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CHARACTERIZATION AND QUALITY ASSESSMENT OF INDONESIAN COMMERCIAL BIOFERTILIZERS

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ABSTRACT

Biofertilizers currently sold in the market are not labeled with a distinct quality standard. As such, farmers may buy low quality biofertilizers, which can reduce their profit and trust on the benefit of biofertilizers. This paper presents the characteristics of various products of commercial biofertilizers as well as farmers' knowledge and experience on the products. The study was carried out in 2004-2006 by collecting and analyzing data on registered commercial biofertilizers, checking their availability at the market (39 agricultural shops), and interviewing farmers on their knowledge and experience on the use of biofertilizers on various farming systems in Bandung District, West Java (86 respondents) and Semarang District, Central Java (77 respondents). The quality of biofertilizers was tested in the laboratory based on microbial density and its functional (phenotypic) traits. The study showed that amongst various brands of biofertilizers commercialized, 41 brands of them have been officially registered as commercial products. Two brands of other biofertilizers found in agricultural shops were registered as organic or inorganic fertilizers. In general, each biofertilizer contained two or more microbial strains and was claimed to have multiple functions. However, most of them (>90%) were not labeled with expiry date information. Macronutrient contents (NPK) of some microbial carriers were almost equal to those of organic fertilizers. Around 38% of respondents in Bandung knew biofertilizers and less than 10% have ever used them. In Semarang, however, familiarity and personal experience of the respondents were much lower, i.e. 10% and 3%, respectively. About 67% and 50% of agricultural shops in Bandung and Semarang sold biofertilizers, respectively. Laboratory analyses showed that microbial density of five biofertilizers tested was lower than that of product specification, although most of them were positive for N-fixing and P-solubilizing traits. Some microbial strains contained less than 10^3 cfu based on the dilution level testing. These figures imply the urgent need to improve the existing quality standard system of biofertilizers including its control mechanisms.

[***Keywords:*** Biofertilizers, macronutrients, microbial density, N-fixing trait, P-solubilizing trait]

INTRODUCTION

To date, various brands of registered and unregistered biofertilizers have been commercialized without labeling of quality standard. As such, the possibility of farmers to buy low quality biofertilizer cannot be avoided, which may reduce farmers' profit, and worst, weaken their trust on the benefit of biofertilizer for agriculture. Without proper attention, this condition will negate promotion to develop environmentally benign agriculture through increase use of non-synthetic agrochemical inputs on agricultural lands.

Actually, a limited quality standard system of biofertilizer has been introduced since 1984 based on the Decree of Director General of Food Crops, Ministry of Agriculture and reestablished in 1991 (Decree no. I.A.5.84.5 and I.HK.050.91.7A). The system was specific for rhizobial inoculants; thereby rendering other biofertilizers uncontrolled although partial quality tests were still conducted as a requirement to register the products. However, recent development in soil microbiological research and inoculant production technology makes it possible to produce various kinds of biofertilizers, including compound biofertilizers that may contain a mixture of strains of the same or different functional groups.

In compound biofertilizer, various strains of bacteria from genus *Rhizobium*, *Azotobacter*, *Azospirillum*, *Bacillus, Lactobacillus,* or *Pseudomonas* had been mixed together with actinomycetes or fungi such as *Streptomyces* or *Aspergillus*. Thus, besides fixing atmospheric N_2 and solubilizing fixed phosphates, a compound biofertilizer is often claimed to promote plant growth or to suppress the growth of pathogens because it contains various plant growth regulator (hormone), antibiotic or other metabolite producing microbes. Although various findings in the last few years showed ample beneficial traits of soil microbes in enhancing plant growth and the possibility of a microbial strain to have more than one of functional traits (Glick 1995; Cattelan *et al*. 1999; Husen 2003), the overall effectiveness of mixed inoculants (compound biofertilizers) in farmers' field and farmers' acceptance on the products had not yet been evaluated.

Enrichment of carrier with macro- and micro-nutrients, hormones, and even fungicides is another important issue of current commercial biofertilizers as reported by Simanungkalit (2001). This practice, according to this author, could conceal or complicate the main effect of inoculant and possibly shorten its effective duration. This also confused the grouping of the products; whether bio, organic or inorganic fertilizers. To date, data on nutrient composition of commercial biofertilizers' carrier are not available. Compilation of the data is important to evaluate whether or not a particular product (inoculant) is appropriate to be categorized as a biofertilizer. The purpose of this study was to evaluate the characteristics of various products of biofertilizer, farmers' knowledge and acceptance, and to test the quality of some representative commercial biofertilizers as a basis to develop a comprehensive quality standard and its control mechanism.

MATERIALS AND METHODS

The study was conducted through field survey, interview of farmers in Bandung (West Java) and Semarang Districts (Central Java), and laboratory tests from 2004 to 2006. The two districts have a high accessibility and are exposed to various introductions of new technologies.

Secondary data (including preliminary laboratory analyses) were collected from Direktorat Pupuk dan Pestisida (2003), Balai Penelitian Tanah (2005a), and Direktorat Sarana Produksi (2006). Biofertilizers for laboratory tests were purchased randomly from agricultural shops and biofertilizers' manufactures.

Data Collection and Analyses

Data collected included a list of registered biofertilizers (status in years 2003 and 2006), registered and unregistered biofertilizers (from agricultural shops), kinds of biofertilizers (single strain or compound), microbial content and functions, carrier characteristics and nutrient composition, kinds of packaging, manufactures, expiry date, etc. Data on the availability of biofertilizers in agricultural shops were obtained through visiting 15 and 22 agricultural shops in Bandung and Semarang, respectively.

Microbial content was listed from the most to the least numerous uses of microbial strains in terms of genus or species and its functional group. The NPK content of biofertilizers' carrier was compared with those of commercial organic fertilizers (liquid and solid forms).

Interview of Farmers' Knowledge and Use of Biofertilizers

Data on farmers' knowledge and use of biofertilizers were obtained through interview of farmers individually. The farmers were selected proportionally from various farming systems, i.e. paddy field, rainfed rice, upland food crops, vegetable crops, and mixed cropping farmings. The number of respondents was 86 and 71 farmers in Bandung and Semarang, respectively. Questionnaire as a tool to gather the information was designed semi-structurally with key questions whether or not the respondents have known biofertilizers and ever used them.

Quality Assessment

Quality assessment was conducted based on microbial density and its phenotypic or functional traits, the commonly used tests for quality evaluation of biofertilizer. Five brands of registered biofertilizers representing 2 solid and 3 liquid forms and 1 single strain and 4 compound biofertilizers (containing 2-5 microbes of different strains or species) were selected. These five biofertilizers were grouped as plant growth enhancers based on manufacturers' claim that they contain dinitrogen fixing bacteria, P-solubilizing microbes and that they improve soil fertility and crop production. Water content and pH were included in the analyses.

Estimation of total viable numbers (microbial density) was conducted by dilution plate count method according to Zuberer (1994). Physiological saline solution (0.85% NaCl) was used as diluents for all ten-fold dilution series. The first ten-fold dilution was agitated using rotary shaker with the speed of 150 rpm for 30 and 60 minutes for the liquid and solid forms, respectively. Granular biofertilizer was pulverized prior to dilution. The aliquots of each dilution were spread onto selective and non-selective agar medium. The number of colony forming unit (cfu) after incubation period (1-10 days) constitutes population density (cfu per g dry weight or cfu per ml of biofertilizer).

The selective media were N-free *Azotobacter* agar for free-living dinitrogen fixing bacteria, yeast mannitol agar (YMA) for *Rhizobium*, Pikovskaya agar for P-solubilizing bacteria, and MRS (Man, Rogosa & Sharpe) agar for *Lactobacillus*. The non-selective media (to grow various functional groups of microbes) included nutrient agar (NA), tryptone-yeast (TY), and complex media for bacteria; M3 agar for actinomycetes; potato dextrose agar (PDA) and Czapex-Dox media for fungi. The use of various non-selective

media was to evaluate their suitability in biofertilizer assay. The composition of each medium was based on Cowan (1974), Somasegaran and Hoben (1994), Wellington and Toth (1994), Alef and Nannipieri (1995), and Subba-Rao (1999).

Quantitative analyses were conducted for indole-acetic acid (IAA) producing and P-solubilizing abilities of the microbes in each biofertilizer by colorimetric method. Media used were minimal salt (MS) medium with L-tryptophan (Frankenberger and Poth 1988) and yeast-glucose (Benizri *et al*. 1998) for IAA-producing microbes and Pikovskaya (Subba-Rao 1999) and hydroxyapatite (Kim *et al*. 1997) for P-solubilizing microbes. One milliliter of aliquot from the first ten-fold dilution of each biofertilizer was inoculated into 50 ml of broth medium and grown for 48 hours at rotary shaker for 150 rpm.

The ability of inoculant (microbes in biofertilizers) to synthesize IAA in broth of MS medium + tryptophan and yeast-glucose was measured colorimetrically according to Gordon and Weber (1951). The measurement was conducted at 0 (control) and after 48-hour incubation periods. Supernatant was separated from microbial cells by centrifugation at 8000 rpm for 10 minutes. One milliliter of supernatant was mixed with 2 ml Salkowski reagent (0.01M $FeCl_3$ in 35% $HClO_4$) and absorbance was measured at 530 nm.

Quantitative analysis of P-solubilizing bacteria was conducted by growing the inoculant in Pikovskaya broth [2.5 g L^{-1} $Ca_3(PO_4)_2$] and media containing 0.4% hydroxyapatite. P released into broth medium was measured based on Olsen and Sommer (1982) and Balai Penelitian Tanah (2005b). Supernatant from broth culture was mixed with phosphate reagent (ascorbic acid + concentrate P-reactant) at ratio of 1 and 5 and the absorbance was measured at 693 nm.

RESULTS AND DISCUSSION

Registered and Commercialized Biofertilizers

Amongst various brands of commercial biofertilizers (the total numbers are not known), about 41 brands of them have been officially registered as commercial products; 35 of them were produced by 23 fertilizer companies and have been registered officially in 2002 (Direktorat Pupuk dan Pestisida 2003), and six brands were new registered in 2006 by four other fertilizer companies (Direktorat Sarana Produksi 2006). Low demand and acceptance, as well as limited knowledge of farmers on the benefit of biofertilizer might be the reasons for the decreased numbers of registration.

Field visit to 15 and 24 agricultural shops in Bandung and Semarang, respectively, revealed that besides the registered biofertilizers, most agricultural shops also sold a few brands of unregistered biofertilizers. Two biofertilizer brands found in agricultural shops in Bandung and Semarang were registered as organic or compound macro-microfertilizers although the labels clearly mentioned that these fertilizers contained microbes and functioned as plant growth enhancer. More than 90% of the manufactures did not state the expiry date of the products. Therefore, the products may not be effective anymore. A strict regulation on the commercialization of biofertilizers should be formulated to protect farmer interests.

Characteristics of Commercial Biofertilizers

Secondary data of laboratory analyses showed that almost all of the commercial biofertilizers were categorized as compound biofertilizers. *Lactobacillus* sp. was the most commonly used species in current commercial biofertilizers instead of *Azotobacter* sp., *Bacillus* sp., *Rhizobium* sp. or *Azospirillum* sp. that were most intensively studied for agriculture (Table 1). Some manufactures preferred to state functional groups of microbes to species in the label and P-solubilizing and N-fixing bacteria were the most and the least frequently stated, respectively. However, none of the products found gave complete description

Table 1. The frequency of microbes in biofertilizers based on microbial species and functional groups (from 39 samples).

Microorganisms	Frequency
Microbial species	
Lactobacillus sp.	12
Azotobacter sp.	9
Bacillus sp.	9
Rhizobium sp.	8
Azospirillum sp.	6
Streptococcus	4
Aspergillus sp.	3
Pseudomonas sp.	3
(Other microbes)	2
Glomus sp.	1
(Other microbes)	1
Microbial functional groups	
P-solubilizing bacteria	10
Photosynthetic bacteria	6
Cellulolytic microbes	3
Nitrifying bacteria	1
N-fixing bacteria	1
Microbial groups	
Fungi	7
Actinomycetes	7
Bacteria	2

of the product in the label. This important information, i.e. microbial species/strains, densities and functions, as well as direction for application in the label should be obligatory in commercializing biofertilizers, otherwise inappropriate use may occur.

Macronutrient content, such as N, P and K, in some biofertilizers' carrier exceeded that in organic fertilizers (Table 2). This is often questioned whether plant growth increase is due to microbial activities or nutrients and other compounds added to the carrier, although, in general, the rates of biofertilizer application is much lower than that of organic fertilizers.

A few manufacturers may have enriched existing commercial biofertilizers with various compounds including fungicides as reported by Simanungkalit (2001). The use of municipal waste compost (rather than peat or charcoals) as biofertilizer carriers, which may contain significant amount of heavy metals, may have happened. These practices are not only confound the effects, but also potentially endanger soil microbes and crop quality. Thus, a strict regulation on carrier composition should be defined in quality specification.

Availability, Farmers' Knowledge and Use of Biofertilizers

About 67% and 50% of agricultural shops in Bandung and Semarang sold biofertilizers, respectively. However, its availability in every agricultural shop was only for one or two most wanted or popular kinds (brands). About 10% and 38% of farmers in Semarang and Bandung, respectively, have recognized biofertilizers although less than 10% have ever used them for farming (Fig. 1). Limited agricultural extension in socializing the benefits and methods of application could be the reason for low adoption of biofertilizers.

Quality of Commercial Biofertilizers

Information on the pH and water content of the biofertilizer products evaluated are limited. These data are important to evaluate whether the physico-chemical properties of the products have changed within a period of shipment and storage.

Test results in Table 3 showed that water content and pH values of granular compound fertilizer CB-2 were not significantly different from those of the product specification or label, meaning that this biofertilizer is well packed and stored. On the other hand, too low pH such as in CB-3 (pH 3.36 and 3.50 based on product specification and test result, respectively) may be translated that a sophisticated technique in carrier preparation has been employed to support the survival of inoculants under this acid condition; or else the value might have changed as shown by CB-4

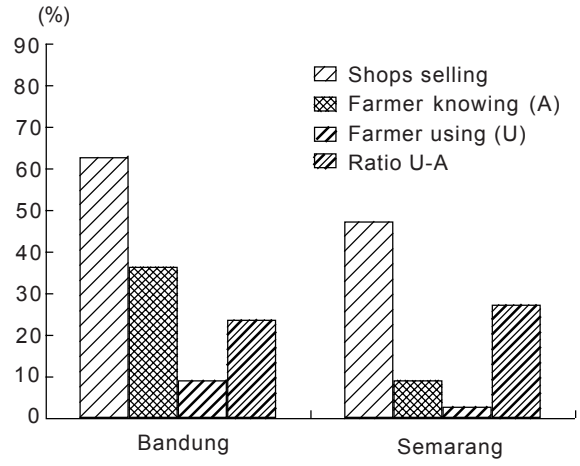

Fig. 1. Percentage of agricultural shops selling, farmers' knowing, farmers' using and the ratio of using and knowing biofertilizers in Bandung and Semarang Districts, 2004.

Table 2. Comparison of macronutrient contents of commercial biofertilizers' carrier and organic fertilizers.

Simple statistics	Biofertilizers			Organic fertilizers		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Liquid form						
Number of samples	19	20	20	4	4	4
Average (%)	0.488	0.027	0.152	3.677	0.043	0.921
Minimum (%)	0.001	0.00002	0.001	2.50	0.001	0.41
Maximum (%)	11.23	7.44	8.12	4.57	0.18	1.73
Solid form						
Number of samples	10	10	10	20	20	20
Average (%)	1.821	0.238	1.582	1.08	2.088	0.912
Minimum (%)	0.45	0.0001	0.55	0.06	0.20	0.06
Maximum (%)	11.50	16.48	9.20	9.07	11.04	8.95

Table 3. Water content and pH values of biofertilizers tested.

Biofertilizers tested[1]	Product specification[2]		Test results	
	Water content (%)	pH	Water content (%)	pH
CB-1 (solid-powder)	na[3]	na	30.4	7.6
CB-2 (solid-granule)	14.07	7.15	14.8	6.9
CB-3 (liquid)	na	3.36	na	3.5
CB-4 (liquid)	na	na	na	3.8
SB-1 (liquid)	na	na	na	4.4

[1]Codes and modifier are not the original names of commercial biofertilizers; CB = compound biofertilizer, SB = single strain biofertilizer.
[2]Source: Direktorat Pupuk dan Pestisida (2003).
[3]na = not available.

and single strain biofertilizer SB-1 (pH values of these two products were not included in the product specification).

Analyses on microbial densities based on genus and functional group approaches showed that biofertilizer CB-1 had the highest population density (Table 4). Total viable cell of *Rhizobium* and P-solubilizing bacteria in selective media and bacteria in non-selective media exceeded those of its product specification. These values in CB-2 were almost comparable with its product specification, especially for total bacteria and fungi using complex media and potato dextrose agar, respectively. On the other hand, microbial density of CB-3 and CB-4 was much lower than their product specification, especially for total *Actinomycetes* and *Lactobacillus* as shown by CB-3. The presence of *Lactobacillus* and fungi on CB-4 as in product specification was not detected in the dilution plate of up to 10^3. Biofertilizer SB-1 was not detected to contain microbes although the manufacture claimed to contain P-solubilizing bacteria as printed on the label of the product.

Since the expiry information of the products was not available, the results presented in this report assumed that all of the sampled biofertilizers were still applicable. The order of quality from the highest to the lowest was CB-1 and CB-2 > CB-3 and CB-4 > SB-1. If quality standard of various single-strain biofertilizers is applied for these compound biofertilizers, such as that used by Ghosh (2001), only biofertilizer CB-1 met the standard because the microbial density, as the most critical characteristics, of $>10^7$ cfu g^{-1} was only met by this biofertilizer.

Defining the critical value for microbial density of compound biofertilizers is a challenge as they contain various functional groups that may compete to grow in agar media. The approach to use functional or physiological groups of microbes (bacteria, actinomycetes, and fungi) in estimating their microbial density is likely workable since the results are quite comparable with species density of the product specification, otherwise other suitable media must be defined for each species.

Quantitative analyses to test the ability of biofertilizers in solubilizing fixed phosphates in broth media of Pikovskaya and hydroxyapatite are shown in Table 5. The amount of P solubilized from Pikovskaya media was much higher than that from hydroxyapatite indicating that the later is less suitable for quantitative analysis of phosphate solubilizing bacteria.

The presence of available P at zero time of incubation implied that the carrier contained available P or has been enriched with P prior to inoculation (injection of the carrier with microbial broth). Except SB-1, other four tested biofertilizers were able to solubilize fixed phosphates as indicated by the increase in available P and the decrease in pH after incubation. The decrease in pH medium is believed as one of the phosphate solubilizing bacteria mechanisms in dissolving fixed phosphates by producing organic acids, such as α-ketoglutarat (Louw and Webley 1959), citric, oxalic, malic, and lactic acids (De Freitas *et al*. 1997; Kim *et al*. 1997). This result verifies previous test that there were no detectable microbes in SB-1.

The ability of biofertilizer to produce plant growth regulator IAA was exhibited only by CB-2 (Table 6). *Azospirillum lipoverum* contained in CB-2 and known as IAA-producer could be the agent of synthesizing IAA from tryptophan added to minimal salt medium as indicated by the increase in IAA concentration after incubation period. Without supplying tryptophan as in yeast-glucose medium, the amount of IAA produced was possibly less than that of IAA degraded (for microbial metabolism). The use of yeast-glucose medium to test IAA synthesis seems unsuitable for biofertilizer analysis.

Both quantitative analyses, P solubilization and IAA production, are the examples of functional trait tests, the results of which were complementary with previous test on microbial density. The critical value of these tests should further be defined also for quality standard of compound biofertilizers as for the single strain biofertilizers.

Table 4. Analyses of microbial density of five selected commercial biofertilizers.

Product specification (form, color, microbial content and density)[1]	Analyses of microbial density		
	Genus and functional groups	Media agar used	cfu g^{-1} or ml^{-1}
CB-1: solid (powder), grayish black			
Rhizobium (1.75×10^8)	*Rhizobium*	Yeast mannitol	1.3×10^9
PSB (2.7×10^8)	PSB[2)]	Pikovskaya	1.6×10^9
	Bacteria	Nutrient agar	4.9×10^6
		Complex medium	2.7×10^9
		Tryptone-yeast	4.1×10^7
CB-2: Solid (granule), gray			
Azotobacter beijerinckii (1.9×10^8)	*Azotobacter*	N-free *Azotobacter*	5×10^6
Aeromonas punctata (5×10^8)	PSB	Pikovskaya	4.5×10^6
Azospirillum lipoverum (1.2×10^8)	Bacteria	Nutrient agar	clp[3)]
		Complex medium	1.3×10^7
		Tryptone-yeast	3.9×10^5
Aspergillus niger (5×10^7)	Fungi	Potato dextrose	5.8×10^7
		Czapex-Dox	1.9×10^5
CB-3: liquid, brown			
PSB (5.7×10^7)	PSB	Pikovskaya	2.5×10^5
Lactobacillus (3.7×10^7)	*Lactobacillus*	MRS	3.0×10^5
Azotobacter (1.7×10^7)	*Azotobacter*	N-free *Azotobacter*	4.1×10^5
Rhizobium (13.3×10^7)	Bacteria	Nutrient agar	clp
		Complex medium	-[4)]
		Tryptone-yeast	-
Actinomycetes (5.8×10^7)	Actinomycetes	M3	9×10^4
CB-4: liquid, brown			
Azotobacter (1.08×10^7)	*Azotobacter*	N-free *Azotobacter*	4.3×10^6
Lactobacillus (4.15×10^7)	*Lactobacillus*	MRS	-
Bacillus (2.37×10^8)	PSB	Pikovskaya	3.5×10^6
Acetobacter (2.13×10^7)	Bacteria	Nutrient agar	clp
		Complex medium	2.2×10^6
		Tryptone-yeast	-
Yeast (3.62×10^6)	Fungi	Czapex-Dox	-
SB-1: liquid, brown			
PSB	PSB	Pikovskaya	-
	Bacteria	Nutrient agar	-
		Complex medium	-
		Tryptone-yeast	-

[1]Codes and modifier are not the original names of commercial biofertilizers (CB = compound biofertilizer; SB = Single strain biofertilizer; Source: Direktorat Pupuk dan Pestisida 2003).
[2]PSB = Phosphate solubilizing bacteria.
[3]clp = Microbial colonies were clumped and not enumerated.
[4]Microbial colonies did not grow at the dilution up to 10^3.

CONCLUSION

Total of 41 brands of officially registered biofertilizers were in the form of compound biofertilizers, the quality of which was distinctly variable and can be misleading. Two brands of other biofertilizers found in agricultural shops were registered as organic or compound macro-microfertilizers although the labels clearly mentioned that these products contained microbes and functioned as plant growth enhancer. Incomplete and inconsistent description of the product specifications for user guidance, such as expiry date information, microbial strains and density, nutrient content, dosage and method of application are common. Thus, the possibility of farmers to get expired biofertilizers cannot be avoided. Low popularity and acceptance of biofertilizers among farmers in Bandung and Semarang might be related to variable quality and limited agricultural extension.

Table 5. Amount of P solubilized and change of pH at Pikovskaya and Hydroxyapatite medium (average of three replications).

Media used and biofertilizers tested[1]	Available P and pH at incubation periods				Difference	
	0 hour		48 hours			
	P (ppm)	pH	P (ppm)	pH	P (ppm)	pH
Pikovskaya [2.5 g L^{-1} $Ca_3(PO_4)_2$]						
CB-1	54.9	5.78	290.0	4.48	235.1	-1.30
CB-2	54.1	5.74	228.5	4.40	174.4	-1.34
CB-3	50.2	5.70	282.5	4.45	232.3	-1.25
CB-4	49.6	5.79	262.7	4.60	213.1	-1.19
SB-1	51.5	5.73	130.1	5.80	78.6	0.07
Hydroxyapatite (4 g L^{-1} HY)						
CB-1	12.0	5.84	40.8	5.28	28.8	-0.56
CB-2	12.2	5.87	49.6	4.49	37.5	-1.38
CB-3	12.4	5.84	30.7	5.63	18.3	-0.21
CB-4	11.8	5.95	34.1	4.90	22.3	-1.05
SB-1	12.5	5.85	16.9	5.91	4.4	0.06

[1]Codes and modifier are not the original names of commercial biofertilizers; CB = compound biofertilizer, SB = single strain biofertilizer.

Table 6. Amount of IAA produced at MSM + tryptophan and yeast-glucose medium (average of three replications).

Media used and biofertilizers tested[1]	Amount of IAA at incubation periods (ppm)		Difference (ppm)
	0 hour	48 hours	
MSM + tryptophan			
CB-1	10.0	6.5	-3.5
CB-2	9.9	14.7	4.8
CB-3	9.6	8.3	-1.3
CB-4	10.7	9.7	-1.0
SB-1	9.8	6.8	-3.1
Yeast-glucose			
CB-1	13.1	9.9	-3.2
CB-2	13.4	8.9	-4.5
CB-3	13.4	10.9	-2.5
CB-4	13.1	9.7	-3.4
SB-1	13.5	9.4	-4.1

[1]Codes and modifier are not the original names of commercial biofertilizers; CB = compound biofertilizer, SB = single strain biofertilizer.

Laboratory analyses of representative five commercial biofertilizers showed that their microbial density was lower than those of product specification, although most of them were positive for N-fixing and P-solubilizing traits. Some microbial strains contained less than 10^3 cfu ml^{-1} based on the dilution level testing. These figures imply the urgent need to improve the existing quality standard system of biofertilizers including its control mechanisms.

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