Enhancing the Nutrient Quality of Compost Produced from Cow Manure and Food Waste Using Indigenous Nitrogen-Fixing Bacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*)

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Abstract

Organic waste recycling through composting represents a sustainable strategy for improving soil fertility while reducing environmental pollution. Bioaugmentation of compost with beneficial microorganisms has recently gained considerable attention as an effective approach for enhancing compost quality and nutrient availability. This study evaluated the effects of two indigenous free-living nitrogen-fixing bacteria, Azotobacter chroococcum and Azospirillum brasilense, isolated from agricultural soils in northwestern Syria, on the physicochemical properties of compost prepared from cow manure and household food residues. Both bacterial isolates were identified using morphological characteristics, Gram staining, selective media, and biochemical tests. Compost was produced through aerobic composting of cow manure and food waste (3:1, w/w) and subsequently inoculated with each bacterial isolate separately. The physicochemical properties of untreated and inoculated compost were evaluated by measuring pH, electrical conductivity (EC), organic matter, total nitrogen (N), available phosphorus (P), and available potassium (K). Data were analyzed using Student's t-test at the 5% significance level. Both bacterial inoculants significantly improved the nutrient composition of compost compared with the uninoculated control. The greatest enhancement was obtained with Azotobacter chroococcum, which markedly increased total nitrogen, available phosphorus, and available potassium while maintaining suitable pH and electrical conductivity values. Azospirillum brasilense also improved compost quality but to a lesser extent than Azotobacter. These findings demonstrate the potential of indigenous nitrogen-fixing bacteria as environmentally friendly bioinoculants for producing nutrient-enriched compost and promoting sustainable organic waste recycling.

Key words: Biofertilizer; Bioaugmentation; Compost quality; Cow manure; Food waste; *Azotobacter chroococcum*; *Azospirillum brasilense*; Nitrogen fixation.

1-Introduction:

The increasing generation of agricultural and household organic wastes has become one of the most pressing environmental challenges worldwide. Improper disposal of livestock manure and food residues contributes to greenhouse gas emissions, nutrient losses, unpleasant odors, and contamination of soil and water resources. Consequently, sustainable management and recycling of biodegradable wastes have become essential components of circular economy strategies and climate-smart agriculture (Bernal et al., 2017; Awasthi et al., 2020).

Aerobic composting is recognized as one of the most efficient biological technologies for converting biodegradable organic wastes into stable, pathogen-free, and nutrient-rich organic fertilizers. During the composting process, diverse microbial communities decompose complex organic compounds into humified materials that improve soil fertility, increase water-holding capacity, enhance soil structure, and supply essential macro- and micronutrients required for plant growth (Li et al., 2021; Sánchez-Monedero et al., 2018).

Besides supplying nutrients, mature compost stimulates soil biological activity by increasing microbial biomass, improving enzymatic activity, and promoting beneficial interactions between soil microorganisms and plants. These effects contribute to enhanced nutrient cycling and improved crop productivity while reducing dependence on synthetic fertilizers (Hargreaves et al., 2008; Awasthi et al., 2020).

Recently, microbial bioaugmentation has emerged as a promising approach for improving compost quality and accelerating the composting process. Bioaugmentation involves inoculating composting materials with selected beneficial microorganisms capable of enhancing organic matter degradation, increasing nutrient mineralization, suppressing pathogens, and improving the agronomic value of the final compost product (Zhang et al., 2021; Wang et al., 2022).

Among beneficial microorganisms, free-living nitrogen-fixing bacteria play an important role in sustainable agriculture because of their ability to convert atmospheric nitrogen into plant-available forms through biological nitrogen fixation. In addition to nitrogen fixation, these bacteria produce phytohormones such as indole-3-acetic acid (IAA), siderophores, vitamins, exopolysaccharides, and several metabolites that stimulate root development and improve nutrient uptake (Bhattacharyya and Jha, 2012; Backer et al., 2018).

Species belonging to the genera Azotobacter and Azospirillum are among the most extensively studied plant growth-promoting rhizobacteria (PGPR). *Azotobacter chroococcum* is an obligate aerobic diazotroph capable of fixing atmospheric nitrogen while producing vitamins, growth regulators, and antifungal compounds that improve soil fertility and plant performance (Mrkovacki and Milic, 2001; Sumbul et al., 2020). Likewise, *Azospirillum brasilense* enhances plant growth through biological nitrogen fixation, phytohormone production, improved root architecture, and increased nutrient acquisition, making it one of the most widely applied biofertilizer microorganisms worldwide (Cassán et al., 2020; Fukami et al., 2018).

Several studies have demonstrated that inoculation of compost with beneficial microorganisms can improve nutrient availability and accelerate organic matter stabilization. Nevertheless, most previous studies focused on commercial inoculants or microbial consortia, whereas relatively limited information is available regarding the use of indigenous nitrogen-fixing bacterial isolates adapted to local environmental conditions for enhancing compost quality. Native isolates are expected to possess greater ecological fitness and survival under local environmental conditions, thereby increasing their effectiveness during compost maturation (Awasthi et al., 2020; Wang et al., 2022).

Therefore, the objective of this study was to isolate and identify indigenous nitrogen-fixing bacteria from agricultural soils and evaluate their effectiveness in improving the physicochemical properties of compost produced from cow manure and household food residues. In addition, the study compared the performance of *Azotobacter chroococcum* and *Azospirillum brasilense* in enhancing nutrient availability and improving compost quality for sustainable agricultural applications..

2. Materials and Methods

2.1 Study Site and Soil Sampling

Agricultural soil samples were collected during the summer of 2019 from cultivated fields located in Baksa village, Lattakia Governorate, northwestern Syria. Three representative sampling sites were selected within the study area. Soil samples were collected from the 5–15 cm surface layer using sterile tools, placed in sterile polyethylene bags, transported immediately to the laboratory, and stored at 4°C until bacterial isolation.

The physicochemical properties of the collected soil were determined before bacterial isolation according to the standard analytical procedures described by FAO (2007).

2.2 Preparation of Compost

Compost was prepared by aerobic composting using fresh cow manure and household food residues at a mixing ratio of 3:1 (w/w). Approximately 15 kg of fresh cow manure and 5 kg of chopped food waste were thoroughly mixed and composted under aerobic conditions.

Moisture content was maintained by periodic watering, while the compost pile was manually turned every three days to ensure adequate aeration and uniform decomposition. The composting process continued for approximately four months until a stable mature compost was obtained, characterized by a dark brown color, earthy odor, and homogeneous texture.

2.3 Isolation of Nitrogen-Fixing Bacteria

Nitrogen-fixing bacteria were isolated from soil samples using serial dilution followed by cultivation on selective nitrogen-free media. Dobereiner's semi-solid malate medium and Congo Red medium were used for the selective isolation of diazotrophic bacteria.

Pure bacterial colonies were obtained through repeated streaking on nutrient agar plates before further characterization.

2.4 Identification of Bacterial Isolates

Bacterial isolates were characterized based on colony morphology, Gram staining, cell morphology, motility, and a series of biochemical tests, including catalase, oxidase, citrate utilization, urease production, gelatin liquefaction, indole production, nitrate reduction, carbohydrate utilization, and hydrogen sulfide production.

Species identification was performed according to Bergey's Manual of Systematic Bacteriology together with standard biochemical identification protocols.

Two nitrogen-fixing bacterial isolates were identified as:

- Azospirillum brasilense
- Azotobacter chroococcum

2.5 Compost Bioaugmentation

After compost maturation, the compost was divided into three experimental treatments:

Treatment	Description
Control	Mature compost without bacterial inoculation
AB	Compost inoculated with *Azospirillum brasilense*
AC	Compost inoculated with *Azotobacter chroococcum*

Each treatment was prepared in triplicate under identical laboratory conditions.

2.6 Physicochemical Analysis of Compost

The physicochemical characteristics of compost were determined before and after bacterial inoculation.

The following parameters were measured:

Parameter	Method
pH	Digital pH meter (1:5 compost-water suspension)
Electrical conductivity (EC)	Conductivity meter
Organic matter	Walkley–Black wet oxidation method
Total nitrogen	Kjeldahl digestion method
Available phosphorus	Murphy and Riley colorimetric method
Available potassium	Flame photometry after ammonium acetate extraction

All analyses were performed following internationally accepted analytical procedures.

2.7 Statistical Analysis

All experimental measurements were performed in triplicate, and results were expressed as **mean ± standard deviation (SD)**.

Differences between treatments were evaluated using **Student's t-test** at a significance level of **$P < 0.05$**.

Statistical analyses were performed using **IBM SPSS Statistics software** (Version 26, IBM Corp., Armonk, NY, USA).

Table 1. Physicochemical properties determined during compost evaluation

Property	Unit	Analytical Method
pH	—	Digital pH meter
EC	dS m^{-1}	Conductivity meter
Organic matter	%	Walkley–Black
Total nitrogen	%	Kjeldahl
Available phosphorus	%	Murphy–Riley
Available potassium	%	Flame photometer

3. Results

3.1 Physicochemical Characteristics of Soil and Mature Compost

The physicochemical characteristics of the agricultural soil used for bacterial isolation and the mature compost produced from cow manure and food residues are presented in **Table 2**.

The soil was classified as clay soil, consisting of 50.3% clay, 26.0% silt, and 23.7% sand. Soil pH was slightly acidic to neutral (6.5), while electrical conductivity was low (0.28 dS m^{-1}), indicating non-saline conditions suitable for microbial growth.

The mature compost exhibited a slightly acidic pH (6.0), relatively high electrical conductivity (0.38 dS m^{-1}), and contained appreciable amounts of organic matter and essential plant nutrients, including nitrogen, phosphorus, and potassium.

Table 2. Physicochemical properties of the agricultural soil and mature compost before bacterial inoculation.

Parameter	Soil	Mature Compost
Sand (%)	23.7	—
Silt (%)	26.0	—
Clay (%)	50.3	—
pH	6.5	6.0
EC (dS m^{-1})	0.28	0.38
Organic matter (%)	0.97	21.0
Total N (%)	0.041	1.53
Available P (%)	0.38	2.50
Available K (%)	1.04	2.80

3.2 Isolation and Identification of Nitrogen-Fixing Bacteria

Numerous bacterial colonies developed on nutrient agar following serial dilution of soil samples. Subsequent purification on selective media produced two morphologically distinct bacterial isolates capable of growing on nitrogen-free media.

The isolates exhibited characteristic growth on Congo Red medium, where colonies developed a dark crimson coloration typical of diazotrophic bacteria.

Microscopic examination following Gram staining revealed that the first isolate consisted of Gram-negative rod-shaped cells, whereas the second isolate consisted of Gram-negative spherical cells.

3.3 Biochemical Characterization of the Bacterial Isolates

The biochemical characteristics of the two bacterial isolates are summarized in **Table 3**.

Both isolates were positive for catalase, oxidase, citrate utilization, urease production, gelatin hydrolysis, glucose utilization, and mannitol fermentation. The isolates also exhibited active motility.

Based on colony morphology, Gram reaction, microscopic observations, and biochemical profiles, the isolates were identified as *Azospirillum brasilense* and *Azotobacter chroococcum*.

Table 3. Biochemical characteristics of the isolated bacteria.

Test	*A. brasilense*	*A. chroococcum*
Gram reaction	–	–
Cell morphology	Rod	Cocci
Motility	+	+
Catalase	+	+
Oxidase	+	+
Gelatin hydrolysis	+	+
Citrate utilization	+	+
Urease	+	+
Indole	–	+
Glucose	+	+
Mannitol	+	+
Maltose	+	–
Sucrose	–	+

3.4 Effect of Bacterial Inoculation on Compost Quality

Both bacterial inoculants improved the physicochemical characteristics of compost compared with the uninoculated control (**Table 4**).

Only minor variations were observed in compost pH and electrical conductivity after inoculation. However, bacterial treatments increased the concentrations of organic matter and essential nutrients.

The highest nutrient concentrations were consistently recorded in compost inoculated with *Azotobacter chroococcum*, particularly for total nitrogen, available phosphorus, and available potassium.

Table 4. Effect of bacterial inoculation on physicochemical properties of compost.

Parameter	Control	*A. brasilense*	*A. chroococcum*
pH	6.05	6.06	6.04
EC (dS m^{-1})	0.30	0.30	0.36
Organic matter (%)	24.6	24.9	24.7
Total N (%)	5.30	5.80	6.20
Available P (%)	5.90	6.40	8.50
Available K (%)	2.00	2.40	2.90

3.5 Comparison of Nutrient Enhancement by the Two Bacterial Isolates

The effectiveness of the two nitrogen-fixing bacterial isolates differed considerably with respect to nutrient enrichment.

Compared with the uninoculated compost, inoculation with *Azospirillum brasilense* increased total nitrogen by approximately **9.4%**, available phosphorus by **8.5%**, and available potassium by **20.0%**.

In contrast, compost inoculated with *Azotobacter chroococcum* exhibited greater improvements, increasing total nitrogen by approximately **17.0%**, available phosphorus by **44.1%**, and available potassium by **45.0%** relative to the uninoculated control.

Student's *t*-test revealed significant differences (**P < 0.05**) between the two bacterial inoculants with respect to nitrogen, phosphorus, and potassium concentrations, indicating that *Azotobacter chroococcum* was more effective than *Azospirillum brasilense* in enhancing compost nutrient quality under the conditions of this study.

4. Discussion

The present study demonstrated that inoculation of mature compost with indigenous nitrogen-fixing bacteria enhanced compost quality by increasing nutrient availability while maintaining favorable physicochemical conditions. The isolated strains were identified as *Azospirillum brasilense* and *Azotobacter chroococcum*, both of which are among the most extensively studied free-living plant growth-promoting rhizobacteria (PGPR).

The agricultural soil used for bacterial isolation exhibited suitable physicochemical characteristics for diazotrophic microorganisms, including a nearly neutral pH, low electrical conductivity, and non-saline conditions. Soil pH is considered one of the principal environmental factors regulating the diversity and activity of nitrogen-fixing bacteria because it directly influences enzyme activity, nutrient availability, and microbial metabolism (Bashan & de-Bashan, 2010; Bhattacharyya & Jha, 2012). Likewise, the mature compost possessed high organic matter content and appreciable concentrations of nitrogen, phosphorus, and potassium, providing a favorable substrate for microbial colonization and activity during the inoculation period.

The successful isolation of *A. brasilense* and *A. chroococcum* confirms the widespread occurrence of free-living diazotrophic bacteria in agricultural soils. Identification based on colony morphology, Gram staining, and biochemical characteristics was consistent with the classical descriptions reported by Bergey's Manual of Systematic Bacteriology (Krieg & Holt, 1984) and with numerous subsequent investigations on plant growth-promoting bacteria (Bashan & de-Bashan, 2010). Both bacterial species exhibited positive reactions for catalase, oxidase, citrate utilization, urease production, and gelatin hydrolysis, indicating high metabolic activity and adaptability to compost environments.

The improvement in compost nutrient composition following bacterial inoculation can primarily be attributed to enhanced microbial mineralization and biological nitrogen fixation. Both bacterial isolates increased total nitrogen content compared with the uninoculated control, confirming their capacity to convert atmospheric nitrogen into biologically available forms. Similar improvements in compost nitrogen have been reported following inoculation with diazotrophic bacteria during compost maturation (Vessey, 2003; Ahemad & Kibret, 2014). Nitrogen fixation not only enriches compost fertility but also stimulates microbial decomposition and accelerates nutrient cycling throughout the composting process.

Phosphorus availability also increased markedly after inoculation, particularly with *Azotobacter chroococcum*. This improvement is likely associated with the production of organic acids, phosphatases, and other metabolites capable of solubilizing insoluble phosphate compounds. Previous studies have demonstrated that both *Azospirillum* and *Azotobacter* possess phosphate-solubilizing abilities that enhance phosphorus bioavailability in organic substrates and agricultural soils (Rodríguez & Fraga, 1999; Richardson et al., 2009). Enhanced phosphorus availability is particularly important because phosphorus is frequently the second most limiting nutrient after nitrogen in crop production systems.

Similarly, potassium concentrations increased following bacterial inoculation. Potassium-solubilizing microorganisms release organic acids and chelating compounds that mobilize potassium from mineral sources into exchangeable forms available for plant uptake (Meena et al., 2016). Although potassium solubilization has received less attention than nitrogen fixation and phosphorus solubilization, increasing evidence suggests that multifunctional PGPR contribute simultaneously to the cycling of multiple essential nutrients.

Among the two isolates evaluated, *Azotobacter chroococcum* consistently produced the greatest improvements in nitrogen, phosphorus, and potassium concentrations. This superior performance may result from its higher capacity for atmospheric nitrogen fixation combined with the production of extracellular enzymes, vitamins, phytohormones, siderophores, and organic acids that collectively stimulate microbial activity and nutrient mineralization (Mrkovacki & Milic, 2001; Ahemad & Kibret, 2014). In contrast, although *Azospirillum brasilense* significantly improved compost quality, its primary ecological role is generally associated with rhizosphere colonization and stimulation of root development rather than maximum nutrient accumulation in compost alone (Bashan & de-Bashan, 2010).

The absence of substantial changes in compost pH and electrical conductivity following bacterial inoculation suggests that the beneficial effects resulted primarily from biological transformations rather than alterations in the chemical environment. Stable pH values are advantageous because extreme acidity or alkalinity may reduce microbial diversity and nutrient availability during compost maturation (Bernal et al., 2009). Likewise, maintaining moderate electrical conductivity minimizes the risk of salt accumulation that could adversely affect subsequent plant growth.

Overall, the findings demonstrate that inoculation of mature compost with indigenous nitrogen-fixing bacteria represents an effective biological strategy for improving compost nutritional quality. The dual capacity of these microorganisms to enhance nitrogen fixation while promoting phosphorus and potassium availability supports their potential use as environmentally friendly biofertilizers capable of reducing dependence on synthetic fertilizers. Future studies employing molecular identification techniques and field validation under different cropping systems are recommended to further optimize bacterial inoculation strategies and evaluate their long-term effects on soil fertility and crop productivity.

5. Conclusion

This study demonstrated that indigenous nitrogen-fixing bacteria isolated from agricultural soil can effectively improve the nutritional quality of mature compost. The two isolates were identified as *Azospirillum brasilense* and *Azotobacter chroococcum* based on their morphological and biochemical characteristics.

Inoculation of compost with both bacterial species enhanced total nitrogen, available phosphorus, and available potassium without causing undesirable changes in compost pH or electrical conductivity. Among the two isolates, *Azotobacter chroococcum* exhibited the greatest capacity to enrich compost with essential nutrients, indicating its superior potential as a biofertilizer inoculant.

These findings highlight the importance of exploiting beneficial indigenous microorganisms to enhance compost quality through biological nutrient enrichment. The application of nitrogen-fixing bacteria represents an environmentally sustainable approach that can reduce dependence on chemical fertilizers while improving nutrient recycling in agricultural production systems.

Future research should focus on molecular identification of the bacterial isolates using advanced genetic techniques, evaluation of their performance under field conditions, and assessment of their long-term effects on soil fertility, crop productivity, and soil microbial communities. Such studies will contribute to the development of efficient microbial biofertilizers for sustainable agriculture.

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