Year: 2014; Volume: 1; Issue: 1
Article ID: BT14 05; Pages: 1-6

BioMed Research

The Open Access Publisher

www.bmrjournals.com

BMR Biotechnology

Research Article

Comparative Study of Vermicast and Charcoal Used as a Carrier Inoculums to the Biofertilizer Preparation

Packialakshmi N and Aliya Riswana T

P.G. and Research Department of Microbiology, Jamal Mohamed College, Trichy-620020, Tamil Nadu, India.

Correspondence should be addressed to Packialakshmi N and Aliya Riswana T.

Received 29 April 2014; Accepted 23 May 2014; Published 23 May 2014

Abstract

The high cost of fertilizer production and environmental pollution caused by the use of these fertilizers makes necessary to use other sources especially biofertilizers. The biomass of the bacterium *Azotobacter* can be used as a biofertilizer due to its ability to fix nitrogen from the atmosphere. The purpose of this research was to study the survival of bacteria *Azotobacter* on different carrier such as vermicast and charcoal. The physical and chemical parameters of the carriers were analyzed to determine the effect of carrier in the survival of bacteria. Bacterial population inoculated in carriers were measured at times 0, 15, 30, 45, and 60 days by colony forming unit. The results of bacterial count after 15 days incubation showed that bacterial population in vermicast was increased and decreased in the charcoal. Considering these results, the use of vermicast as carrier could increase the survival of bacteria.

Keywords: *Azotobacter*, Vermicast, Charcoal and Biofertilizer.

Introduction

Application of biofertilizer for crop production is environmental friendly and sustainable for ecological system. Several types of biofertilizer have been developed from bacteria, particularly *Rhizobium* species, *Azospirillum* species, *Azotobacter* species and used in the production of various plants [6]. Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants uptake of nutrients by their interactions in the rhizosphere when applied through seed or to soil. Biofertilizer from nitrogen fixing bacteria come in three forms: liquid, solid and lyophilized. For liquid and lyophilized ones, only solution medium is used, but for solid form, carriers such as peat, activated charcoal and chicken dung are needed. *Azotobacter* is a free living nitrogen

fixing bacteria is a beneficial biofertilizer which has profitable effects on plants and soil fertility. *Azotobacter* is gram-negative, motile, pleomorphic aerobic bacterium which produces catalase, oval or spherical that form thick walled cysts and may produce large quantities of capsular slime[3, 4].

Presently lignite powder is being used as carrier material by most of the bioinoculant producing units in India. Often it has also been found that its availability is also made difficult, as it is being used as fuel by thermal power stations, etc. Availability of quality lignite powder is also in doubt because of adulteration by agents and improper mesh size in the pulverizing unit. Several scientists have suggested compost as carrier material for biofertilizers. But the role of good compost in maintaining microbial population has not been studied much. The existing studies exhibit that the earthworm casts is ideal material for carrying microbial culture from the agriculture point of view[2]. In the present study, the effect of charcoal and vermicast as carrier in maintaining the shelf life of *Azotobacter* in powder inoculum at room temperature.

Materials and Methods

Preparation of carriers

Charcoal and vermicast were used in this study. The raw material were ground, sieved with 0.5 cm mesh screen and dried in a hot air oven at 60°C for two days. The materials were autoclave at 121°C at a pressure of 15 lb for 30 minutes.

Analysis of physical and chemical properties

The carriers were analyzed for physical and chemical properties including pH, temperature, moisture, total nitrogen (N_{tot}), total phosphorus (P_{tot}) and total potassium (K_{tot}).

Collection of soil samples

Soil samples were collected from Semmedu-Kolli hill forest, Namakkal, Tamil Nadu.

Isolation of *Azotobacter* from soil sample:

Azotobacter was isolated from the soil sample by serial dilution as 1.0g of air dried samples was dissolved in 99ml of distilled water. The soil suspension was further diluted up to 10^{-6} level. The diluted soil suspension (0.1ml) was spread on the surface of Jensen agar medium which is a selective medium for isolating *Azotobacter*. The pH of the medium was adjusted to 7.0 with the help of 1N HCl/ 1N NaOH. The plates were incubated at 28°C for 5-7 days and the colonies were observed. Strains of *Azotobacter* were picked out and purified by repeated streaking on Jensen medium and were preserved as slant culture for further usage.

Identification of isolates

The isolate was identified by Gram staining and the biochemical characterization of the isolate was carried out by using standard method.

Cultivation of *Azotobacter* powder inoculum and packaging

A loopfull of *Azotobacter* pure culture was transferred into 250ml Erlenmeyer flask containing 100ml of Ashby's broth and incubated at 28°C on 120 rpm rotary shaker for 72 hours. After incubation, 10ml of the inoculums was transferred to 1000ml of respective broth and kept in shaking incubator for mass multiplication. 750ml was mixed thoroughly with 1000g of each sterile carrier, adjusted the moisture content to 75% water holding capacity, packed in polyethylene bags, sealed and incubated under room temperature. The inoculums were repacked in sterile polyethylene bags and stored at room temperature for further usage.

Evaluation for survival of the *Azotobacter* during storage at room temperature:

The survival of *Azotobacter* was determined after the inoculum was subjected to different carriers at room temperature. Ten grams of each sample was taken for estimating viable cells at the initial day, 15, 30, 45 and 60 days after storage using dilution plating method on Ashby's agar and incubated at 28°C for 5-7 days. The number of apparent *Azotobacter* colonies after incubation from both carriers was counted and calculated into viable cells.

Results

Physical and chemical properties of carriers

Some physical and chemical properties of the carriers are shown in (Table 1). The pH of vermicast was nearly neutral compared to charcoal. The highest moisture content was found in vermicast, while that of charcoal was the lowest. There was a significantly difference among the physical and chemical properties of the carriers.

Isolation of *Azotobacter*

Azotobacter was isolated from rhizosphere soil by serial dilution method. *Azotobacter* strain was recovered from the soil samples collected using Jensen agar medium. This media is very specific for the isolation of Azotobacter.

Colony morphology

Large mucoid opaque colonies were observed.

Gram staining

The smear was examined under the microscope, the isolates does not retain violet colour and it was to be confirmed as Gram-negative and it showed rod or spherical shape thick walled cyst in the cell morphology.

Characterization of *Azotobacter* on various biochemical tests

The confirmation of *Azotobacter* was done through various biochemical tests. Isolates was showed positive results to MR, Citrate, Urease, Oxidase, Catalase and Nitrate where they expressed negative result to Indole and VP. The isolates were efficient in hydrolyzing Starch (Table 2).

Survival of *Azotobacter* in the carriers at room temperature

After 60 days of inoculants maintenance, the population of the bacteria was determined through Colony Forming Unit method. At initial days the bacterial population was higher in charcoal. After 15 days of incubation, the bacterial population in the charcoal was intensively declined (Figure 1). While the population of bacteria in the vermicast increased after 15 days (Figure 2). As listed in Table.3, the best carrier was treatment of vermicast. The weakest carrier was charcoal.

Table 1: Physical and chemical properties of carriers

Parameter	Carrier materials	
	Vermicast	Charcoal
pH	7.15	7.45
Moisture (%)	18.18	15
Water uptake (mins)	18(mins)	28(mins)
N_{tot} (%)	8.5	0.83
P_{tot} (%)	6.0	0.39
K_{tot} (%)	5.0	0.24

Table 2: Characteristics of *Azotobacter*

Characteristics	Observations
Shape	Rod or spherical
Gram's reaction	Negative
Indole production	-
Methyl red	+
Voges proskauer	-
Citrate utilisation	+
Nitrate reduction	+
Urease	+
Catalase	+
Oxidase	+
Starch hydrolysis	+
TSI	Alkaline slant, Acid butt

Table 3: Population of inoculants during storage at room temperature

Days	Carrier	Colony forming unit/gram	
		10^{-4}	10^{-5}
0	Charcoal	350	197
	Vermicast	132	108
15	Charcoal	243	129
	Vermicast	142	117
30	Charcoal	158	109
	Vermicast	159	128
45	Charcoal	117	87
	Vermicast	179	140
60	Charcoal	62	20
	Vermicast	192	165

Figure 1: Survival of *Azotobacter* inoculants in charcoal carrier on different days of incubation (0, 15, 30, 45, and 60 days)

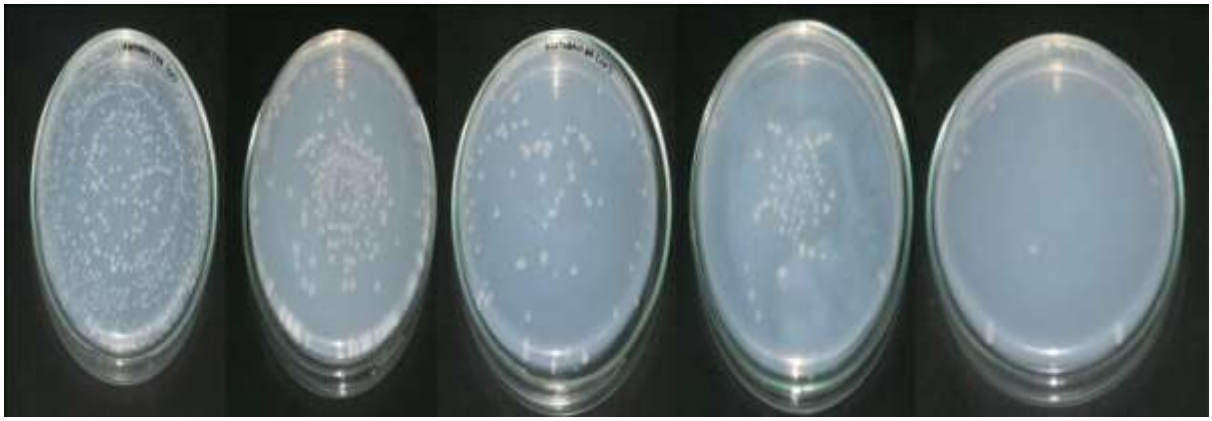
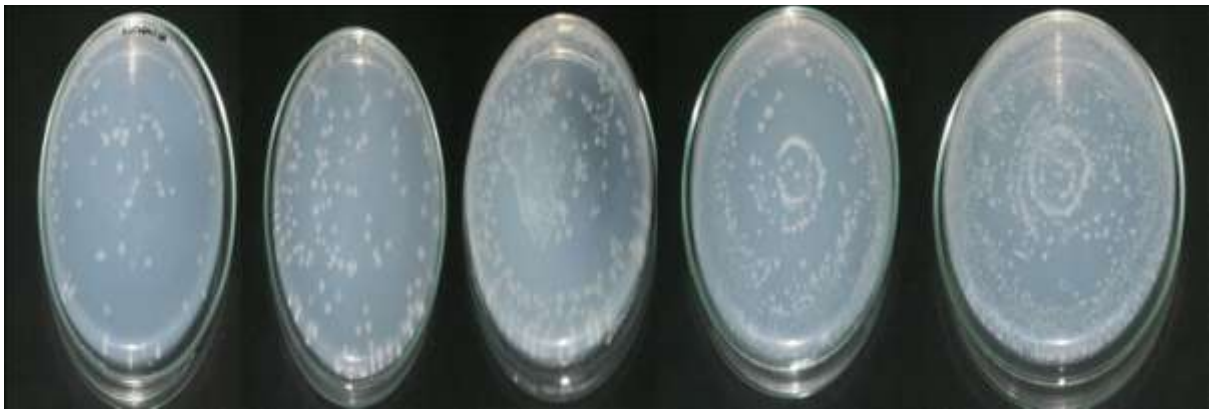


Figure 2: Survival of *Azotobacter* inoculants in vermicast carrier on different days of incubation (0, 15, 30, 45, and 60 days)



Discussion

Previous studies have suggested that high temperature could cause growth and increase of the bacterial population, it produced wastes that were not only toxic for bacteria, but also changed the pH of medium that could be the reason of population reduction and death of bacteria[1]. Mendez and Videira (2005) stated that bacterial maintenance at 28°C for 41 days caused an increase in number of viable bacterial cells on all carriers so that the population reached nearly 10^9 bacteria per gram of carrier[7].

The study by Kalra *et al.* (2008) that the granular vermicompost as a carrier is capable of holding 10^8 viable bacteria after 180 days[5].Sekar and Karmegam (2010) reported that vermicasts from *E. euginae* as a carrier material which supports the survival of more than $1x10^7$ g^{-1} viable cells of *A. chroococcum*, *B. megaterium* and *R. leguminosarum* till the end of 10th month which is longer than observed in lignite (a commercial carrier material)[9]. Saleh *et al.* (2001) reported that the population of *Azotobacter vinelandii* A1 in rice husk carrier rise up to 128% from the initial population after storing at 30°C. At 30 days after storage, the bacterial population increased slightly in Pt and ptLC stored at 16°C, and Pt, PtCC, PtMC and PtLC at 5°C[8]. The vermicasts when used as carrier material for biofertilizers supported the survival rate for more than one year[9]. The findings of the present study also showed similar results when the vermicast used as carrier for the survival and viability of the

biofertilizer *Azotobacter* inoculant for long period during storage than the charcoal as a carrier.

Conclusion

Use of organic sources of fertilizers improved the soil chemical properties through increasing the content of macro and micronutrients and organic carbon in the subtropical climate of India. The biological property of soil with regard to microbial count was strengthened when Azotobacter was applied along with vermicast. The vermicast prepared from the earthworm *Perionyx excavatus* is the most suitable inoculum carrier after storage for 60 days. Vermicast inoculum can be stored at room temperature for a period 60 days and still maintained the bacterial population when compared to the *Azotobacter* inoculated in charcoal.

Acknowledgements

Authors are thankful to the Tamil Nadu government state council for science and technology to the supported this research work through scholarship sanctioned.

References

1. Dearmon, IA, Orlando MD, Rosenwald AJ, Albert FK, Ralph LF, Paul RM: Viability and estimation of shelf-life of bacterial populations. Journal of Applied Microbiology 1962, 10: 422-427.
2. Gandhi A, Sivakumar K: Impact of vermicompost carrier based bioinoculants on the growth, yield and quality of rice (*Oryza sativa* L.) C.V. NLR 145. An International Quarterly Journal of Environmental Sciences 2010, 4(1): 83-88.
3. Gomare KS, Mese M, Shetkar Y: Isolation of Azotobacter and cost effective production of biofertilizer. Indian Journal of Applied Research 2013, 3(5): 54-56.
4. Holt JG, Novel RK, Peter HAS, James TS, Stanley TW: Bergey's Manual of Determinative of Bacteriology (9th ed.). Lippincott Williams and Wilkins, USA 2000.
5. Kalra A, Chandra M, Khanuja SPS: A novel vermicompost based formulation for bioinoculants with added plant and microbial growth promoting natural products. In: Fuchs JG, Kupper T, Tamm L, Schenk K (ed) Proceedings of the International congress on compost and Digestate: Sustainability, Benefits, Impacts for the Environment and for Plant Production. Research institute of Organic Agriculture FiBL, Ackerstrasse 2008, CH-5070 Frick, 193-194.
6. Marisa Phiromtan, Thongchai Mala, Peerasak Srinives: Effect of various carriers and storage temperatures on survival of *Azotobacter vinelandii* NDD-CK-1 in powder inoculants. Modern Applied Science 2013, 7(6):81-89.
7. Mendes F, Videira I: Residues of the cork Industry as carriers for the production of Legume Inoculants. Av. da República, Quinta do Marquês, OEIRAS 2005, 2780-159.
8. Saleh EA, Nassar FR, Yassen AM: Survival o microorganisms of multi-strains inoculant on different carriers. Annals of Agricultural Science 2001, 39: 2163-2169.
9. Sekar KR, Karmegam N: Earthworm casts as an alternate carrier material for biofertilizers: assessment of endurance and viability of *Azotobacter chroococcum*, *Bacillus megaterium* and *Rhizobium leguminosarum*. Scientia Horticulturae 2010, 124(2):286-289.