



# Azospirillum Bacteria and Cultivation of Food Crops

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## ABSTRACT

*Azospirillum* is one of the bacteria that can be used as biological fertilizer because the bacteria is known as a producer of PGPR (Plant Growth Promoting Rhizobacteria) which can increase crop production in a sustainable manner. Another potential possessed by *Azospirillum* is as a biocontrol agent for controlling plant pathogens. The application of *Azospirillum* in agriculture, especially food crops, is still very limited on a small scale. The purpose of this paper was a literature review that reviews the potential of *Azospirillum* as a biofertilizer and biocontrol of pathogens in food crops with a focus on its application and impact on the productivity sector. The methodology used a search for literature related to the topics listed in the search for current and relevant information about the use of bacteria of the genus *Azospirillum* in the cultivation of food crops. Among the studies, selected reports on the use of bacteria from the genus *Azospirillum* in food crops, which may contain results obtained in the field or in a controlled environment. The conclusion was that field inoculation has not been a major area of *Azospirillum* research at present, recent field and greenhouse experiments, especially in cereals and rice plant, have once again shown promising potential. The use of *Azospirillum* bacteria to improve the quality and quantity of food crop products and develop environmentally friendly practices is a reality. However, the development of research that determines the best strategy for the use of this technology in food crops should continue, aiming at the best conditions for producers.

**Keywords:** *Azospirillum*, application, food crop.

## 1. INTRODUCTION

*Azospirillum sp.* are spiral-shaped gram-negative bacteria that can fix nitrogen in the atmosphere and live in association with plants by colonizing plant roots and utilizing exudate released by these roots and multiplying especially in the area of root extension and root hair base [1]. These bacteria are microaerophilic when grown in N-free medium and turn into aerobic when they get nitrogen. Activity and growth of *Azospirillum sp.* will be optimal at acidic pH conditions and temperatures around 32 to 40 °C [2].

*Azospirillum sp.* which was first isolated was named *Spirillum lipoferum* and grouped in the genus *Pseudomonadales* which was later identified as *Azospirillum lipoferum* [3]. Characteristics of *Azospirillum sp.* is the development of a thin white, dense and swelled pellicle on a semi-solid medium containing malate [1]. Their preferred energy source is organic acids such as malic, succinic, lactic, and pyruvic [4].

*Azospirillum* is one of the bacteria that can be used as a biological fertilizer because the bacteria are known as producers of PGPR (Plant Growth Promoting Rhizobacteria) which can increase crop production in a sustainable manner [5,6,7]. The use of *Azospirillum* as a biological fertilizer is one solution to reduce the use of chemical fertilizers and pesticides, reduce the cost of chemical fertilization, and reduce environmental pollution caused by the use of chemical fertilizers and pesticides. Several studies have been carried out by several world researchers to determine the potential possessed by *Azospirillum* related to the use of these bacteria as biological fertilizers. The use of *Azospirillum* as a biological fertilizer can reduce the use of chemical fertilizers by up to 20% without reducing the yield obtained. In addition, biofertilizers can be produced at a low cost so as to increase farmers' profits [8,9].

Biological fertilizer containing bacteria *Azospirillum sp.*, *Pseudomonas sp.* and *Bacillus sp.* are able to increase nutrient uptake, plant growth and productivity, it was further stated that *Azospirillum sp.* is the best to

be developed as a biofertilizer in plants because it contains IAA which can increase root elongation, increase root hairs, and plant root surface area [10,11,12,13,14].

Another potential possessed by *Azospirillum* is as a biocontrol agent for controlling plant pathogens. *Azospirillum* is able to produce several compounds that can inhibit the growth of plant pathogens such as siderophores and cyanide. *Azospirillum* can act as a moderate biological control against several diseases, such as wounds caused by *Agrobacterium tumefaciens* and some leaf and tissue diseases caused by bacteria [13]. The application of *Azospirillum* in agriculture, especially food crops, is still very limited on a small scale. This paper is a literature review that reviews the potential of *Azospirillum* as a biofertilizer and pathogen biocontrol in food crops with a focus on its application and impact on the productivity sector. Search for literature related to the topics listed in the search for current and relevant information about the use of bacteria of the genus *Azospirillum* in the cultivation of food crops. Among the studies, selected reports on the use of bacteria from the genus *Azospirillum* in food crops, which may contain results obtained in the field or in a controlled environment.

## 2. POTENTIAL OF AZOSPIRILLUM AS BIOFERTILIZER IN FOOD PLANT

Biofertilizer are fertilizers that contain microorganisms whose presence can be single or in the form of a combination of several types called consortia [15]. *Azospirillum* is one of the bacterial species that is used as one of the leading fundamental biological fertilizers in nature, which is involved in the process of fixing atmospheric nitrogen into most of the world's staple food crops such as rice, corn, sorghum, wheat and millet [16]. Inoculation of *Azospirillum* in several food crops such as rice, wheat, sorghum, and corn can increase yields compared to controls [5].

*Azospirillum sp.* is a non-symbiotic nitrogen-fixing soil bacterium, including a group of rhizobacteria that has the potential as a biological fertilizer [17]. These bacteria live freely in the soil, which are around or close to the roots [18]. From the results of research *Azospirillum sp.* has many benefits in soil and plants, so it is often used as a biofertilizer, because *Azospirillum* can produce ethylene [19]. In the plant body, ethylene is widely needed in morphophysiological processes, such as root initiation, inhibition of root elongation, acceleration of fruit ripening, acceleration of seed germination, and activation of the biosynthesis of other phytohormones [20].

Several studies that have been carried out in testing *Azospirillum* on food plants are still at the testing stage in the laboratory or greenhouse, only a few in the field.

The results of the study using nitrogen fixing inoculants and phosphate solvents, either as single or mixed inoculants, were generally able to increase the growth and yield of maize plants. The single inoculants of *Azospirillum sp.* can able to stimulate the growth of corn plants [13]. This is in line with the results of research [21] that single inoculants of *Azospirillum sp.* and mixed inoculants with *Azotobacter sp.* and *Aspergillus sp.* are able to stimulate the growth of corn.

The results of research [22] on maize plants in a greenhouse using sterile sand, a source of isolate from maize roots, it is known that *Azospirillum* inoculation can increase plant growth and plant total N content, in addition to increasing the efficiency of N fertilization. This is in line with research conducted by [10] who were also in a greenhouse with isolate sources from weed roots, showed the results of *Azospirillum sp.* are able to increase the growth of corn plants on sterile sand medium.

The results of the study of biological fertilizers containing *Azospirillum* were able to fix 30% nitrogen in corn plants [23]. Nitrogen is needed to stimulate the vegetative growth of plants as a whole, especially the growth of roots, stems and leaves, also plays a role in the formation of leaf green matter (chlorophyll) which is very important for the photosynthesis process. The results of further research inoculation of *Azospirillum sp.* on corn can increase the total dry weight of the plant by 52.55% and increase the yield of corn by 108.84% [21].

The research results of [24], activities in experimental gardens with acid soil, source of isolate from the Research and Development Center for Isotope & Radiation Technology BATAN, Srikandi corn varieties seeds inoculated with *Azospirillum* ( $10^7$  cells/plant) increased maize yield 16.16% compared with without inoculation. Meanwhile, research on Bisma corn varieties with *Azospirillum* ( $10^7$  cells/plant) can increase corn yields up to 18% compared to those without inoculation [25].

Application of nitrogen fixing biological fertilizers such as *Azospirillum sp.* in several studies it was able to reduce the use of urea, improve soil health and rice yields [26], so it can be considered for application to lowland rice. Increased production of lowland rice through the application of biological fertilizers requires isolates of indigenous *Azospirillum sp.*, this indicates that these bacteria are able to adapt and have a high capacity to fix nitrogen in paddy fields with half wet conditions. The population of *Azospirillum* decreases when rice fields are inundated [27].

According to [27], activities in greenhouses using sterile paddy soil medium, isolate sources from rice roots then inoculation of *Azospirillum* bacteria were carried out immediately after planting rice seedlings, in

the results of this study there was no significant. Then research conducted by Riyanti and Listanto [28] where the source of *Azospirillum* isolates and mutants came from the collection of the Microbiology Laboratory of BB Biogen, showed that the results in the generative phase could increase the number of panicles and grain weight per clump. Meanwhile, the results of research [29], his activities in a greenhouse with sterile swamp soil media, and the isolation source from swamp soil, showed that inoculation of *Azospirillum* and phosphate solubilizing bacteria was able to increase vegetative growth of rice plants and increase N and P levels in rice plants and land.

Several research results on rice plants, showed the application of *Azospirillum sp.* are able to significantly increase the nitrogen uptake of rice plants [30]. IAA produced by *Azospirillum* can stimulate the development of wheat roots [31] and the improvement of rice roots [32]. The results of other studies, *Azospirillum* inoculation in several crops such as rice, wheat, sorghum, and corn can increase crop yields compared to controls [5].

Several other reports also indicated the positive effect of *Azospirillum* on various food crops such as barley (a type of wheat), legumes (beans, peanuts, peas, and soybeans). The positive effect of *Azospirillum* on various types of plants is still being studied, both using existing *Azospirillum* strains and local isolates obtained/isolated from the local soil environment (indigenous).

Currently, several products containing *Azospirillum* have been commercialized. In Indonesia, various types of bacteria including *Azospirillum* are found in various types/brands of commercial compound biofertilizers (compound biological fertilizers containing more than one type/strain of microbes). Among them are free-living N-fixing bacteria and P solubilizing bacteria which are also capable of producing growth hormone. The results of research conducted by Ezward [33] application of bioboost fertilizer (containing *Azotobacter*, *Azospirillum*, and *Bacillus sp.*) to sorghum plants can increase plant height, increase N availability, and increase nutrient uptake and productivity.

The bio-fertilizer BIOST (Bio Organic Soil Treatment) contains the microbes *Azotobacter*, *Azospirillum*, *Mycorrhizae*, *Bacillus*, and *Tricoderma*. BIOST biofertilizers that improve soil properties can reduce soil damage. The microbial activity contained in it can also help decompose nutrient elements that are strongly bound and directly (non-symbiotically) able to take N from the free air, so as to reduce the use of N fertilizer. BIOST fertilizer is an example of a biological fertilizer that can improve soil structure and fertility [34].

In general, the limited use of some biofertilizer products is related to the inconsistency of the effectiveness of rhizobacteria in the field [35]. The variety of environmental conditions (soil type, level of soil management, climate, and cultivated plants) with a short field testing period and inappropriate application techniques are obstacles that still need to be researched for its successful use in the future. Saikia [36] said that the use of *Azospirillum* as a biological fertilizer is very beneficial because it can increase crop production and improve environmental quality by reducing the use of chemical fertilizers and can improve and improve soil health to achieve sustainable agriculture. The evolution of research trends that examine the use of *Azospirillum* bacteria from time to time in maize and rice plants can be seen in Tables 1 and 2.

### 3. POTENTIAL OF AZOSPIRILLUM AS BIOCONTROL AGENT IN FOOD PLANT

*Azospirillum* belongs to a group of bacteria that have been used not only as biological fertilizers but also as active ingredients for pesticides [37]. The real progress obtained from research on the use of *Azospirillum* for plants has increased the enthusiasm of researchers to popularize it as an important agent in environmentally friendly agricultural production systems, because the use of *Azospirillum* will reduce the use of excessive synthetic chemical compounds, both in the provision of plant nutrients (biofertilizers) and in controlling pathogens soil borne (bioprotectants).

The presence of *Azospirillum* in plant roots is able to produce amino acids, organic acids, sugars, and other aromatic compounds that can function to protect plants from root pathogens. The strains from *Azospirillum* genera are not classical agents for bio-control, but some of them can help by their secretion to control some pathogens [39]. *Azospirillum* as a biocontrol agent in food crops has not been widely reported. The results of Tortora [38] showed that *Azospirillum brasilense* can be used as a biocontrol agent to treat anthracnose in strawberry plants by inhibiting the use of Fe by pathogens by producing siderophores. The production of siderophores by *Azospirillum brasilense* is thought to inhibit the metabolic activity of the pathogenic fungus *Colletotrichum acutatum* which is the cause of anthracnose disease in strawberry plants.

**Table 1.** Some research results in testing *Azospirillum* on corn plants in Indonesia

No.	How to apply	Media	Isolate Source	Research sites	Research result	Reference
1.	Inoculation This was done by giving 1 ml of <i>Azospirillum</i> bacteria suspension to the roots of corn.	sterile sand	Isolation from corn plant roots	Greenhouse	<i>Azospirillum</i> inoculation increases plant growth and total N content plants, in addition to increasing efficiency fertilization N.	Gandanegara et al. [22]
2.	Bisma variety corn seeds were inoculated with isolates <i>Azospirillum</i> with sterile peat carrier, so that each seed gets $10^6$ cfu.	Latosol sterile soil	Isolate <i>Azospirillum</i> selected by the Research Center for Biotechnology-LIPI	Greenhouse	Plant growth was not significantly different from the control	Gandanegara et al. [22]
3.	Corn seeds of Srikandi variety were inoculated with <i>Azospirillum</i> ( $10^7$ cells/plant)	sour soil	Research and Development Center for Isotope & Radiation Technology BATAN	Field (Experimental Garden)	<i>Azospirillum</i> inoculation can increase corn yield 16.16% compared to no inoculation	Mustikawati et al. [24]
4.	Corn seeds of Bisma variety were inoculated with <i>Azospirillum</i> ( $10^7$ cells/plant)	sour soil	Research and Development Center for Isotope & Radiation Technology BATAN	Field (Experimental Garden)	<i>Azospirillum</i> inoculation can increase corn yield 18% compared to no inoculation	Mustikawati and Gandanegara, [25]
5.	<i>Azospirillum</i> liquid inoculum ( $10^8$ cfu ml <sup>-1</sup> ) sprinkled around the roots using a micropipette.	sterile sand	Isolation from weed roots	Greenhouse	<i>Azospirillum</i> spp. Are able to increase the growth of corn plants on sand medium sterile	Oedjijono et al. [10]

**Table 2.** Some research results in testing *Azospirillum* on rice plants in Indonesia

No.	How to apply	Media	Isolate Source	Research sites	Research result	Reference
1.	Inoculation <i>Azospirillum</i> bacteria is carried out immediately after planting rice seedlings	sterile paddy soil	Isolation from rice plant roots	Greenhouse	there is no significant increase significantly in root length and plant height due to inoculation of <i>Azospirillum</i> sp.	Danapriatna, [27]
2.	Rice seeds of Ciherang variety were inoculated with <i>Azospirillum</i> at a cell density of $10^6$ cells/ml in a	Soil	<i>Azospirillum</i> isolates and mutants from the collection of the Microbiology	Greenhouse	In the generative phase, it can increase the number of panicles and the weight of grain per clump	Riyanti and Listanto [28]

	seedling tank.		Laboratory, BB Biogen			
3.	<i>Azospirillum</i> inoculation on rice seeds	sterile swamp soil	Isolation from swamp soil	Greenhouse	<i>Azospirillum</i> and phosphate solubilizing bacteria are able to increase plant vegetative growth rice and increase the levels of N and P in soil.	Wuriesyliane, [29]

## CONCLUSION

1. Inoculation of *Azospirillum* in food crops in the field has not been the main area of research at this time.
2. Several recent field and greenhouse experiments, specifically for rice and maize, have shown promising potential.
3. The use of *Azospirillum* bacteria to improve the quality and quantity of food crop products and to develop environmentally friendly practices is a reality.
4. However, the development of research that defines the best strategy for the use of this technology should be carried out continuously, aiming at the best conditions for producers.
5. *Azospirillum* research specifically and integrated on food crops is still very much needed to increase the effectiveness of its use in the field.

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