APPLICATION OF BACILLUS SPECIES CULTURED ON DIFFERENT LOW COST ORGANIC SUBSTRATES FOR THE CONTROL OF ROOT-KNOT NEMATODE INFECTION ON OKRA (ABELMOSCHUS ESCULENTUS MOENCH)

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Abstract

Low cost organic substrates such as rice grain, chickpea, wheat grains, rice husk, chickpea husk and wheat bran were used to culture Bacillus subtilis and B. firmus. Vegetative cell counts of both bacterial species generally increased on majority of substrates in initial 5 days and declined in subsequent incubation. Spore counts also increased with time but declined at 10th day. Bacterial species cultured on various substrates were inoculated in soil before sowing of okra in a pot culture experiment. The growth of okra plants was significantly increased in all substrates except chickpea husk, as compared to control. Root-knot nematode infection severity was measured in term of galls/ root system and egg masses/ root system. Both bacterial species showed varying effectiveness when grown on different organic substrates, but they controlled root-knot nematodes infection in okra in comparison to the control.

Introduction

Bacillus species are widely distributed and well known plant growth promoting bacteria, commonly present in the rhizosphere. They increase plant growth by vanishing plant pathogens by their unique antimicrobial activities, including production of antibiotics (Awais et al., 2007) and toxins (Mukry et al., 2010) to compete with pathogenic organisms. Some strains are also known to induce systemic resistance in plants against variety of pathogens. Application of Bacillus species as biocontrol agents against plant pathogens is increasingly interesting these days (Bargabus et al., 2004, Han et al., 2005, Liu et al., 2009, Yánez-Mendizábal et al., 2012). It is a common practice to introduce Bacillus species on/around plant roots by multiplying them on culture media like nutrient and Luria Bertani agar/broth. Generally a single culture type is employed for biocontrol or improvement of plant growth and yield. Bacillus species are ubiquitous in nature found around the diverse habitats and have ability to grow and multiply on different types of organic substrates including plant and animal materials. Bacillus species can multiply on a variety of substrates, including cheap agricultural byproducts (Bishop, 2002). Solid state fermentation is helpful for production of bacterial metabolites at low cost on large scale (Bora & Kalita, 2009). It is reported that growth in nutrient rich media alters the biochemical properties of Bacillus species (Bizzarri et al., 2008). The production of different hydrolytic enzymes, including α-amylase and β-galactosidase increases on different organic substrates such as corn, wheat and rice as compared to commercial medium (Konsoula & Liakopoulou-Kyriakides, 2007).

The present study report a) growth and multiplication rate of two different Bacillus species namely Bacillus subtilis and B. firmus on different organic substrates including Rice, Rice husk, Chickpea, Chickpea husk, Wheat grains and Wheat bran for varying incubation period and also b) the effectiveness of these Bacillus species grown on these organic substrates to control root-knot nematodes and the growth of okra plants.

Materials and Methods

Growth of Bacillus species on organic substrates:

Bacteria were cultured on Luria Bertani (LB) broth for 24 hours at 37°C in shaking incubator. Cultures were centrifuged at 4000xg for 10 min., and bacterial cells were re-suspended in sterilized distilled water, after washing twice with sterilized distilled water. Six different organic substrates were selected for the study viz., Rice, Rice husk, Chickpea, Chickpea husk, Wheat grains and Wheat bran. Twenty gram from an organic substrate was taken on each flask; moisture was maintained at sixty percent with distilled water and autoclaved at 121°C at 15 psi for 15 minutes. 0.1 mL of cell suspension was added in each flask, which contained log 9.53557 CFU of B. subtilis and log 9.36008 CFU of B. firmus. Total colony forming units (CFU) and endospores /g of substrate was recorded by serial dilution technique after 5, 10 and 15 days of incubation at 28°C. Two series of dilutions were made for each sample at the time of observation, one set of dilutions were treated with heat at 80°C for 15 minutes in water bath (for endospore count), the other series was not heated (for total cell count). Appropriate dilution of 0.1 mL was taken in sterilized Petri plate and about 10 mL slightly warm LB agar was poured (Petras & Casida 1985). Data was transformed to log10 before statistical analysis.

Green house experiment:

Soil was collected from the field of the Department of Botany, University of Karachi. A two factor experiment was conducted in small plastic pots under greenhouse conditions. Two Bacillus species (Bacillus subtilis and B. firmus) were applied in soil after growing for 10 days on different organic substrates (Rice, Rice husk, Chickpea, Chickpea husk, Wheat grains and Wheat bran). Another set of pots was also arranged that contained organic substrates without bacteria. About 3g of substrate was inoculated in 300 g soil for each plastic pot. One g of each substrate contained bacterial colony forming units (Log10) as given in Table 1. The untreated pots were served as control. Five seeds were sown in each pot one day after inoculation and two seedlings per pot were...
maintained after germination. After seedlings have established, freshly hatched 2000 second stage juveniles of root-knot nematodes were released in soil around the roots of each plant. The pots were randomly placed on green house benches and watered daily. The plants were carefully harvested after 45 days of nematodes inoculation. Data on fresh shoot weight and plant height was recorded immediately after harvesting. To determine the severity of root-knot nematodes infection, the roots were collected from the plants and spread on large glass Petri plates and number of galls/root system and egg masses/root system were counted under stereomicroscope (4X). Large roots were cut into two or more pieces before counting.

Table 1. Population sizes of Bacillus species cultured on various organic substrates (log10 CFU g-1) at the time of their application in soil

<table>
<thead>
<tr>
<th>Organic substrates</th>
<th>Population log10 CFU g-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Rice</td>
<td>7.8906</td>
</tr>
<tr>
<td>Rice husk</td>
<td>9.7866</td>
</tr>
<tr>
<td>Chickpea</td>
<td>8.7854</td>
</tr>
<tr>
<td>Chickpea husk</td>
<td>9.2997</td>
</tr>
<tr>
<td>Wheat</td>
<td>9.0184</td>
</tr>
<tr>
<td>Wheat husk</td>
<td>8.0918</td>
</tr>
</tbody>
</table>

Results and Discussion

Bacterial populations including spore count significantly increased at 5 days of incubation. The maximum Bacillus subtilis population was observed in wheat bran (log10 CFU:10.8343), followed by chickpea (log10 CFU: 10.7645) on 5th day of incubation. The minimum growth of B. subtilis was recorded on chickpea husk. Total cell count declined at 10th day of incubation on most of the substrates except chickpea husk and rice husk. The spore count showed similar trend as we observed in total cell count. On chickpea husk and wheat grain at 15th day of incubation, total cell count and spore count was almost equal, indicating sporulation in all cells (Fig. 1). Bacillus firmus successfully multiplied on all substrates and population increased on all organic substrates. The maximum total spore count was observed in rice to be 11.5311 log10 CFU at 5 days of incubation, followed by chickpea (10.2014 log10 CFU) and wheat grain (10.1704 log10 CFU). A decline in total cell count was observed on 10th days of incubation in all organic substrates except in rice husk. In chickpea husk total cell count increased after 10 days of incubation. Similar trend of endospore count was noticed (Fig. 2). The two Bacillus species showed growth rates varying on different substrates. B. subtilis showed the maximum growth on wheat bran, while, B. firmus on rice. Bacillus species have been reported to multiply rapidly on different low cost organic substrates (Khan et al., 2011). The growth of Bacillus circulans has been reported to rapidly increase on rice straw during initial 24 hours (Dhillon et al., 2000). Wheat bran has been reported the best organic substrate for the growth and increased enzymatic activity of Bacillus species (Haq et al., 2003, Poopathi & Kumar, 2003). Bacillus thuringiensis strains was successfully multiplied and produced on rice and rice bran, exhibited insecticidal and nematicidal properties (Khan et al., 2011; Lee & Seleena, 1991). Chickpea and rice are rich source of Carbohydrates, proteins, minerals and vitamins, provides bacterial cells a suitable environment to multiply and produce biologically active metabolites. In Bacillus species sporulation takes place from 24 hours and time of sporulation depend on type of medium or substrate used; under some favorable conditions sporulation may take a week or more (Donnellan et al., 1964; Jinks et al., 1985).

All organic substrates significantly increased growth of okra plants. The maximum plant height was observed in plants treated with Bacillus firmus (45.7 cm) followed by that on chickpea (Fig. 3). Chickpea husk alone and after growth with bacteria and control showed least plant growth. Fresh plant weight most effectively increased on plants treated with B. firmus after growing on chickpea (5.2 g). However, chickpea husk found ineffective to increase plant fresh weight. Bacteria grown on different organic substrates significantly decreased the galling intensity on roots of okra. Minimum number of galls/ root system (35 galls) was recorded in plant treated with B. firmus and B. subtilis grown on rice, wheat husk and wheat grain, respectively. Most of the organic substrates when applied alone showed increased galling intensity compared to applied with bacteria except chickpea husk. Maximum infection was observed in non treated control (Fig. 4). B. firmus after growing on rice most significantly reduced egg mass formation (20 egg masses), followed by B. subtilis after growing on chickpea husk (22 egg masses). Bacillus species have been reported as Plant Growth Promoting Rhizobacteria (PGPR). It was observed that B. firmus showed improved plant growth when applied in soil as compared to B. subtilis. However, plant growth promotion varies with substrates used for the growth of strains. Organic amendments in soil to improve plant growth and to control variety of soil borne pathogens have been studied extensively. Further more presence of organic material continuously increase bacterial inoculum after incorporation in soil. However, growth rate and metabolite production especially proteases also vary according to presence of nutrients in the medium (Hageman et al., 1984). Bacillus species have been reported to produce antibiotics, proteases and other metabolites having nematicidal activity (Saxena et al., 2000; Terefe et al., 2009, Khan et al., 2010). Another important purpose was to develop Bacillus inoculum on low cost substrates which can be applied in soil on large scale. Wheat bran, chickpea and rice were found very good solid substrates for the development Bacillus inoculum. Chickpea husk yield lesser growth of B. subtilis during early time of inoculation.
Fig. 1. Populations of *Bacillus subtilis* on different Organic substrates up till 15 days.
Fig. 2. Populations of *Bacillus firmus* on different Organic substrates up till 15 days.
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Fig. 3. Effect of Bacillus species on growth of Okra following application in soil after multiplication on different organic substrates.

Fig. 4. Effect of Bacillus species on root-knot nematodes in Okra following application in soil after multiplication on different organic substrates.

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References


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