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Short communication

Poultry manure and banana waste are effective biofertilizer carriers for promoting plant growth and soil sustainability in banana crops

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ABSTRACT

The aims of our study were to compare the effectiveness of poultry manure (PM) and banana waste (BW), with regard to their use as inoculant carriers of a bacterial consortium constituted by strains of *Azospirillum*, *Azotobacter* and P-solubiliser bacteria and to establish the most efficient dose of biofertilizer for a soil cultivated with banana (*Musa paradisiaca* AAA Simmonds), with respect to improving plant performance and soil physical and microbiological properties. Six months after planting, plant growth had increased with increase in dose of the biofertilizers applied. The biofertilizer prepared on BW enhanced the density of P-solubiliser bacteria, the concentrations of available P and foliar P to a greater extent than with the biofertilizer prepared on PM. The increases produced by the biofertilizer prepared on PM for the soil aggregate stability, enzymatic activities and the labile carbon fractions were highly correlated to the dose applied. Both biofertilizers can be considered potentially useful as inoculant carriers of PGPR but the usefulness of BW appears to be restricted to moderate doses of application ($\leq$3%).

The banana (*Musa paradisiaca* AAA Simmonds) c.v. 'Dwarf Cavendish' is an important food crop in tropical areas of Mexico, with a cultivated area of 76,313 ha and an annual production of 2,196,155 Tm (SIAP-SAGARPA, 2006). In particular, about 19% of surface cultivated with banana is located in the state of Tabasco (SE Mexico). The system production is based on intensive use of inorganic fertilizers and pesticides that yields 7–70 Mg ha^{-1} of banana per year (Orozco-Romero and Pérez-Zamora, 2006); this can lead to a loss of organic matter, reduction of soil water holding capacity and soil structural stability and an increase in acidity and alkalinity (Roldán et al., 2005).

Biofertilizers based on rhizospheric beneficial microorganisms have emerged as an alternative to chemical fertilizers to increase soil fertility and crop production in sustainable agroecosystems (Wu et al., 2005). Beneficial, free-living soil bacteria are usually referred to as plant growth-promoting rhizobacteria or PGPR (Kloepper et al., 1989). PGPR participate in many key ecosystem processes, such as those involved in the biological control of plant pathogens, N fixation, solubilisation of nutrients and phytohormone synthesis (Vessey, 2003), and therefore deserve particular attention for sustainable agriculture. PGPRs are important components in the agroecosystems because not only can they contribute to nutrient availability in the soil, but they can also bind soil particles into stable aggregates, which improve soil structure and reduce erosion potential (Kohler et al., 2006).

Development of a successful inoculant involves the selection of a suitable carrier substrate in order to support the growth of the target organism and maintain a high number of inoculant bacteria. An appropriate carrier should contain a high organic matter and optimum nitrogen contents and should also be inexpensive and non-toxic. Incorporation of microorganisms in a carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers. Although peat is the carrier of choice, it is not ubiquitously distributed and a more readily available substrate may be required. Alternative carriers have been investigated including various clays, animal manure such as poultry manure (PM), composted plant materials or other complex organic matrices (Stephens and Rask, 2000). Because animal manure possibly contains pathogens and antibiotic, serious soil degradation and phytotoxicity effects may be associated with the application of un-composted PM to soil. We hypothesise that the banana waste (BW) may be useful and convenient as a carrier for the production and application of bacterial inoculum, thereby contributing to the sustainability of the agroecosystem.

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Table 1
Changes in growth parameters and foliar nutrients of *Musa paradisiaca* AAA Simmonds in response to different doses of biofertilizers, six months after planting ($n = 6$)

	Shoot (g dry wt)	Root (g dry wt)	Foliar N (g kg^{-1})	Foliar P (μg g^{-1})	Avail. P (μg g^{-1})
Control	122^{a}	121^{a}	12.2^{a}	779^{a}	52^{a}
Bio-PM (1%)	189^{bcd}	360de	18.4^{ab}	1159^{b}	60^{ab}
Bio-PM (2%)	234de	332^{d}	26.5^{bc}	1354^{bc}	76^{bc}
Bio-PM (3%)	207^{cd}	406^{e}	27.3^{bc}	1626^{d}	113^{d}
Bio-PM (4%)	281^{e}	542^{f}	28.6^{c}	1585^{cd}	115^{d}
Bio-BW (1%)	145^{ab}	155^{a}	10.6^{a}	1689de	82^{c}
Bio-BW (2%)	180^{bc}	175^{b}	11.1^{a}	1557^{cd}	129de
Bio-BW (3%)	199^{cd}	232^{bc}	11.3^{a}	1893^{ef}	145^{e}
Bio-BW (4%)	264^{e}	244^{c}	14.5^{a}	2092^{f}	192^{f}

For each parameter, values in columns followed by the same letter are not significant different (Tukey, $P < 0.05$).

Table 2
Two-factor ANOVA (type of biofertilizer and dose of biofertilizer) for all parameters studied in the rhizosphere soil of *Musa paradisiaca* AAA Simmonds six months after planting

	Biofertilizer (B)	Dose (D)	Interaction (B × D)
Shoot biomass	NS	<0.001	<0.001
Root biomass	<0.001	<0.001	<0.001
Shoot/root	<0.001	<0.001	0.015
Foliar N	<0.001	0.011	NS
Foliar P	<0.001	<0.001	0.012
Available P	<0.001	<0.001	<0.001
Azospirillum sp.	<0.001	<0.001	<0.001
Azotobacter sp.	NS	<0.001	<0.001
P-solubilizers	<0.001	<0.001	<0.001
Water-soluble C	0.011	<0.001	<0.001
Water-soluble carbohydrates	NS	<0.001	<0.001
C-biomass	<0.001	<0.001	<0.001
Aggregate stability	<0.001	<0.001	<0.001
Dehydrogenase	<0.001	<0.001	<0.001
Urease	NS	<0.001	<0.001
Protease	<0.001	<0.001	<0.001
Phosphatase	<0.001	<0.001	<0.001
β-Glucosidase	<0.001	<0.001	<0.001

P significance values.

The objectives of this study were to select suitable carrier (PM or BW) for inoculating a bacterial consortium and to establish the most efficient dose of biofertilizer application to a soil cultivated with banana (*M. paradisiaca* AAA Simmonds, with respect to improving plant performance and soil quality and functioning). Soil quality was assessed as the labile C fractions (water-soluble C, water-soluble carbohydrates and microbial biomass), enzyme activities (dehydrogenase, protease-BAA, urease, phosphatase and β-glucosidase) and soil aggregate stability.

We collected an agricultural soil (Eutric Fluvisol), used to cultivate banana, at the El Eden experimental field, near Tabasco (SE Mexico). The plant used in the experiment was banana (*M. paradisiaca* AAA Simmonds) 'Dwarf Cavendish'.

A bacterial consortium composed of native strains of *Azospirillum*, *Azotobacter* and P-solubiliser bacteria and two carriers, PM and BW, were used to prepare the biofertilizers. The bacterial strains were isolated from the agricultural soil using standard techniques following serial dilution of the soil and were identified using molecular methods, based on PCR denaturing gradient gel electrophoresis followed by 16S rDNA cloning and sequencing.

The experiment was a mesocosm assay, conducted as a completely randomised factorial design with two factors. The first factor had three levels: without biofertilization, fertilization with either biofertilizer prepared on PM or with biofertilizer prepared on BW. The second factor comprised the addition of biofertilizer at a rate of 0, 1, 2, 3 or 4%. Six replicates per treatment were carried out, making a total of 54 pots.

Six months after planting, fresh and dry (105 °C, 5 h) mass of shoots and roots were recorded. The foliar P was determined by colorimetry (Murphy and Riley, 1962). The foliar N was determined with the Kjeldahl method. Water-soluble carbon was measured with an automatic Carbon Analyser for liquid samples and water-soluble carbohydrates were determined by the method of Brink et al. (1960). Microbial biomass C was determined using the fumigation–extraction method (Vance et al., 1987). Dehydrogenase, urease, *N*-α-benzoyl-L-argininamide (BAA) hydrolyzing protease, phosphatase and β-glucosidase activities were assayed following the procedure described in Tabatabai (1982). The percentage of stable aggregates was determined by the method described in Lax et al. (1994). The population size of *Azotobacter*, *Azospirillum* and phosphate-solubilising bacteria was estimated using the dilution plating technique (Ranganayaki et al., 2006).

Biofertilizer addition, dose of biofertilizer and their interactions effects on measured variables were tested by a two-way analysis of variance and comparisons among means were made using Tukey's test calculated at $P < 0.05$.

Both biofertilizers prepared, using either PM or BW as the carrier for the bacterial inoculant, increased significantly shoot and root biomass of banana (Tables 1 and 2). Plant growth increased with increasing dose of biofertilizer application. The two biofertilizers showed similar levels of effectiveness in improving the performance of banana plants, independent of the type of carrier used.

The observed benefits on growth of banana by application of both biofertilizers seem likely to be due to the supply of nutrients to the crop. Foliar P was increased by both biofertilizers (Tables 1 and 2). At the highest dose of biofertilizer prepared on BW, foliar P was increased by about 169% with respect to the control plants. Phosphate solubilisers are able to degrade agrowastes of a lignocellulosic nature, such as BW, and excrete organic acids, which increase

Table 3
Changes in carbon fractions and microbial populations of rhizosphere soil of *Musa paradisiaca* AAA Simmonds in response to different doses of biofertilizers, six months after planting ($n = 6$)

	Azospirillum sp. (10^3 cfu g^{-1} dry soil)	*Azotobacter* sp. (10^3 cfu g^{-1} dry soil)	PSB (10^3 cfu g^{-1} dry soil)	WSC (μg g^{-1})	WSCH (μg g^{-1})	C-biomass (μg g^{-1})	Aggreg. stability (%)
Control	14.2^{a}	2.9^{a}	0.3^{a}	182^{a}	38^{a}	58^{a}	40.2^{a}
Bio-PM (1%)	32.7^{ab}	15.6^{ab}	3.0^{b}	219^{b}	43^{ab}	90^{ab}	47.8^{b}
Bio-PM (2%)	89.5^{cd}	19.2^{b}	6.8^{bc}	221^{b}	55^{bc}	149^{bc}	47.0^{b}
Bio-PM (3%)	105.4^{de}	19.2^{b}	7.5^{bc}	235^{b}	71^{d}	183^{c}	58.1^{c}
Bio-PM (4%)	310.1^{f}	78.9^{d}	11.3^{c}	309^{c}	86^{e}	495^{d}	58.8^{c}
Bio-BW (1%)	73.7^{cd}	18.2^{b}	19.8^{d}	219^{b}	64^{cd}	162^{c}	62.2^{cd}
Bio-BW (2%)	71.6^{cd}	26.7^{b}	20.8^{d}	239^{b}	67^{cd}	176^{c}	65.3^{cd}
Bio-BW (3%)	65.0^{bc}	42.1^{c}	28.2^{e}	230^{b}	67^{cd}	187^{c}	69.0^{d}
Bio-BW (4%)	140.1^{e}	45.3^{c}	42.6^{f}	240^{b}	68^{cd}	189^{c}	43.9^{ab}

WSC: water-soluble C; WSCH: water-soluble carbohydrates.
For each parameter, values in columns followed by the same letter are not significant different (Tukey, $P < 0.05$).

Table 4
Changes in enzyme activities of rhizosphere soil of *Musa paradisiaca* AAA Simmonds in response to different doses of biofertilizers, six months after planting ($n = 6$)

	Dhase (μg INTF g^{-1} soil)	Urease (μmol NH_3 g^{-1} h^{-1})	Protease (μmol NH_3 g^{-1} h^{-1})	Phosphatase (μmol PNP g^{-1} h^{-1})	β-Glucosidase (μmol PNP g^{-1} h^{-1})
Control	10^{a}	2.21^{a}	1.95^{a}	5.72^{a}	0.20^{a}
Bio-PM (1%)	31^{e}	3.45^{b}	3.10^{bc}	5.32^{a}	0.33^{b}
Bio-PM (2%)	36^{f}	3.83^{bc}	3.34^{bcd}	3.05^{c}	0.53^{c}
Bio-PM (3%)	38^{f}	4.51^{bc}	4.71^{e}	10.02^{b}	0.43^{bc}
Bio-PM (4%)	39^{f}	7.65^{e}	6.76^{f}	11.01^{b}	0.73^{d}
Bio-BW (1%)	8^{a}	4.30^{bc}	2.59^{ab}	9.28^{b}	1.17^{e}
Bio-BW (2%)	15^{b}	5.05^{cd}	3.45^{bcd}	9.74^{b}	1.20^{e}
Bio-BW (3%)	21^{c}	6.22^{d}	4.13^{de}	9.83^{b}	1.25^{e}
Bio-BW (4%)	26^{d}	3.74^{b}	3.54^{cd}	10.85^{b}	0.72^{d}

For each parameter, values in columns followed by the same letter are not significant different (Tukey, $P < 0.05$).

the concentration of phosphorus in solution by mechanisms involving chelation and exchange reactions (Vessey, 2003). In the present study, the population density of phosphate-solubilising bacteria was higher in the soil amended with the bacterial consortium inoculated on BW (Table 3). The greatest PSB population was correlated positively with the highest levels of available P in the soil and the foliar P in the plants inoculated with this type of biofertilizer. In contrast, N uptake by the plants followed a different trend to P uptake in response to application of the biofertilizers. Thus, N accumulation in the shoot was highest in the soil amended with the biofertilizer prepared on PM (Table 1). In this treatment, the population density of N-fixing bacteria like *Azotobacter* and *Azospirillum* was increased considerably with the highest dose of biofertilizer, reaching higher values than the soil amended with bacterial consortium inoculated on BW (Table 3). It has been demonstrated that *Azospirillum* are metabolically versatile and can grow vigorously in the presence of nitrogenous compounds in the soil, but as soon as the external nitrogen supply is exhausted the bacteria switch to diazotrophy. The increased growth of banana could also be due to nutrient supplementation among the inoculated organisms, which might have mutually enhanced their efficiencies of N fixation, by *Azotobacter* and *Azospirillum*, and P-solubilisation, by PSB (Rudresh et al., 2005).

The reactivation of microbial activity in the rhizosphere can increase plant nutrient availability, since the soil microbial community mediates the processes of organic matter turnover and nutrient cycling. In particular, a higher rhizodeposition of soluble C fractions is expected to stimulate microbial activity in the rhizosphere. The concentrations of water-soluble carbon and carbohydrates and C-biomass were higher in the soil amended with the highest dose of biofertilizer prepared on PM (Table 3). A direct measurement of the total microbial activity is the dehydrogenase activity. This enzyme is sufficiently sensitive to indicate perturbations caused by microbial inoculation (Alguacil et al., 2005). In this study, dehydrogenase activity was significantly higher in the soils amended with the biofertilizers, particularly with the biofertilizer prepared on PM (Table 4). Our results also show that both biofertilizers significantly stimulated hydrolases related to the cycle of N (urease and protease-BAA activities), P (phosphatase activity) and C (β-glucosidase activity). In the soil inoculated using the PM-based carrier, all hydrolases increased proportionally to the dose of biofertilizer. In contrast, it is worth noting that the soil amended with the highest dose of biofertilizer prepared on BW had lower urease and β-glucosidase activities than the soil inoculated at a rate equal to or less than 3%. Perhaps the release of lignin, as a consequence of BW mineralisation, could have had adverse effects on the functional activity of microflora when the highest dose was applied.

Soil structure largely determines soil quality and fertility, which in turn favours the plant growth (Roldán et al., 2006). Soil structural stability was significantly improved by the addition of both biofertilizers (Tables 2 and 3). The biological transformations that the banana waste and PM underwent during preparation of the bacterial inoculants increased the quantity of aggregate-stabilising agents. These include the microorganisms themselves and the polysaccharides from microbial synthesis and decomposition of the organic materials used as carriers (Six et al., 2006). Likewise, stimulation of microbial activity prompted by the root release of organic C into the rhizosphere soil of plants treated with the biofertilizers would have led to increased levels of bacteria and, particularly, of fungal populations, which are principally responsible for the formation of aggregates larger than 0.2 mm (Roldán et al., 1994). The quality of the substrate used as the carrier also alters fungal:bacterial ratios, with low-quality substrates (high C/N), such as banana waste, favouring fungi and high-quality substrates (low C/N), such as PM, favouring bacteria (Bossuyt et al., 2001). The biofertilizer prepared on BW was more effective with regard to improving aggregate stability when applied at a rate equal to or less than 3%. However, this effect disappeared when the dose of this biofertilizer was greater than 3%. This could be related to the adverse effects of lignin on the activity of microflora, as mentioned above, counteracting the beneficial effects that the application of a substrate with a higher concentration of carbon is able to produce on the stabilisation of structural aggregates.

In conclusion, the combined addition of strains of *Azospirillum*, *Azotobacter* and P-solubiliser bacteria, using either PM or BW as carrier, stimulated the growth of *M. paradisiaca* AAA Simmonds under greenhouse conditions. The effectiveness of the biofertilizer prepared on BW was related to its ability to support the growth and survival of phosphate-solubilising bacteria, with a subsequently enhanced P uptake. Both biofertilizers were effective in improving soil quality parameters, particularly inducing an improvement of structural stability. The beneficial effects of the biofertilizer prepared with PM on the soil physical, chemical, microbiological and biochemical quality were correlated highly with the dose applied. The beneficial use of banana waste as a carrier appears to be restricted to moderate doses of application ($\leq$3%).

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References

Alguacil, M.M., Caravaca, F., Roldán, A., 2005. Changes in rhizosphere microbial activity mediated by native or allochtonous AM fungi in the reafforestation of a Mediterranean degraded site. Biology and Fertility of Soils 41, 59–68.

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16, 195–208.

Brink, R.H., Dubach, P., Lynch, D.L., 1960. Measurements of carbohydrates in soil hydrolyzates with anthrone. Soil Science 89, 157–166.

Kloepper, J.W., Liftshitz, K., Zablotowitcz, R.M., 1989. Free-living bacterial inocula for enhancing crop productivity. Trends in Biotechnology 7, 39–43.

Kohler, J., Caravaca, F., Carrasco, L., Roldán, A., 2006. Contribution of *Pseudomonas mendocina* and *Glomus intraradices* to aggregates stabilisation and promotion of

biological properties in rhizosphere soil of lettuce plants under field conditions. Soil Use and Management 22, 298–304.
Lax, A., Díaz, E., Castillo, V., Albaladejo, J., 1994. Reclamation of physical and chemical properties of a salinized soil by organic amendment. Arid Soil Research and Rehabilitation 8, 9–17.
Murphy, J., Riley, J.P., 1962. A modified single solution method for determination of phosphate in natural waters. Analytica Chimica Acta 27, 31–36.
Orozco-Romero, J., Pérez-Zamora, O., 2006. Tensión de humedad del suelo y fertilización nitrogenada en plátano (*Musa*-AAA Simmonds) CV. Gran Enano. Agrociencia 40, 149–162.
Ranganayaki, N., Tilak, K.V.B.R., Manoharachary, Ch, Mukerji, K.G., 2006. Methods and techniques for isolation, enumeration and characterization of rhizosphere microorganisms. In: Mukerji, K.G., Manoharachary, C., Singh, J. (Eds.), Microbial Activity in the Rhizosphere. Springer Verlag, Berlin, Heidelberg, Germany, pp. 17–38.
Roldán, A., García-Orenes, F., Lax, A., 1994. An incubation experiment to determine factors involving aggregation changes in an arid soil receiving urban refuse. Soil Biology and Biochemistry 26, 1699–1707.
Roldán, A., Salinas-García, J.R., Alguacil, M.M., Díaz, E., Caravaca, F., 2005. Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. Geoderma 129, 178–185.
Roldán, A., Carrasco, L., Caravaca, F., 2006. Stability of desiccated rhizosphere soil aggregates of mycorrhizal *Juniperus oxycedrus* grown in a desertified soil amended with a composted organic residue. Soil Biology and Biochemistry 38, 2722–2730.
Rudresh, D.L., Shivaprakash, M.K., Prasad, R.D., 2005. Effect of combined application of *Rhizobium*, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L.). Applied Soil Ecology 28, 139–146.
SIAP-SAGARPA, 2006. Sistema de Información Agroalimentaria de Consulta 1980–2006 (SIACON). México DF Available from: <http://www.siapsagarpa.gob.mx>.
Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to C-sequestration in agroecosystems. Soil Science Society of America Journal 70, 555–569.
Stephens, J.H.G., Rask, H.M., 2000. Inoculant production and formulation. Field Crops Research 65, 249–258.
Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, E.M., Keeney, D.R. (Eds.), Methods of Soil Analysis. American Society of Agronomy and Soil Science Society of America, Madison, WI, pp. 501–538.
Vance, E.D., Brookes, P.C., Jenkinson, D., 1987. An extraction method for measuring microbial biomass carbon. Soil Biology and Biochemistry 19, 703–707.
Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255, 571–586.
Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C., Wong, M.H., 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125, 155–166.