

# **INTERNATIONAL JOURNAL OF AGRICULTURE, MANAGEMENT AND TECHNOLOGY**



**Vol 8(1) 2024**

**Published by**  
Federal College of Agriculture,  
Ishiagu Ebonyi State

## EFFECT OF ZINC-SOLUBILIZING RHIZOBACTERIA ON ZINC ACCUMULATION, MICROBIAL ACTIVITY, AND GROWTH OF CASSAVA

<sup>1</sup>Amajor, E.E., <sup>2</sup>Nwachukwu, E., and <sup>2</sup>Achi, O.K.

<sup>1</sup>National Root Crops Research Institute, Umudike, Abia State

<sup>2</sup>Michael Okpara University of Agriculture Umudike, Abia State.

Corresponding email: [essyjohn2006@gmail.com](mailto:essyjohn2006@gmail.com)

### ABSTRACT

Zinc (Zn) is an essential micronutrient for plant growth, but its limited bioavailability in soils affects cassava productivity. Zinc-solubilizing rhizobacteria (ZSR) can enhance Zn availability, promoting better plant uptake and growth. This study evaluates the effectiveness of *Pseudomonas* and *Bacillus* spp. in improving Zn solubilization, microbial respiration, and Zn accumulation in cassava (*Manihot esculenta*). The experiment was conducted at the National Root Crops Research Institute (NRCRI), Umudike, Nigeria, under screen house conditions. A completely randomized design was used with eight treatments and three replications. *Pseudomonas* (P), *Bacillus* (B), ZnSO<sub>4</sub> only, *Pseudomonas* + *Bacillus* (P+B), *Pseudomonas* + Zn (P+Z), *Bacillus* + Zn (B+Z), *Pseudomonas* + *Bacillus* + Zn (P+B+Z), and a control. Bacterial isolates were screened for Zn solubilization ability, and their growth was monitored in different Zn sources and time. CO<sub>2</sub> respiration rates were measured to assess microbial activity, while Zn accumulation in cassava roots, stems, and leaves was determined at harvest using atomic absorption spectrophotometry. Results showed that *Pseudomonas* sp and *Bacillus* sp showed higher solubilization efficiency during the screening with 300% and 280% respectively. The ZnSO<sub>4</sub> treatment exhibited the highest turbidity bacterial growth rate (OD<sub>600</sub>) of 2.5nm and 2.0nm under *pseudomonas* and *bacillus* sp and P+B+Z treatment showed a significant increase in CO<sub>2</sub> respiration level at 8MAP (52.6mg/kg), indicating enhanced microbial activity. Zinc accumulation followed the trend: P+B+Z > P+Z > B+Z > P+B > Zn only > Control, with the highest Zn concentrations of 3.93 mg/kg and plant height of 150.13cm. In conclusion, the combined application of *Pseudomonas* and *Bacillus* (ZSR) with ZnSO<sub>4</sub> significantly improved Zn solubilization, microbial activity, and Zn uptake in cassava. These findings suggest that ZSB-based biofertilization could enhance cassava nutrition and productivity. Field validation is recommended to optimize bacterial formulations for large-scale application.

**Keywords:** Zinc-Solubilizing Rhizobacteria (ZSB), Cassava, Zinc Uptake and Biofortification.

### Introduction

Zinc (Zn) is an essential micronutrient for various physiological functions in plants, including enzyme activation, protein synthesis, and growth regulation. Despite its importance, Zn deficiency is prevalent in many agricultural soils, leading to stunted growth and reduced crop yields. In Nigeria, cassava (*Manihot esculenta*) serves as a staple food for over 180 million people. However, cassava's edible storage roots are low in micronutrients like Zn, contributing to widespread dietary deficiencies in regions reliant on this crop (Gegios *et al.*, 2010).

Traditional Zn fertilization methods often face challenges such as low bioavailability due to soil fixation and leaching. An eco-friendly alternative is the utilization of zinc-solubilizing rhizobacteria (ZSR), which can convert insoluble Zn compounds into plant-accessible forms. These bacteria, including species like *Bacillus*, *Pseudomonas*, and *Rhizobium* spp. They solubilize Zn through the production of organic acids and other metabolites (Saravanan *et al.*, 2007).

Studies have demonstrated that ZSR inoculation can enhance Zn uptake and promote growth in various crops. For instance, isolates from the rhizosphere soils of peanuts, sweet potatoes, and cassava have shown high Zn solubilization efficiencies, indicating their potential as bioinoculants (Ramesh *et al.*, 2014). Similarly, ZSR isolated from the rhizosphere soil in India exhibited significant Zn solubilization, highlighting their role in sustainable agriculture (Tamboli, 2019).

Despite these advancements, the application of ZSR in cassava cultivation remains underexplored. This study aims to evaluate the potential of ZSR in enhancing Zn uptake in cassava, thereby contributing to sustainable agriculture and addressing micronutrient deficiencies in cassava-consuming populations.

### Objective of this study

1. Screening, Characterization and Identification of ZSR Isolates
2. To ascertain the estimation time of cell density for microbial growth with different zinc

compounds

3. To determine the rhizobacteria respiration ( $\text{CO}_2$ ) rates after application of  $\text{ZnSO}_4$
4. To determine the accumulation of zinc in the root, stem and leaves of cassava at Harvest. (Plant analysis).

## Materials and Methods

### Greenhouse Experiment

A greenhouse study was set up using a Complete Randomized Design (CRD) with three replicates. Cassava stem cuttings were planted in feed bags containing soil (30kg). Cassava variety used was TME 419. Number of treatments = 8 (Total of  $8 \times 3$  replications = 24 treatments). Treatments included: 1. *Bacillus subtilis* (BS) only. 2. *Pseudomonas aerogenosa* (PA) only. 3. Zinc (Zn) only. 4. *Bacillus* + *Pseudomonas* (Consortium of BS+PA). 5. Zinc + *Pseudomonas aerogenosa* (Zn + PA). 6. Zinc + *Bacillus subtilis* (Zn + BS). 7. Zinc + *Pseudomonas aerogenosa* + *Bacillus subtilis* (Zn + PA + BS). 8. Control (C) (No treatment) (Gegios *et al.*, 2010). Bacterial inoculants were imported from IITA and were screened.

Rhizosphere soil samples were collected from cassava (*Manihot esculenta*) fields in National Root Crops Research Institute Umudike. 10 grams of soil from the root zone (10–15 cm depth) were aseptically collected into sterile bags and transported to the laboratory for further soil analysis (Ramesh *et al.*, 2014).

### Screening, Characterization and Identification of ZSR Isolates

Morphological and biochemical characterizations of imported ZSR isolates were performed on the important isolates including Gram staining, catalase activity, and starch hydrolysis tests. The colony morphology and Gram staining of zinc-solubilizing rhizobacteria were conducted following the methodology described by Vincent (1970). Pure cultures of the zinc-solubilizing strains were streaked on PVK agar and incubated at  $28 \pm 2^\circ\text{C}$  for 24 hours. Gram staining was performed under a light microscope to observe the gram reaction. The strains were cultured in PVK broth overnight and adjusted to an OD600 of 0.5 for catalase and starch hydrolysis test. Catalase activity was determined by adding 3% hydrogen peroxide to bacterial cultures grown on PVK medium (Chacko, Pramod, and Suchit, 2009) while starch hydrolysis capability was tested using starch agar plates according to Aneja (2003).

### Estimation Time of Cell Density for Microbial Growth with Different Zinc Compounds

*Bacillus* and *Pseudomonas* cultures were inoculated into PVK broth containing various zinc compounds ( $\text{ZnSO}_4$ ,  $\text{ZnO}$ ,  $\text{ZnCO}_3$ ). Each broth was inoculated with a standardized microbial culture and incubated at  $37^\circ\text{C}$  with shaking at 200–250 rpm. At 4-hour intervals, the optical density at 600 nm (OD600) was measured using a spectrophotometer which was zeroed with a

blank containing the corresponding zinc concentration without microbes. OD600 values were recorded over time to create growth curves. These curves were analysed to compare growth rates and final cell densities across different zinc compounds. Higher OD600 indicates more growth, while lower values suggest inhibition by zinc. The experiments were carried out in triplicate with control for accuracy and to assess baseline growth. (Madigan *et al.*, 2014).

### Determination of Zinc Solubilizing Rhizobacteria (ZSR) Respiration Levels ( $\text{CO}_2$ )

$\text{CO}_2$  emissions were quantified using the substrate-induced respiration method (Horwath and Paul, 1994; Lupwayi *et al.*, 2001; Alef and Nannipieri, 1995). In this method, 300 mg of glucose was dissolved in 4.5 ml of water and added to 50 g of air-dried soil to stimulate microbial respiration. The soil samples were then incubated in 1-liter jars for 3 hours at  $22^\circ\text{C}$ , and the accumulated  $\text{CO}_2$  in the headspace was measured using gas chromatography.

### Assessment of Plant Growth and Zinc Uptake

After 12 weeks, cassava were harvested, and growth parameters such as plant height and leaf number were recorded. Plant tissues were oven-dried, ground, and digested using a mixture of nitric and perchloric acids. Zinc concentration in the digests was determined using atomic absorption spectrophotometry (Ramesh *et al.*, 2014).

### Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) to assess the significance of treatments on plant growth and Zn uptake. Means were compared using the least significant difference (LSD) test at a 5% significance level (Tamboli, 2019).

### Discussions

#### Screening, Characterization and Identification of ZSR Isolates

In table 1, the screened zinc-solubilizing rhizobacteria (ZSR) demonstrated varying zinc solubilization efficiencies, with *Pseudomonas aeruginosa* (300%) and *Pseudomonas fluorescens* (280%) showing the highest efficiency, followed by *Bacillus* spp. (*B. subtilis* and *B. megaterium*) at 210–250%. These results align with previous studies highlighting *Pseudomonas* and *Bacillus* as efficient zinc solubilizers due to their organic acid production (Ramesh *et al.*, 2014; Saravanan *et al.*, 2007). The biochemical tests confirmed the identity of the isolates, with catalase-positive results for most species, except *Rhizobium* spp., which is typically catalase-negative. The starch hydrolysis test was positive for *Bacillus* and *Rhizobium* spp., suggesting their ability to produce extracellular enzymes that aid nutrient mobilization.

### Estimation Time of Cell Density for Microbial Growth with Different Zinc Compounds

Figure 1 shows a time line graph of the rate of growth of different isolates under different zinc compounds. The growth rate was measured under OD600 at 4-hours intervals from 8<sup>th</sup> hour to 24<sup>th</sup> hour. All the result showed a progressive increase in the growth of the isolates from 8<sup>th</sup> hour to 16<sup>th</sup> hour and declined at 24<sup>th</sup> hour. The result at 16<sup>th</sup> hour showed that zinc sulphate (ZnSO<sub>4</sub>) for both isolates (*Pseudomonas* and *Bacillus* sp) had the highest turbidity rate of 2.5nm and 2.0nm respectively.

Zinc sulphate (ZnSO<sub>4</sub>) is highly soluble, providing a readily available source of zinc ions which can be easily utilized by *Pseudomonas* sp for various metabolic processes. Zinc is a vital trace element that acts as a cofactor for numerous enzymes in bacterial cells, enhancing their growth (Bach *et al.*, 2007). High solubility ensures that zinc ions are more bioavailable, which likely contributed to the rapid initial growth observed for *Pseudomonas* sp. While Zinc oxide (ZnO) is less soluble compared to Zinc sulphate (ZnSO<sub>4</sub>) leading to a slower release of zinc ions. *Pseudomonas* species have shown the ability to solubilize ZnO through the production of organic acids, which can help in slowly releasing zinc ions (Saravanan *et al.*, 2003).

#### **Determination of Zinc Solubilizing Rhizobacteria (ZSR) Respiration Levels (CO<sub>2</sub>)**

Zinc solubilizing Rhizobacteria (ZSR) respiration refers to the metabolic process by which ZSR consume organic carbon sources and release carbon dioxide (CO<sub>2</sub>) as a byproduct of their energy production activities. The results (Table2) showed that the total respiration in all treatments increased across the months after planting (MAP). Among all the treatments, the combination of *Pseudomonas*, *Bacillus*, and zinc (PBZ) gave a significant increase in respiration levels of 28.6, 34.6, 50.0, and 52.6 mg/kg at 2, 4, 6, and 8 MAP, respectively. This was followed by the *Bacillus* + Zinc (BZ) treatment, which also showed a significant increase of 4.04mg/kg at 6 months after planting (6 MAP).

The increase in respiration with months after planting is likely due to the progressive growth and establishment of microbial communities in the rhizosphere, which enhance organic matter decomposition and nutrient cycling, thus elevating CO<sub>2</sub> production (Blagodatskaya and Kuzyakov, 2013). Additionally, as cassava plants grow, they exude more root exudates, providing additional substrates for microbial activity and further stimulating microbial respiration over time (Cheng *et al.*, 2003).

The observed results highlight the synergistic effects of *Pseudomonas* and *Bacillus* species in enhancing zinc uptake and biomass production in cassava. The combined treatment of *Pseudomonas*, *Bacillus*, and

zinc (PBZ) resulted in the highest increases in respiration rates and total biomass, indicating enhanced microbial activity and nutrient cycling. These findings align with previous studies that demonstrate the role of Zinc Solubilizing Rhizobacteria (ZSR) in improving nutrient availability and plant growth (Vessey, 2003; Lugtenberg and Kamilova, 2009).

#### **Assessment of Plant Growth and Zinc Uptake**

The results in table3 showed that, the treatment combination of *Pseudomonas*, *Bacillus* isolates and zinc (PBZ) gave the highest content of zinc in its traits (3.93mg/kg) for the root. The treatment combination of *Pseudomonas*, *Bacillus*, and zinc (PBZ) significantly increased all cassava traits, with measurements of 150.13 cm for plant height and 32.0 for the number of leaves. This was followed by the *Bacillus* and zinc (B+Z) treatment, which also showed significant increases, with values of 142.97 cm for plant height and 24 for the number of leaves.

Increased zinc uptake in the *Pseudomonas*+ *Bacillus*+ zinc (PBZ) treatment can be attributed to these synergistic interactions, leading to improved root architecture and greater nutrient mobilization. This effect is particularly important for cassava, which is often grown in zinc-deficient soils. Moreover, the application of zinc in conjunction with these bioinoculants likely enhances the bioavailability of zinc, facilitating its transport and storage within the plant tissues (Lugtenberg and Kamilova, 2009). Zinc is an essential micronutrient for plant growth and human health, contributing to various physiological processes in plants and improving nutritional value in crops (Cakmak, 2008). *Pseudomonas* and *Bacillus*, as plant growth-promoting rhizobacteria, facilitate nutrient uptake and enhance plant vigor through mechanisms such as hormone production and nutrient solubilization (Vessey, 2003; Lugtenberg and Kamilova, 2009).

#### **Conclusion**

This approach is environmentally sustainable compared to traditional zinc fertilization methods. It also offers a cost-effective alternative for farmers, reducing dependency on chemical fertilizers. This biofortification method can play a crucial role in combating micronutrient malnutrition, particularly in developing countries where zinc deficiency is prevalent and also promotes sustainable agricultural practices, improving soil health and reducing the environmental impact of chemical fertilizers.

Therefore, this study shows that a ZSR can provide multiple functions that benefit plant health thus highlights the importance of ZSR in soil health and plant growth.

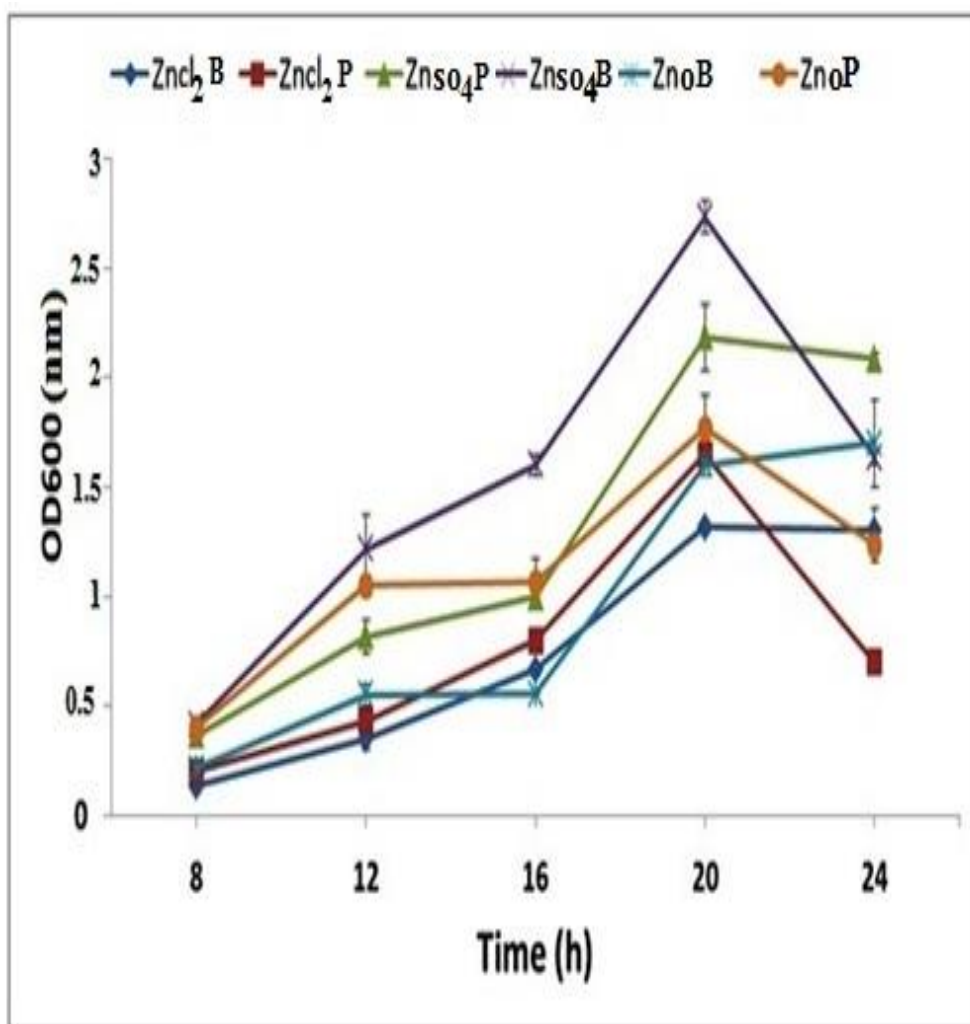
**Table1:** Biochemical Screening of Zinc Solubilizing Rhizobacteria (ZSR).

Colony Morphology	Gram Reaction	Zinc Solubilization efficiency	Catalase Test	Starch Hydrolysis	Probable Identity
Gram circular	Gram+ve	210 ±15	+	+	<i>Bacillus subtilis</i>
White mucoid	Gram-ve ( <i>Pseudomonas</i> ) <i>sp</i>	280 ±18	+	–	<i>Pseudomonas Flourescence</i>
Yellow Irregular	Gram-ve ( <i>Rhizobium sp</i> )	190 ± 12	–	+	<i>Rhizobium spp</i>
Gram circular	Gram +ve	250 ±17	+	+	<i>Bacillus Megaterium</i>
Green smooth	Gram-ve ( <i>pseudomonas sp</i> )	300 ± 20	–	–	<i>Pseudomonas aerogenosa</i>

**Table2:** Zinc Solubilizing Rhizobacteria Respiration (CO<sub>2</sub>)

Samples	2MAP	4MAP	6MAP	8MAP
	(Mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
<b>B</b>	15.4±1.23cd	23.6±0.26e	36.6±0.75d	42.4±0.51c
<b>B+Z</b>	22.6±0.67b	28.9±0.4b	40.4±0.15b	44.8±0.06b
<b>Ctr</b>	4.9±0.5f	9.7±0.31h	15.2±0.1g	19.5±0.52g
<b>P</b>	9.7±0.92e	21.3±0.17f	32.7±0.35e	36.8±0.06e
<b>P+B</b>	15.2±0.1d	25.5±0.31d	35.7±0.68d	41±0.42d
<b>P+B+Z</b>	28.6±0.1a	34.6±0.36a	50±1.1a	52.6±0.68a
<b>P+Z</b>	17.3±0.55c	27.2±0.25c	38.5±0.21c	42.9±0.46c
<b>Z</b>	8.2±0.05e	13.8±0.46g	24.2±0.4f	28±0.42f

Means with the same alphabet are not significantly difference. LSD=0.05



**Figure1:** Microbial growth of ZSR at 4hours intervals under OD600

**Table 3:** The effect of ZSR on growth and Zinc concentration of cassava root.

Samples	Pant height (cm)	Number of. leaves	Zinc concentration (Mg/kg)
B	133.57±1.52d	14.33±1.52e	06.67±1.52de
B+Z	142.97±1.2b	24±1.2b	1.5±1.2b
C	120.7±1.15f	6±1.15g	0.03±1.15g
P	132.03±0.86d	12.67±0.86ef	04.83±0.86ef
P+B	132.7±0.5d	17±0.5d	2.03±0.5d
P+B+Z	150.13±0.35a	32±0.35a	3.93±0.35a
P+Z	137.73±2.2c	20.67±2.2c	1.17±2.2c
Z	126.33±2.29e	10.67±2.29f	03.67±2.29f

Means with the same alphabet are not significantly difference. LSD=0.05



## References

- Alef, K. and Nannipieri, P. (1995) Soil respiration. In 'Methods in applied soil microbiology and biochemistry'. (Eds :Alef .K) pp. 214–219.(Academic Press: London).
- Anje, K. R. (2003). Experiments in microbiology, plant pathology and biotechnology. 4th ed., New age international publishers, New Delhi, pp.74-128
- Blagodatskaya, E., and Kuzyakov, Y. (2013). Active microorganisms in soil: Critical review of estimation criteria and approaches. *Soil Biology and Biochemistry*, 67, 192-211.
- Cakmak, I. (2008). Enrichment of cereal grains with zinc: Agronomic or genetic biofortification *Plant and Soil*, 302(1-2), 1-17.
- Chacko, J., Pramod, A. B., and Suchit, R. (2009). Practical Microbiology. Tata McGraw-Hill Education.
- Cheng, W., Zhang, Q., Coleman, D. C., Carroll, C. R, and Hoffman, C. A. (2003). Is available carbon limiting microbial respiration in the rhizosphere: *Soil Biology and Biochemistry*, 35(4), 465-472.
- Gegios, A., Amthor, R., Maziya-Dixon, B., Egesi, C., Mallowa, S., Nungo, R., Gichuki, S., and Manary, M. J. (2010). Children consuming cassava as a staple food are at risk for inadequate zinc, iron, and vitamin A intake. *Plant Foods for Human Nutrition*, 65(1), 64–70. ([link.springer.com](http://link.springer.com))
- Horwath, W. R., and Paul, E. A. (1994). Microbial biomass. In Methods of Soil Analysis. Part 2.
- Lugtenberg, B. J., and Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, 63, 541-556.
- Lupwayi, N. Z., Rice, W. A., Clayton, G. W., and Grant, C. A. (2001). Soil microbial biomass and diversity respond to tillage and sulphur fertilization. *Canadian Journal of Soil Science*, 81(4), 577-586.
- Madigan, M. T., Martinko, J. M., and Parker, J. (2014). Brock Biology of Microorganisms (14th ed.). Pearson.
- Ramesh, A., Sharma, S. K., Sharma, M. P., Yadav, N., and Joshi, O. P. (2014). Inoculation of zinc solubilizing *Bacillus aryabhatai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Applied Soil Ecology*, 73, 87–96.
- Saravanan, V. S., Madhaiyan, M., and Thangaraju, M. (2007). Solubilization of zinc compounds by the diazotrophic plant growth promoting acterium *Gluconacetobacter diazotrophicus*. *Chemosphere*, 66(9), 1794–1798. ([annalsmicrobiology.biomedcentral.com](http://annalsmicrobiology.biomedcentral.com))
- Saravanan, V. S., Subramoniam, S. R., and Raj, S. A. (2003). Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Brazilian Journal of Microbiology*, 34(2), 121-125.
- Tamboli, R. R. (2019). Isolation and characterization of zinc solubilizing bacteria from rhizosphere soil of Latur district, Marathwada, India. *Biosciences Biotechnology Research Asia*, 16(4). ([biotech-asia.org](http://biotech-asia.org)).
- Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255(2), 571-586.
- Vincent, J. M. (1970). A Manual for the Practical Study of the Root-Nodule Bacteria. Blackwell Scientific Publications.