Biochar as a Carrier for Microbial Inoculants

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Soil Inoculation Technology

0 AD Romans use soil from legumes to inoculate new fields.

1896 Basic methods using peat as a carrier developed by Nobbe and Hiltner.

1930s Large scale field inoculation with Azotobacter in Russia begun and then abandoned in 1950s. Likewise, use of *Bacillus megaterium* for phosphate solubilization begun on large scale in Eastern Europe, but failed.

1950s Large scale use of Rhizobium inoculants begins, Widespread problems with contamination, inconsistent performance.

1970s Government agencies established to regulate inoculum quality established in Australia, USA, Canada

1980s Mycorrhizal inoculants developed for ecosystem restoration.

1990s Soil inoculation technologies extended for plant growth promoting microorganisms and bioremediation. Field trials mostly fail.

2000 New inoculation technologies. DNA based monitoring of inoculants.

2010 Commercialization and widespread use of microbial inoculants for agricultural biotechnology and environmental cleanup begins as serious global enterprise.
Thousands of studies have shown the utility of soil inoculants for plant growth promotion and bioremediation in the laboratory and the greenhouse. Field studies are less consistent due to poor understanding of soil ecology and monitoring of inoculant survival and activity.

Control - No PGPR

Inoculated with PGPR

Enhanced Legume Growth following Inoculation with Plant Growth Promoting Rhizobacteria (PGPR).
Trichoderma Inoculants for Plant Growth Promotion
Plant Growth Promoting Bacteria: Azotobacter

Azotobacter on Peanut (India)
Bacillus “crop enhancers”
Improved root growth
water use efficiency
fertilizer use efficiency
Improved soil structure
Control of root diseases

Bacillus cereus
Functions of Plant Growth Promoting Bacteria in the Rhizosphere

Yang et al. 2009 Trends in Plant Science
Characterization of individual bacterial isolates for PGPR activities

Nadeem, Shaharoona, Crowley, Unpublished data 2011
Ethylene Inhibition of Root Growth and Role of PGPR with ACC Deaminase Activity

PGPR Bacteria
ACC deaminase

\[ \text{ACC} \rightarrow \text{PGPR Bacteria} \rightarrow \text{ACC deaminase} \rightarrow \alpha\text{-ketoglutarate} + \text{NH}_4 \]

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Drought Tolerance and Long Term Selection of PGPR:
The 11,700 Year-Old, King Clone of *Larrea tridenta*
Lucerne Valley, San Bernadino County, California
Sampling of the Rhizosphere from the World’s Oldest Living Plant

Metagenomic Analysis of the Rhizosphere from the 11,700 Year Old “King Clone”

The King Clone
*Larrea tridenta* (Creosote bush)

Collection of Rhizosphere Bacteria
January 2010
Crowley & Jorquera
Properties of Selected PGPR Isolates from the King Clone
( ) = percent increase in comparison to sterilized control soil
Inoculant Technology

Seed inoculants

Soil Inoculum - Carriers
peat, coal, soil,
calcined clay, vermiculite, perlite
rock phosphate
polymers, alginate beads,
plant waste products (corn cobs)
slurries, liquids, powders

**Biochar?**

Desired characteristics
Sterile carrier media, defined cultures
High cell density
Long shelf life (months to years)
Low cost
Inoculum Production Methods

1. Selection of the most appropriate inoculant carrier
   - Modification of the carrier to obtain the appropriate conditions for supporting rhizobial growth:
     - Neutral pH
     - Appropriate particle size and water content

2. Packaging of the carrier

3. Sterilization of the carrier by heating or gamma irradiation

4. Inoculation
   - Incubation of inoculated bags for further rhizobial growth (maturing period)

5. Storage of bags until delivery to farmers

Rhizobial cultures (>10^3 cells/mL)

Quality control

- Gram staining
- Cell counting by microscopy
- Viable cells counting

Rodriguez-Navarro, Agron Sustain. Dev. 2010
Importance of Inoculum Density (Cell Numbers/Gram) for Rhizobium nodule formation and Relationship to Grain Yields

Figure 1. Relationship between numbers (log_{10}) of rhizobia on the narrow-leaved lupin seed at sowing and % plant nodulated and grain yield (data from Roughley et al. 1993).
# Potential Applications of Microbial Inoculants for Agricultural Biotechnology

<table>
<thead>
<tr>
<th>Biofertilizers</th>
<th>Biocontrol</th>
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<tbody>
<tr>
<td>Mycorrhizae</td>
<td>Plant root diseases</td>
</tr>
<tr>
<td>Phosphate solubilizing bacteria</td>
<td>Insects</td>
</tr>
<tr>
<td>Phosphate solubilizing fungi</td>
<td>Weeds</td>
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<tr>
<td>N fixing bacteria</td>
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<td>Selenium</td>
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<table>
<thead>
<tr>
<th>Plant Growth Enhancers</th>
<th>Bioremediation</th>
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<tbody>
<tr>
<td>Root growth promotion</td>
<td>Oil</td>
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<tr>
<td>Hormone production</td>
<td>Pesticides</td>
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<tr>
<td>Removal of cyanide</td>
<td>Munitions</td>
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<tr>
<td>Ethylene suppression</td>
<td>Azo dyes</td>
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<td></td>
<td>Solvents</td>
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<td></td>
<td>Metals</td>
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</tbody>
</table>
Studies in Japan in the 1990s show increases in plant nutrient uptake, mycorrhizae formation, and yields following inoculation with rhizobium and the addition of charcoal.

Table 1. Effect of *Rhizobium* inoculation and charcoal application on the growth of alfalfa, root infection with VAM fungi and nutrient uptake by the plant

<table>
<thead>
<tr>
<th></th>
<th>Fresh weight of shoots (mg/plant)</th>
<th>Root area infected with VAM fungi (%)</th>
<th>Nutrients absorbed in shoots (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38 days*</td>
<td>58 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38 days</td>
<td>58 days</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>227±27a</td>
<td>1,250±60c</td>
<td>22</td>
</tr>
<tr>
<td>F + R</td>
<td>249±41a</td>
<td>1,400±60c</td>
<td>32</td>
</tr>
<tr>
<td>F + C</td>
<td>396±29b</td>
<td>1,360±30c</td>
<td>39</td>
</tr>
<tr>
<td>F + R + C</td>
<td>2,390±70d</td>
<td></td>
<td>45</td>
</tr>
</tbody>
</table>

* Days after seeding. ± S.E. of triplicate means.

F: Fertilizer alone, F + R: *F + Rhizobium*, F + C: *F + charcoal*, F + R + C: *F + Rhizobium + charcoal*. Different letters refer to the values which were significantly different at P = 0.01 by Duncan’s multiple range test.

Source: Nishio and Okano 1991
Terra Preta and Bocashi soils are composed of a mixture of biochar that has reacted with clay, minerals and biomass in the presence of micro-organisms to produce organo-mineral agglomerates. These soils are rich in elements such as P, Mg, Zn, Ca, Fe, Mg, Ti and Mn. They exhibit higher water holding capacity than the surrounding soil, higher pH, and higher cation exchange capacity (CEC).

Examples of commercial products

Charcoal Green® BIOCHAR PLUS (Bio-Charcoal) is a management product that rejuvenates the biodiversity of soils and replaces the essentials organisms that were lost. When the right set of organisms are present and performing their functions both plant health and profitability soar.

2005 CHO CORN TRIALS

BEFORE

AFTER - only 7 lbs per acre

From old growth forests to the most productive cropland of the world, plant health is directly linked to a diverse community of organisms that inhabit the soil. With modern management practices, many beneficial species have been eliminated. This reduces crop performance, nutrition and productivity.

Charcoal Green® BIOCHAR PLUS contains beneficial soil microorganisms and enriched substrates. When added to growing media and native soils, research has shown that it promotes improved plant growth.

Field trials have shown that Charcoal Green® BIOCHAR PLUS can improve plant health and allow growers to reduce input costs.

http://www.buyactivatedcharcoal.com/biochar_plus_info
Charcoal Green® BIOCHAR PLUS

Inoculated Biochar
Charcoal Green® BIOCHAR PLUS - a biochar inoculated with beneficial soil microorganisms and enriched substrates.

Charcoal Green® Biochar Plus helps bind organic toxins (such as herbicides) from soil to provide a safer environment for new or existing root systems.

Charcoal Green® Biochar Plus provides the following plant health benefits:
- Improved Soil Drainage
- More Neutral pH
- Reduced Soil Compaction
- Increased Nutrient Cycling
- Greater Retention of Water In Dry Soils
- Improved Germination
- Improved Plant Resistance To Fungal Disease, Root Feeding Nematodes and Insect Infestations

Charcoal Green® Biochar Plus is made by a team of horticultural professionals that specialize in the production and use of carbon-based soil amendments. With six years of field research in turf and agricultural applications, these products have made numerous achievements in crop health and productivity.

GROWING GIANT PUMPKINS & WATERMELONS (2009 & 2010 RECORDS) - see links below!

Effects of Biochar Plus on yield of Irish Potato, Sweet Corn and Tomato
* 10% increase in Sweet Corn yield
* 30 lb./acre savings in nitrogen for Irish Potatoes
* 22% increase in Tomato yield
* 47% increase in Tomato yield

Yield of tomatoes was achieved by adding -
2 CUPS of BIOCHAR PLUS per 5 GALLONS of the TRANSPLANT POTTING MEDIA

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SKU: BIOCHAR-PLUS
GREEN:
- 10-lbs 2gal. pail ($38.50)
- 25-lbs 5gal. pail (0.7cu. ft.) ($94.50)
- 50-lbs poly sack ($141.00)

Qty: 

Add to Cart
Challenges for Successful Use of Soil Inoculants:

Inoculum
- Low cell numbers
- Contamination with undesired microorganisms
- High cost for culture media
- Poor survival in storage
- High cost of transporting inoculum to the field
- Practical methods to incorporate inocula into the soil

Physiology
- Starvation
- Predation
- Rapid cell death
- Low activity

Ecology
- Competitive exclusion from the rhizosphere
- Poor adaptation of inoculum to local soil conditions pH
- No methods to easily monitor survival and activity
The BioJect system offers a novel approach to applying microbes - it grows them on site! The BioReactor unit is a fermentation vessel in which beneficial microbes multiply to high concentrations and are injected on a daily basis into the irrigation system for distribution over the entire course. In this way the BioJect is able to consistently treat large areas while conserving valuable labor resources.

Adaptation of Batch Culture Bioreactor for Inoculation of Biochar: Custom Production of Microbial Cultures

The BioReactor unit is a fermentation vessel in which beneficial microbes multiply to high concentrations and are injected on a daily basis into the irrigation system for distribution over the entire course. In this way the BioJect is able to consistently treat large areas while conserving valuable labor resources.
Methods for On-Site Automated Inoculum Production
Changing Paradigms: Purpose of Inoculation

Single and Mixed Inoculants:

Introduce a missing function (eg N fixation), or to temporarily increase the population of plant growth promoting bacteria to an effective population size, generally $10^4$ to $10^6$ cells gram

New Paradigm:

Shape the microbial community structure to promote optimal soil biological functions associated with plant health. Includes use of management practices, soil amendments (char and composts) and use of inoculants – tailored to individual crops and soils.
Use of Artificial Neural Network Models for Monitoring Microbial Community Responses to Soil Management Practices (Biochar, Microbial Inoculants, Soil Type...)

Microbial community
% Clay
Soil pH
Biochar

Input

Hidden

Output

Disease status
Yields

Artificial Neural Network Analysis of Factors Affecting Microbial Community Structures in Arid Zone Agricultural Soils of SW USA
(Ma, Ibekwe, Yang, Crowley, unpublished data)

Sampling:
34 fallow soils
conventional vs organic

Microbiological Analyses:
58,000 16S rRNA gene sequences
Illumina high throughput sequencing
sequences sorted by taxon (family)

Survival of E. coli

Soil Analyses:
N, P, K, Ca,…
Management: organic vs conventional
pH, salinity, texture, bulk density
organic carbon, total and water soluble
Sensitivity analysis of output variable to selected input variables

Prediction of dependent variable values in relation to all independent variables
Effects of Soil Variables on Microbial Community Structure on Arid Zone Agricultural Soils in Southern California
Predicted changes in Proteobacteria classes with water soluble organic carbon and salinity.
Summary

The use of biochar as a soil amendment offers new opportunities to develop improved biofertilizer formulations that provide value-added enhancement of commercial biochar products.

Research is in progress to determine optimal pyrolysis substrates and conditions to optimize biochar properties as an inoculum carrier. Much can be learned from previous research on rhizobium.

Quality control and assurance requires testing of the efficacy of biochar products for modifying soil microbial community structures and PGPR populations.

Decision support tools based on ANN models are now being developed to predict the effects of management practices, biochar amendments and soil inoculants on plant yields in different soils and climates.
Development Process for Use of Biochar as an Inoculum Carrier

- Determine relationship between pyrolysis temperature, char properties, and suitability as a habitat for growth and long term survival of inoculants.

- Consider mixtures of chars

- Pretreatment of char to obtain desired granule size, pH, moisture conditions prior to inoculation.

- Incorporation with composts, rock phosphate, other additives.

- Application of quality control, quality assurance criteria for biochar-inoculant products.
Properties of Biochar as an Inoculum Carrier

Large internal surface area provides protected habitat 2 – 20 μM pore space
For growth of bacteria and fungi in internal spaces

Production process leads to pre-sterilized medium

Adsorbs nutrients and growth factors
Table 2. Effects of storage temperature, peat treatment and rhizobial strain on numbers of viable rhizobia after 52 weeks of storage. Values are rhizobial nos/g peat (× 106) (data from Boonkerd 1991).

<table>
<thead>
<tr>
<th>Temperature/peat</th>
<th>USDA110 (soybean)</th>
<th>THA205 (groundnut)</th>
<th>THA301 (mungbean)</th>
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</thead>
<tbody>
<tr>
<td>10°C</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>irradiated</td>
<td>5500</td>
<td>1230</td>
<td>2140</td>
</tr>
<tr>
<td>autoclaved</td>
<td>3890</td>
<td>270</td>
<td>930</td>
</tr>
<tr>
<td>non-sterile</td>
<td>1320</td>
<td>12</td>
<td>66</td>
</tr>
<tr>
<td>30°C</td>
<td></td>
<td></td>
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<tr>
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<td>11</td>
<td>830</td>
<td>1100</td>
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<tr>
<td>autoclaved</td>
<td>250</td>
<td>580</td>
<td>316</td>
</tr>
<tr>
<td>non-sterile</td>
<td>6</td>
<td>45</td>
<td>115</td>
</tr>
</tbody>
</table>

Fig. 1. Quantification of growth promotion in A. thaliana with exposure to airborne chemicals released from six growth-promoting bacterial strains compared with a nongrowth-promoting E. coli strain DH5α and water treatment alone; representative examples of 10-day-old A. thaliana seedlings grown on plates with airborne exposure to bacteria strains and water treatment are shown in inset. The plates were prepared as gnotobiotic systems so that the inoculated bacteria were the only microorganisms present.
DEEP BANDED MALLEE CHARCOAL

1 t/ha at broad-pace rate for row spacing of 600 mm

6 t/ha in 100 mm wide band

visible effect at 6 t/ha rate

Paul Blackwell, Syd Shea, Paul Storer, Zakaria Solaiman, Mike Kerkmans, and Ian Stanley

Improving wheat production with deep banded Oil Mallee Charcoal in Western Australia, IAI Conference Terrigal 2007

[PDF] Bring Biochar to the Market - 2009 Bioeconomy Conference
High Resolution Analysis of Bacterial Species in Amazon Forest Soils
By Oligonucleotide Fingerprinting of 16S rDNA Clones

Terra Preta

- Proteobacteria
- Acidobacterium
- Nitrospira
- Chloroflexi
- Bacillus
- Proteobacteria

Preserved Forest

- Acidobacterium
- alpha-Pro
- Actinobacteria
- Acidobacterium
- Chloroflexi
- Delta-Pro
- Verrucomicrobia
- Actinobacteria
- gamma-Pro
- beta-Pro

Verrucomicrobia
Actinobacteria
Proteobacteria
Planctomycete
Verrucomicrobia
Actinobacteria

Scale: 0.05
Repetitive Applications of the Biocontrol Agent
*Pseudomonas putida* 06909-rif/nal and Effects on Populations
of *Phytophthora parasitica* in Citrus Orchards
Repetitive Applications of the Biocontrol Agent
*Pseudomonas putida* 06909-rif/nal and Effects on Populations of *Phytophthora parasitica* in Citrus Orchards

**Treatments:**

Biocontrol agent: *Pseudomonas putida*
- once per year – manual application
- once per week – Bioject field fermenter
- water control

Pesticide control: Ridomil

**Inoculum density**
- Annual application trtmt
  - $10^8$ cfu/ml, 100 ml/tree

- Bioject
  - $10^7$ cfu/ml 20 liters/tree
  - sufficient for 50 hectares

Steddom et. al. Phytopath. 2002
2011 Ecological Solutions Bioject System II

Features:

- 2000 liter dual fermentation units, sequential operation
- System delivers $10^7$ cfu/ml irrigation water for 100 hectares
- Inoculum produced in sterile, hot-water disposable bags
  - Low cost, no contamination issues, no waste
- Computer operated selection of inocula for selected functions
  - PGPR for plant growth promotion
  - Specific inocula for biocontrol of different diseases and pests
- Fermentation system housed in mobile trailer
- Remote control and monitoring of system operations
- Remote monitoring of security using video cameras
Results of field trials for control of Phytophthora root rot using the Bioject System

Phytophthora populations suppressed by 84% in 1998

Biocontrol equivalent to chemical control for 1998 and 1999

Bacteria delivered to 75 cm depth

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<tr>
<td>Water control</td>
<td>0.66 a</td>
<td>0.40 a</td>
<td>0.67 a</td>
<td>0.53 a</td>
<td>0.57 a</td>
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<td>Yearly applications</td>
<td>0.58 a</td>
<td>0.45 a</td>
<td>0.60 a</td>
<td>0.53 a</td>
<td>0.55 a</td>
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<tr>
<td>Weekly applications</td>
<td>0.74 a</td>
<td>0.07 b</td>
<td>0.40 a</td>
<td>0.23 b</td>
<td>0.40 ab</td>
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<td>Fungicide/nematicide</td>
<td>0.05 b</td>
<td>0.12 b</td>
<td>0.55 a</td>
<td>0.33 ab</td>
<td>0.24 b</td>
</tr>
</tbody>
</table>

![Graph showing soil populations (log cfu/gm) at different depths (25, 50, 75 cm) with bars for Yearly applications and Weekly applications, indicating significant differences at certain depths.](image)