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Conversion of cassava wastes for biofertilizer production using phosphate solubilizing fungi

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ABSTRACT

Two fungi characterized as *Aspergillus fumigatus* and *Aspergillus niger*, isolated from decaying cassava peels were used to convert cassava wastes by the semi-solid fermentation technique to phosphate biofertilizer. The isolates solubilized $Ca_3(PO_4)_2$, $AlPO_4$ and $FePO_4$ in liquid Pikovskaya medium, a process that was accompanied by acid production. Medium for the SSF fermentation was composed of 1% raw cassava starch and 3% poultry droppings as nutrients and 96% ground (0.5–1.5 mm) dried cassava peels as carrier material. During the 14 days fermentation, both test organisms increased in biomass in this medium as indicated by increases in phosphatase activity and drop in pH. Ground cassava peels satisfied many properties required of carrier material particularly in respect of the organisms under study. Biofertilizer produced using *A. niger* significantly ($p < .05$) improved the growth of pigeon pea [*Cajanus cajan* (L.) Millsp.] in pot experiments but product made with *A. fumigatus* did not.

1. Introduction

Phosphorus (P) is only second to nitrogen as a mineral nutrient required for plant growth. Because of their nature, tropical and subtropical soils are extremely deficient in this nutrient. In many countries, a massive increase in the rate of application of chemical fertilizers has been adopted to ameliorate this deficiency. In sub Saharan Africa, for example, chemical fertilizer consumption by the year 2015 is projected to grow by 63% of its 1997–1999 rates (Anon., 2006). Apart from its high price in developing countries, the supply of phosphorus for agriculture by the application of chemical fertilizers poses other problems. First is the observation that once in the soil, a large proportion of the phosphorus content of chemical fertilizers is quickly transformed to the insoluble form, thereby making them unavailable to plants. Secondly, there are global concerns that the unbalanced use of chemical fertilizers has a role in environmental degradation and climate change (Omar, 1998; Gyaneshwar et al., 2002).

Reviewing the role of phosphate-solubilizing microorganisms (PSM) in sustainable agriculture, Khan et al. (2007) concluded that in spite of the variations in their performance in situ, P supply through biological means, remains a viable alternative to the use of chemical fertilizers. However, many published reports about the applications of PSM in agriculture, have been done with pure microbial inocula making it difficult for adoption in developing countries. The availability of these inocula as biofertilizers will make them easier to handle and distribute among small scale farmers. Biofertilizers hold great promises for improving world food security through the enhancement of agricultural yield in developing countries such as Africa and Asia, which together hold 50% and 74% of the total land mass and population of the globe, respectively. For example, unlike chemical fertilizers, they can be cheaply produced anywhere and utilizing a wide range of raw materials including wastes from agricultural processing.

One example of such a material is cassava peel, which not only remains unexploited, but additionally constitutes a nuisance in the environment. Nigeria currently produces about 39 million metric tons of cassava (Adeniji, 2006). While advancements in utilizing effluents have been recorded, utilization of the solid fractions particularly the cassava peel (about 10% of the wet weight of the roots) remain limited because of its low digestibility and toxicity from high levels of hydrocyanic acid (Ubalua, 2007).

The objective of this study is to develop a low technology process, which can be adapted by the small scale industry in developing countries, to utilize cassava peels or other suitable farm wastes and suitable inocula to make phosphate biofertilizer. Such a product will provide an affordable means of distributing inocula to the small scale farmer and in addition present a viable means of disposing farm wastes.

2. Methods

2.1. Isolation and selection of microorganisms

In order to obtain phosphate-solubilizing microorganisms (PSM) capable of utilizing cassava wastes as a source of nutrients,

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the search for suitable isolates for this project was performed on decaying cassava peels. Ten-gram quantities of samples were extracted in 90 ml sterile distilled water from, which 0.1 ml aliquots were plated on Pikovskaya (1948) agar. The medium was composed of glucose, 10 g; $(NH_4)_2SO_4$, 0.5 g; NaCl, 0.2 g; KCl, 0.2 g; $MgSO_4{\cdot}7H_2O$, 0.1 g; $MnSO_4{\cdot}7H_2O$, 0.5 g; $FeSO_4{\cdot}7H_2O$, 0.5 g; yeast extract, 0.5 g; 15 g, agar powder and 5 g $Ca_3(PO4)_2$, in 1 L distilled water. Plates were incubated at 30 °C for five days.

Isolates showing zones of clearing were screened for their ability to utilize cassava starch as sole carbon source. Isolates which demonstrated good utilization of starch were subsequently screened for utilization of cellulose. The assays for utilization of starch and cellulose were done by growing selected isolates on mineral salts medium composed per litre of: K_2HPO_4, 2 g; $(NH_4)_2SO_4$, 2 g; NaCl, 2 g; $MgSO_4{\cdot}7H_2O$ 2 g; agar powder, 15 g and supplemented with 10 g raw cassava starch and CMC (Sigma), respectively. After incubation, 30 °C/48 h for starch and 30 °C/2 weeks for cellulose, plates were sprayed with iodine and Congo red, respectively to show zones of digestion of the substrates. Indices for phosphate solubilization and the utilization of starch and cellulose by the isolates were calculated using the formula; clearing zone diameter/colony diameter.

Two molds, which were finally selected, were identified by studies of their colonial and microscopic characteristics according to Smith (1971) and Patterson and McGinnis (2009). Isolates were stored on Sabouraud's dextrose agar (SDA) slants at 4 °C during the period of study.

2.2. Phosphate solubilization characteristics of selected organisms

The ability of selected organisms to solubilize different insoluble phosphates in liquid medium was studied in Pikovskaya (PVK) medium similarly composed as that used for PSM isolation but without the addition of agar. Tricalcium phosphate was substituted with other appropriate insoluble phosphates ($AlPO_4$ or $FePO_4$) as necessary. To perform the test, a spore suspension of test organism containing about 6×10^6 spores was inoculated into 250 ml of medium. Uninoculated flasks were set up as controls and all flasks incubated at 30 °C for seven days. Cultures were then centrifuged (Centurion Scientific Ltd.) at 5000 rpm for 15 min and the concentration of soluble phosphate in the supernatant determined using the photometric chlorostannous reduced phosphomolybdic acid blue method as described by Jackson (1967) on a Jenway 6405 UV/Vis Spectrophotometer. *p* values were deduced from a standard curve of soluble phosphate (KH_2PO_4) prepared under similar experimental conditions.

Salinity and pH of soil are important factors in the ability of PSM to solubilize phosphates. The effect of salt concentration was tested by amending Pikovskaya medium, containing $Ca_3(PO4)_2$ with NaCl (0.5–2%). The influence of pH was tested by adjusting the pH of same medium between 4 and 10. Inoculation of test organisms, incubation and then determination of concentration of soluble phosphate released under the various conditions were determined as previously described.

2.3. Production of biofertilizer by semi-solid fermentation (SSF)

The SSF medium was designed to incorporate both nutrients for the growth of biofertilizer organism during the fermentation (cassava starch, 1% and poultry droppings, 3%) as well as carrier (Ground cassava peel, 96%). The carrier was prepared from sundried cassava peels, which was ground coarsely in a domestic mill to granular form (0.5–1.5 mm). Cassava starch consisted of sundried effluent from a 'garri' processing industry.

Fermentation was performed by inoculating about 6×10^6 spores of test PSM suspended in 18 ml of sterile distilled water into 30 g of autoclaved media contained in 250 ml Erlenmeyer flasks. Fermentation lasted 14 days after, which the biofertilizer was dried in the sun until moisture content of 30–35% was attained.

2.4. Chemical and microbiological analyses

Moisture content was determined by drying samples at 105 °C to a constant weight. The pH of the SSF was determined on 5 g quantities of sample homogenized in 15 ml of distilled H_2O using a Hanna HI 991001 pH/Temperature meter. Mold growth was estimated by measurement of acid phosphatase activity (Eivazi and Tabatabai, 1997) of mycelia and enmeshed coarse particles of the SSF medium. Material for this assay was prepared by washing 2 g of the SSF held on Whatman No. 1 filter paper with sterile distilled water until the filtrate was clear. Biofertilizer shelf life at room temperature (about 30 °C) was determined by plating aliquots of a 10^{-4} dilution of sample on PDA and counting of colonies developing within 24 h.

2.5. Efficacy of biofertilizer

Soil for this experiment was sandy loam in nature with a pH of 5.2 and contained 268 mg/kg total P. About 10 kg of soil was thoroughly mixed and distributed in 500 g portions into polyethylene bags. A local variety of a legume, pigeon pea [*Cajanus cajan* (L.) Millsp.] was used as test plant. The experimental design was the completely randomized design with three replicates. Treatments were: T0 control (No biofertilizer) T1 (Biofertilizer applied by seed inoculation) and T2 (Biofertilizer applied by 1% soil inoculation). Seed inoculation was performed by mixing seeds with 5 g biofertilizer suspended in 10 ml of a 1% gel of starch. The plants were placed in a green house and watered regularly for 35 days. Plants were carefully uprooted at the end of this period, washed clean of sand particles and their whole weight recorded after they had been dried at 50 °C for 72 h. The results were subjected to analysis of variance (ANOVA) and the Tukey HSD (.05) test using the VassarStats (Lowry, 1999–2009) online statistical software.

2.6. Statistical analyses

All experiments were replicated trice and subjected to simple statistical analysis using Microsoft Excel. Results reported are means with, when necessary standard deviations.

3. Results and discussion

3.1. Identification of selected microorganisms and determination of ability to utilize cassava wastes

Two molds were selected from several organisms screened. The first isolate grew rapidly on SDA at 30 °C as dark smoky green velvety colonies with a slight yellow reverse, which grew darker with age. Microscopy of this culture revealed smooth-walled conidiophores, which terminated in flask shaped vesicles, fertile on two-thirds with phialades borne directly on them. The phialades bore rough globose conidia. This isolate was identified as *Aspergillus fumigatus*. The second isolate also showed rapid growth on SDA with colonies initially white but quickly becoming black. Reverse side of colonies was pale yellow. Microscopic examination revealed radiate conidial heads initially, which later split into columns. Long conidiophores were terminated in globose vesicles covered by metulae and phialides bearing rough, globose conidia. This fungus was identified as *Aspergillus niger*.

The starch and cellulose utilization indices for *A. fumigatus* were 1.3 ± 0.1 and 1.3 ± 0.08 and for *A. niger*, 1.6 ± 0.1 and 1.3 ± 0.07, respectively. The starch plates were read after two days of incuba-