of other bacteria applied to different plants.