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NOTE

Determination of Carbon Source Utilization of *Bacillus* and *Pythium* Species by Biolog® Microplate Assay

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The carbon utilizations of *Bacillus* species and *Pythium* species were investigated by using a Biolog® microplate assay to determine if there are differences in the carbon utilizations of selected strains of these species. It may be possible to afford a competitive advantage to bacterial biological control agents by providing them with a substrate that they can readily use as a carbon source, for example, in a seed coating formulation. Microplates, identified as SFP, SFN and YT were used to identify spore-forming bacteria, nonspore-forming bacteria, and yeast, respectively. Bacterial and mycelial suspensions were adjusted to turbidities of 0.10 to 0.11 at 600 nm. One hundred microliters of each of the bacterial and mycelial suspension were inoculated into each well of each of the three types of microplates. L-arabinose, D-galactose, D-melezitose and D-melibiose of the 147 carbohydrates tested were found to be utilized only by bacteria, and not by *Pythium* species, by Biolog® microplate assay, and this was confirmed by traditional shake flask culture. Thus, it indicated that the Biolog® microplate assay could be readily used to search for specific carbon sources that could be utilized to increase the abilities of bacterial biological control agents to adapt to contrived environments.

Key words: *Bacillus species*, Biolog® microplate assay, carbon utilization, *Pythium* species, seedling disease

The biological control of plant disease using bacteria has been extensively studied, and some agents have been developed as commercial products (Weller, 1988). However, temporary dry conditions present a primary constraint in the utilization of bacterial biological control agents (Parke, 1991). Dry conditions may cause bacterial populations to decline to below the levels required for disease control. Planting rice seeds into a rice paddy field filled with water offers an ideal system for the application of bacterial biological control agents (Schneider, 1994). In this case, seeds could be inoculated with bacteria and then planted immediately in flooded fields, which would circumvent the problems associated with drying conditions.

Although water-seeded rice offers an ideal system with the continued presence of moisture for the introduction and maintenance of bacterial populations, other factors also must be considered in order to optimize the process. It is presumed that an introduced strain inoculated via seeds into soil must be able to establish itself and maintain an effective population for a minimum time at the sites of infection. Chun and Schneider (1998) established that, in rice, *Pythium* spp. attacks embryonic tissue (shoot, radicle and scutellum) and that this embryonic tissue is susceptible for 2 to 4 days after planting. Thus, an effective population of biocontrol agents needs to be maintained for only a relatively short time, and developing roots need not be colonized by the introduced strains. In light of the above, it was concluded that it might be possible to give introduced bacterial strains a competitive advantage by providing them with a substrate that they can readily utilize as a carbon source via a seed coating formulation. This strategy becomes implementable only if a substrate can be identified that is utilized by the introduced strain but not by the pathogen.

Janisiewicz *et al.* (1992) examined the nutritional enhancement of biological control agents in a study of blue mold on apples. In their work, they screened nitrogen sources preferentially utilized by the yeast biocontrol agents, but not by *Penicillium* species by using traditional shake culture methods. However, shake flask culture is very laborious for the screening of many C or N sources, for example, more than 100 carbohydrates may have to be

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Table 1. Substrates utilized as carbon sources by *Bacillus* species as determined by Biolog® microplate assays

Substrate[y]				
B. megaterium (strain 91-13b)	*B. megaterium* (strain 91-21)	*B. megaterium* (strain 91-51)	*B. cereus* (strain 91-12)	*B. cereus* (strain 91-126)
2-keto-D-gluconic acid[z]	2-keto-D-gluconic acid	2-keto-D-gluconic acid	adonitol	2'-deoxy adenosine
α-D-glucose	α-D-glucose	α-D-glucose	α-cyclodextrin	adenosine
α-D-lactose	α-D-lactose	α-D-lactose	α-D-glucose	adenosine-5'-monophosphate
dextrin	α-methyl-D-galactoside	α-methyl-D-galactoside	citric acid	arbutin
dextrin+D-xylose	α-methyl-D-glucoside	dextrin	cellobiose	α-cyclodextrin
D-fructose	dextrin	D-fructose	cis-aconitic acid	α-cyclodextrin
D-galactonic acid lactone	D-fructose	D-galactonic acid lactone	D-fructose	cellobiose
D-galacturonic acid	D-galactonic acid lactone	D-galctose	D-mannose	dextrin
D-gluconic acid	D-gluconic acid	D-gluconic acid	D-trehalose	D-fructose
D-mannitol	D-melezitose	D-mannitol	D,L-lactic acid	D,L-α-glycerol phosphate
D-melezitose	D-melibiose	D-melezitose	gentiobiose	D-malic acid
D-melibiose	D-raffinose	D-melibiose	glucose-6-phosphate	D-ribose
D-raffinose	D-trehalose	D-trehalose	glycogen	fructose-6-phosphate
D-serine	gentiobiose	D-raffinose	glycyl-L-glutamic acid	glucose-1-phosphate
D-trehalose	glycogen	gentiobiose	inosine	glucose-6-phosphate
gentiobiose	L-arabinose	glycogen	malonic acid	glycyl-L-glutamic acid
glycogen	L-asparagine	lactulose	maltose	inosine
lactulose	L-glutamic acid	L-arabinose	L-alanyl-glycine	L-lactic acid
L-arabinose	L-malic acid	L-asparagine	L-malic acid	L-serine
L-asparagine	L-pyroglutamic acid	L-glutamic acid	N-acetyl L-glutamic acid	maltotriose
L-malic acid	maltose	L-malic acid	N-acetyl-D-glucosamine	N-acetyl L-glutamic acid
maltose	maltotriose	maltose	N-acetyl-D-galactosamine	N-acetyl-D-galactosamine
maltotriose	methyl-pyruvate	maltotriose	pyruvic acid	pyruvic acid
N-acetyl-D-glucosamine	N-acetyl-D-glucosamine	methyl-pyruvate	succinic acid	thymidine
palatinose	pyruvic acid	N-acetyl-D-glucosamine	sucrose	thymidine-5'-monophosphate
qunic acid	qunic acid	palatinose	thymidine	tween 40
salicin	salicin	stachyose	thymidine-5'-monophosphate	uridine
stachyose	sucrose	sucrose	tween 40	uridine-5'-monophosphate
sucrose	turanose	uranose	uridine	
turanose			uridine-5'-monophosphate	

B. filicolonicus (strain 91-19)	*B. filicolonicus* (strain 91-23)	*B. thuringiensis* (strain 91-26)	*B. brevis* (strain 91-110)
α-cyclodextrin[z]	α-cyclodextrin	2'-deoxy adenosine	adenosine-5'-monophosphate
α-D-glucose	α-D-glucose	α-cyclodextrin	α-D-glucose
arbutin	adenosine	α-methyl-D-glucoside	amygdalin
α-cyclodextrin	arbutin	α-cyclodextrin	α-keto-glutaric acid
α-methyl-D-galactoside	α-methyl-D-galactoside	cellobiose	α-methyl-D-glucoside
cis-aconitic acid	cellobiose	dextrin	bromo succinic acid
cellobiose	cis-aconitic acid	D-fructose	cellobiose
dextrin	citric acid	D-malic acid	dextrin
dextrin+D-xylose	dextrin	D-mannose	D-melezitose
D-fructose	D-fructose	D-trehalose	D-trehalose
D-gluconic acid	D-galactonic acid lactone	glucose-1-phosphate	glycogen
D-mannitol	D-mannose	glucose-6-phosphate	glycerol
D-mannose	D-melibiose	glycogen	glycyl-L-glutamic acid
D-melibiose	D-psicose	glycyl-L-aspartic acid	L-glutamic acid
D-melibiose+D-xylose	D-raffinose	glycyl-L-glutamic acid	maltose
D-psicose	D-trehalose	L-asparagine	maltotriose
D-raffinose	lactulose	maltose	methyl pyruvate
D-ribose	L-asparagine	maltotriose	N-acetyl-D-glucosamine
D-trehalose	L-aspartic acid	methyl-pyruvate	sucrose
glycogen	L-glutamic acid	N-acetyl L-glutamic acid	thymidine
inosine	L-malic aicd	N-acetyl-D-galactosamine	turanose
L-glutamic acid	L-proline	N-acetyl-D-glucosamine	tween 40
N-acetyl-D-glucosamine	maltose	pyruvic acid	uridine
maltose	N-acetyl L-glutamic acid	thymidine	uridine-5'-monophosphate
maltotriose	sucrose	thymidine-5'-monophosphate	
palatinose	quinic acid+D-xylose	tween 40	
pyruvic acid	salicin	uridine-5'-monophosphate	
salicin	urocanic acid		
sucrose			

[y]Each well in Biolog® microplates contained approximately 0.35 to 0.84% carbohydrates and approximately 0.16% for all other C sources (Ziva Abraham, personal communication, Biolog, Inc, USA).

[z]Turbidity in each well was measured at 600 nm in an automated microplate reader after 48 h incubation at 28°C. Utilization of each compound was ranked, and the first and second rankings are listed (Data for all compounds not shown).

investigated. The Biolog® system (Biolog, USA) has been used for the identification of bacteria and yeast on basis of their oxidation reductase and carbon utilization patterns. However, to our knowledge, the Biolog® system has never been used to search for specific carbon sources, which are differently utilized by bacteria and fungi. The objectives of the present study were to identify differences in the carbon utilizations of selected *Bacillus* spp., used as biological control agents, and *Pythium* spp., which cause seedling disease in rice. The carbon sources utilized preferentially by *Bacillus* spp. would then be evaluated as adjuncts to seed coating formulations.

Carbon utilization was determined in 96-well plastic microplates, as designated by the manufacturer as SFP, SFN and YT, which were used to characterize or identify spore-forming bacteria, nonspore-forming bacteria, and yeasts, respectively. All wells in each plate were pre-filled by the manufacturer with a nutrient base and each well contained a different carbon source (approximately 0.35 to 0.84% for carbohydrates and approximately 0.16% for all other substrates) (Ziva Abraham, personal communication). The SFP and SFN plates did not contain a tetrazolium indicator.

The following *Bacillus* and *Pythium* species and strains were included: *B. megaterium* (91-51, 91-21, 91-13b), *B. cereus* (91-12, 91-126), *B. filicolonicus* (91-19, 91-23), *B. thuringiensis* (91-26), *B. brevis* (91-110), *P. arrhenomanes* (1398), *P. dissotocum* (1374) and *P. myriotylum* (1397).

Single colonies of each of the strains grown on nutrient agar were suspended in 300 µl of sterilized buffered (pH 7.0, 1.0 mM phosphate) water. These suspensions were then spread on fresh nutrient agar plates and incubated at 28°C overnight. The next day bacterial lawns were swabbed from the plates with sterile cotton swabs and suspended in sterile buffered water. The bacterial suspensions were adjusted to turbidities of 0.10 to 0.11 at 600 nm. Mycelia of *Pythium* spp. were harvested from potato dextrose broth cultures after 7 to 10 days of incubation on a rotary shaker (100 rpm) at room temperature. Mycelia were collected on a 1 mm mesh sieve and washed five times with sterile buffered water before being mixed for 10 sec in a blender set at low speed. The suspension was passed through a 1 mm mesh sieve and the optical density of the mycelial suspension was adjusted to 0.1 at 600 nm with sterile buffered water.

One hundred microliters of each of the bacterial and mycelial suspensions was inoculated into each well of each of the three types of Biolog® microplates. Turbidities in each well were determined at 590 nm using an automated plate reader (Model EL311, Bio-Tek Instruments, USA) after 48 h incubation at 28°C. Data from the microplate reader were transferred to a computer for mathematical manipulations and statistical analyses. Blank plates were prepared by adding 100 µl of sterile, buffered water to each well of each of the three Biolog® plate types. These plates were incubated and read after 48 h along with the inoculated plates, and absorbance values for each of the blank wells were subtracted from the corresponding inoculated wells. Three replicates of each plate type and each strain were prepared.

The reliability of the Biolog® plates as indicators of carbon utilization by bacteria and fungi is unknown. Therefore, experiments were conducted to compare the Biolog® results with those obtained from traditional shake flask cultures using a defined basal medium. Claus' (Sneath, 1986) basal medium, [KH_2PO_4 (0.8 g), K_2HPO_4 (0.2 g), $CaSO_4 \cdot 2H_2O$ (0.05 g), $MgSO_4 \cdot 7H_2O$ (0.05 g), NH_4NO_3 (0.058 g)], was modified by adding 10 ml of Schmitthener's (1962) minor element solution, [30 mg KH_2PO_4, 30 mg K_2HPO_4, 20 mg $MgSO_4 \cdot 7H_2O$, 0.56 mg $CaCl_2$, 2.88 mg $MnCl_2$, 1.67 mg $ZnCl_2$, 0.10 mg $FeCl_2$, and 11.60 mg of the disodium salt of ethylenediaminetetraacetic acid (EDTA) in one liter of deionized water]. On the basis of the Biolog® microplate test results, which characterized organic compounds as good or poor C sources for *B. megaterium* and *P. arrhenomanes*, the following compounds were chosen for evaluation in shake flask culture: L-arabinose, D-melezitose, D-galactose, D-melibiose, lactulose, Tween 40, L-rhamnose, L-serine, L-histidine, L-leucine, maltose, cellobiose, α-D-glucose, and sucrose. Solutions (10%, w/v) were prepared for each of these compounds, filter-sterilized (0.25 µl pore size), and then added to the basal medium to a concentration of 10 g/l for *B. megaterium* and 5 g/l for *P. arrhenomanes*. One hundred microliters of suspensions of *B. megaterium* and *P. arrhenomanes*, prepared as described above, were inoculated into each 125 ml Erlenmeyer flask containing 25 ml of the defined medium. Bacterial growth was determined by measuring turbidities at 600 nm after 72 h of incubation on a rotary shaker (100 rpm) at room temp. Fungal growth also was measured turbidimetrically after 7 days of incubation on the rotary shaker. Mycelia of *Pythium* spp. in the defined media were triturated for 10 sec in a blender set at low speed and the turbidities (600 nm) of the homogenates was determined immediately. Absorbance values for each of the non-inoculated test media were subtracted from each of the respective values for the inoculated media. Tests were performed in triplicate.

Until recently, several fungicides, such as Vitavax 200 FF, thiram and copper, have been used to control rice seedling disease in Louisiana. In addition, farmers presoaked rice seeds one day before planting in order to stimulate emergence. However, the Louisiana Department of Environmental Quality and the U.S. Environmental Protection Agency has now banned the use of these compounds and the cultural practice of presoaking seeds, because the water used for presoaking seeds was regarded as an environmentally contaminating waste by the U.S. EPA. For these reasons, there is an urgent need to develop

Table 2. Substrates utilized as carbon sources by *Pythium* species as determined by Biolog® microplate assays

Substrate[y]		
P. arrhenomanes (strain 1398)	*P. myriotylum* (strain 1397)	*P. dissotocum* (strain 1374)
2'-deoxy adenosine[z]	α-D-glucose	alaninamide
2, keto-D-gluconic acid	α-D-lactose + D-xylose	α-D-glucose
adenosine	α-methyl-D-glucoside	α-methyl-D-glucoside
adenosine-5'-monophosphate	cellobiose	cellobiose
α-cyclodextrinz	cis-aconitic acid	dextrin
α-D-glucose	dextrin	dextrin+D-xylose
α-methyl-D-glucoside	dextrin+D-xylose	D-fructose
α-cyclodextrin	D-alanine	D-mannose
cellobiose	D-raffinose	D-raffinose
dextrin	D-trehalose	D-trehalose
dextrin+D-xylose	D,L-lactic acid	D,L-lactic acid
D-fructose	gentiobiose	gentiobiose
D-mannitol	glycyl-L-aspartic acid	glycerol
D-melezitose	glycyl-L-glutamic acid	glycogen
D-melibiose	L-alanine	glycyl-L-glutamic acid
D-raffinose	L-alanyl-glycine	L-alanine
D-trehalose	L-arabinose	L-alanyl-glycine
fumaric acid	L-asparagine	L-asparagine
gentiobiose	L-glutamic acid	L-glutamic acid
glycogen	L-lactic acid	L-histidine
glycyl-L-glutamic acid	L-malic acid	L-malic acid
inosine	L-proline	L-ornithine
maltose	L-pyroglutamic acid	L-phenylalanine
maltotriose	maltose	maltose
sorbitol	maltotriose	maltotriose
sucrose	malonic acid	α-amino butyric acid
tween 40	salicin	succinic acid
urocanic acid	succinic acid	sucrose
	tween 40	tween 40
		tween 80

[y]Each well in Biolog® microplates contained approximately 0.35 to 0.84% carbohydrate and approximately 0.16% of other C sources.
[z]Turbidity in each well was measured at 600 nm in an automated microplate reader after 48 h incubation at 28°C. The consumption of each compound was ranked, and the first and second rankings are listed (Data for all compounds is not shown).

a technology of the biological control of rice seedling disease. However, in order to accomplish this, detailed etiological information on the disease is required, i.e. when and for how long are seedlings susceptible and exactly which portions of the seed and seedling are susceptible. Results from etiological studies described in a previous study (Chun and Schneider, 1998) suggested that seedling disease in water-seeded rice could be biologically controlled using biocontrol agents, because the infection court was restricted primarily to the embryo, and the embryo is susceptible for a very short period of time.

The present study shows that the Biolog® microplate assay can be readily used to search for the specific carbon sources preferentially used by biological control agents but not by a plant pathogen. Tables 1 and 2 list the carbon substrates utilized by *Bacillus* and *Pythium* species. Three types of plates were used in the study, i.e., the SFP, SFN, and YT plates, and several of the C sources were included in more than one type of plate. In such cases, results from the different plates involved were combined for statistical analyses.

There were large differences in the C utilizations of *Bacillus* and *Pythium*, which were arranged according to the order of best C sources utilized by *Bacillus megaterium* strain 91-51 (Fig. 1). Many C sources, such as N-acetyl-D-glucosamine, L-arabinose, and D-galactose were preferred by *B. megaterium* strain 91-51, which was one of the best biocontrol strains (Fig. 1). Glucose, maltose and sucrose were utilized well by most of the *Bacillus* species and by all *Pythium* species (Tables 1 and 2 and Fig. 1). Many C sources were not utilized by either *Bacillus* or *Pythium* (Fig. 1). The C sources utilized preferentially by the biological control agent, *B. megaterium* strain 91-51 could significantly increase biological control efficacy (to a level comparable with that of normal fungicide treatment) compared to a C source unamended control when coated onto rice seeds in a formulation containing more than 8% of carbohydrates (Chun, 1997). However, C sources utilized well by the biological control agent and by the pathogen rather decreased the efficacy of biological control (Chun, 1997).

According to the Biolog® assays, the following substrates were found to be good C sources for *B. megaterium* strain 91-51: L-arabinose, D-melezitose, D-galactose, D-melibiose and lactulose. Likewise, *P. arrhenomanes* grew well on maltose, cellobiose, D-glucose or sucrose. *B. megaterium* did not grow well on tween 40, L-histidine, L-serine, L-rhamnose or L-leucine and *P. arrhenomanes* did not grow well on L-arabinose, D-melezitose, D-galactose, D-melibiose or lactulose. All of these compounds were tested in liquid shake flask cultures. In general, those compounds identified as good C sources by the Biolog® analyses also performed well in the shake flask cultures for both *B. megaterium* and *P. arrhenomanes* (Table 3). Moreover, those substrates identified as poor C sources by the Biolog® microplate assay were also validated by shake flask culture experiment for *B. megaterium* and *P. arrhenomanes*. Differences between *Pythium* spp. and *Bacillus* spp. in terms of carbon source utilization were found to be determined reliably with the Biolog® microplate assay. Results were based on a 48 h incubation of the inoculated microplates because this time allowed better adequate car-

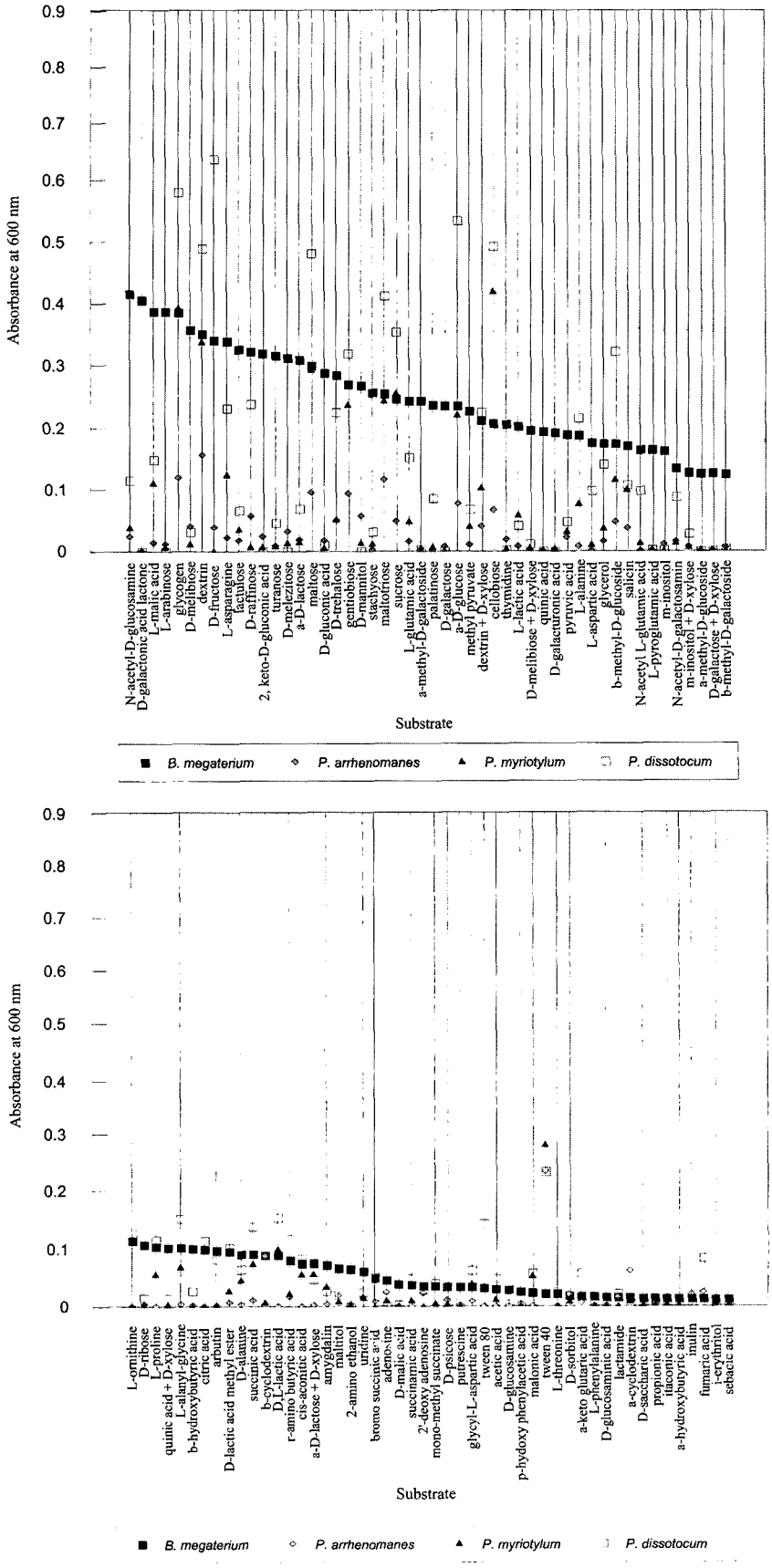
Absorbance at 600 nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
N-acetyl-D-glucosamine
D-galactonic acid lactone
L-malic acid
L-arabinose
glycogen
D-melibiose
dextrin
D-fructose
L-asparagine
factulose
D-raffinose
2, keto-D-gluconic acid
turanose
D-melezitose
a-D-lactose
maltose
D-gluconic acid
D-trehalose
gentiobiose
D-mannitol
sucrose
maltotriose
L-glutamic acid
a-methyl-D-galactoside
palatinose
D-galactose
a-D-glucose
methyl pyruvate
dextrin + D-xylose
cellobiose
thymidine
L-lactic acid
D-melibiose + D-xylose
quinic acid
D-galacturonic acid
pyruvic acid
L-alanine
L-aspartic acid
glycerol
b-methyl-D-glucoside
salicin
N-acetyl L-glutamic acid
L-pyroglutamic acid
m-inositol
N-acetyl-D-galactosamin
m-inositol + D-xylose
a-methyl-D-glucoside
D-galactose + D-xylose
b-methyl-D-galactoside
Substrate
B. megaterium
P. arrhenomanes
P. myriotylum
P. dissotocum
Absorbance at 600 nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
L-ornithine
D-ribose
D-proline
quinic acid + D-xylose
L-alanyl-glycine
b-hydroxybutyric acid
citric acid
arbutin
D-lactic acid methyl ester
D-alanine
succinic acid
b-cyclodextrin
D,L-lactic acid
r-amino butyric acid
cis-aconitic acid
a-D-lactose + D-xylose
amygdalin
mallitol
2-amino ethanol
uridine
bromo succinic acid
adenosine
D-malic acid
succinamic acid
2'-deoxyadenosine
mono-methyl succinate
D-psicose
putrescine
glycyl-L-aspartic acid
tween 80
acetic acid
D-glucosamine
p-hydoxy phenylacetic acid
malonic acid
tween 40
L-threonine
D-sorbitol
a-keto glutaric acid
L-phenylalanine
D-glucosaminic acid
lactamide
a-cyclodextrin
D-saccharic acid
propionic acid
itaconic acid
a-hydroxybutyric acid
inulin
fumaric acid
i-erythritol
sebacic acid
Substrate
B. megaterium
P. arrhenomanes
P. myriotylum
P. dissotocum

Fig. 1. Con'd

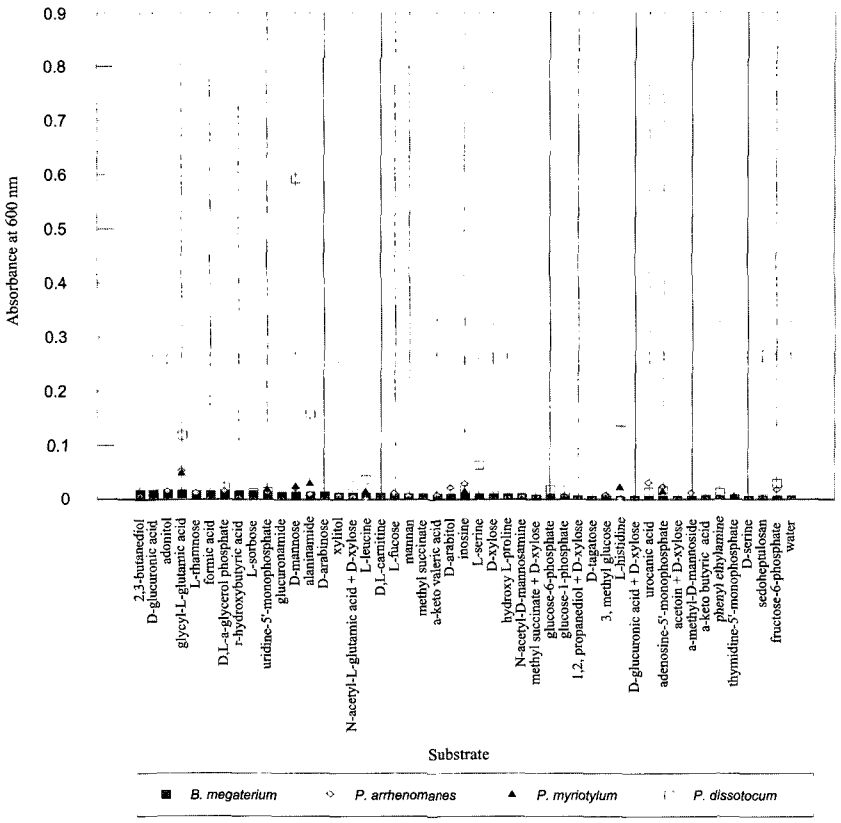

Fig. 1. Comparative carbon utilization by *B. megaterium* and *Pythium* species. Carbon utilization was determined using Biolog® microplates. Bacteria were inoculated into wells and turbidity readings were made at 48 h. Entire plates were used as water blanks, and readings for blank wells were subtracted from the respective inoculated wells. Carbon substrates with starting the letters a-, b-, or r- denote α-, β-, or γ-, respectively.

Table 3. Comparison of Biolog® microplate assays and liquid shake cultures for determining carbon utilization by *B. megaterium* 91-51 and *P. arrhenomanes* 1398

Carbon source	*B. megaterium* (91-51)		*P. arrhenomanes* (1398)	
	Biolog® ranking[x]	Shake flask culture[y]	Biolog® ranking	Shake flask culture[y]
L-arabinose	1	0.706±0.015	4	0.000±0.000
D-melezitose	1	0.722±0.006	2	0.004±0.000
D-galactose	1	0.766±0.006	5	0.013±0.000
D-melibiose	1	0.586±0.041	2	0.006±0.001
lactulose	1	0.595±0.004	3	0.000±0.000
Tween 40	6	0.000±0.000	1	0.004±0.002
L-rhamnose	8	0.000±0.000	4	0.021±0.012
L-serine	9	0.000±0.000	8	0.024±0.014
L-histidine	10	0.000±0.000	9	0.021±0.012
L-leucine	8	0.000±0.000	9	0.877±0.506
Maltose	2	0.222±0.013	1	1.118±0.025
Cellobiose	nd[z]	nd	1	1.513±0.025
α-D-glucose	2	0.289±0.051	1	1.312±0.012
Sucrose	2	0.215±0.002	2	0.998±0.051
Glycogen	nd	nd	1	1.351±0.036

[x]On a scale of 1-10, smaller numbers indicate better C sources in the Biolog assays (Data not shown). Correlation coefficients (r) between Biolog® rankings and growth in shake flask cultures were -0.86 (P=0.0002) and -0.40 (P=0.15) for *B. megaterium* (91-51) and *P. arrhenomanes* (1398), respectively.
[y]Growth in shake flask cultures was measured as turbidity at 600 nm after incubation in a defined medium for 3 and 7 days at room temp (22-30°C) for *B. megaterium* and *P. arrhenomanes*, respectively. (Mean±SEM)
[z]Not done.

bon utilization than a 24 h.

The Biolog® plates used for the present study did not contain redox dyes, as used by others (Winding, 1994). We used turbidity, as opposed to colorimetry assays based on tetrazolium dye reduction, as a measure of cell growth, because dye reduction (oxidation-reduction reactions) may proceed in the absence of growth. It was particularly interesting to observe that there were the distinctive patterns of carbon utilization among *Pythium* spp., which suggested that the Biolog® plate assays might be useful for the identification of *Pythium* spp., as the identification of *Pythium* species by microscopy is very difficult unless the microbiologist concerned is suitably experienced. Therefore, the present results suggest that the Biolog® microplate assay could be readily used, not only for the study on carbon source utilization but also for the identification and classification of *Pythium* species.

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