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Effects of *Klebsiella planticola* SDF20 on soil biota and wheat growth in sandy soil

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Abstract

The potential for ecological effects to occur after the release of genetically engineered microorganisms is a global concern and the release of biotechnology products must be assessed on a case-by-case basis. In this research, a genetically engineered strain of *Klebsiella planticola* (SDF20) bacteria was added to microcosms containing sandy soil and wheat plants to assess the potential for effects on soil biota and plant growth. One half of the soil treatments in this study contained wheat plants to compare some effects on growing rhizosphere communities in the experimental system. When SDF20 was added to soil with plants, the numbers of bacterial and fungal feeding nematodes increased significantly, coinciding with death of the plants. In contrast, when the parental strain, SDF15 was added to soil with plants, only the number of bacterial feeding nematodes increased, but the plants did not die. The introduction of either SDF15 or SDF20 strains to soil without plants did not alter the nematode community. No effects were observed on the activity of native bacterial and fungal communities by either SDF15 or SDF20. This study is evidence that SDF20 can persist under conditions found in some soil ecosystems and for long enough periods of time to stimulate change in soil biota that could affect nutrient cycling processes. Further investigation is needed to determine the extent these observations may occur in situ but this study using soil microcosms was the first step in assessing potential for the release of genetically engineered microorganisms to result in ecological effects. © 1999 Elsevier Science B.V.

Keywords: Genetically engineered microorganisms (GEMs); *Klebsiella planticola*; Soil ecology; Ecological effects

1. Introduction

Doyle et al. (1995) in a review on the potential for genetically engineered microorganisms (GEMs) to disturb microbial populations in natural environments, has concluded that GEMs require study on a case-by-case basis. This would include not only assessing the growth and survival of the GEM, but also some trophic interactions in environments where they are released. This review noted that, GEMs can cause a variety of changes in different habitats, often unpredictable, that included increased enzymatic activity (Crawford et al., 1993), increased culture respiration rates (Trevors and Grange, 1992) and in some cases, the loss of a fungal component from the soil biota (Short et al., 1991). However, these studies did not include assessment of multiple components of soil biota essential for

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nutrient cycling processes, plant growth, and disease incidence. Our research focused on understanding some changes in many soil biota after introductions of GEMs and potential effects to ecological processes in soil.

We became interested in *Klebsiella planticola* SDF20 because of its potential to be released to the environment after use to produce ethanol from agricultural wastes in fermentors. This Gram negative, lactose-fermenting bacterium was engineered to produce increased ethanol concentrations up to 85% of the theoretical maximum (Tolan and Finn, 1987; Feldmann et al., 1989). After removal of ethanol from the fermentor, the remaining residue would be available for use as an organic soil amendment. In preliminary studies, with this GEM, we could recover approximately 0.002% ethanol in the headspace of our culture system using soil microcosms (Holmes, 1995). It is possible that prolonged exposure to this concentration of ethanol can affect soil biota including the numbers of bacterial predators that mineralize nitrogen retained in bacterial biomass (Ingham et al., 1986) and thus a short period of increase nitrogen availability for plant growth (Hunt et al., 1987). Low concentrations of ethanol was also reported to have negative effects on biological systems (Jones, 1989).

In this study, we sought to determine whether the ethanol producing strain of *Klebsiella planticola* SDF20 can induce changes in soil biota, and to define potential mechanisms for those results. A low organic matter, sandy soil similar to that used by Short et al. (1991) was used, and changes in soil biota and plant growth were used endpoints to assess effects. Low molecular weight compounds secreted from plant roots are important inputs to soil for bacterial metabolism (Paul and Clark, 1989; Lynch, 1990; Killham, 1994). Spring wheat (*Triticum aestivum* L.) was planted in soil microcosms to provide a rapidly growing root mass so that the effects of carbon inputs from plant roots on the survival of SDF20 and potential effects to other organisms in soil could be assessed.

2. Materials and methods

2.1. Bacterial cultures

The parent (SDF15) and GEM (SDF20) cultures of *K. planticola* were provided by Dr. Georg A. Sprenger (Institut für Biotechnologie, Federal Republic of Germany). Constructs of strains SDF15 (parental) and SDF20 (GEM) were described by Feldmann et al. (1989). Presence of the pZM15 plasmid that encodes novel genes important for growth and ethanol production by SDF20 were previously described using microcosms identical to this study (Holmes, 1995).

To evaluate the production of ethanol by SDF15 and SDF20, 10^7 bacteria per gram of soil were added to soil microcosms. After 1 week incubation in microcosms with different soil conditions, the concentration of ethanol was determined using gas chromatography (Shimadzu, Kyoto, Japan) with flame ionized detection (8-ft Porpak-Q column at 150°C and 200°C injection port temperatures). Standard concentrations of ethanol were also analyzed at the beginning of each analysis to determine retention times and concentrations of ethanol in samples. Ethanol was detected only in the headspace of microcosms containing HOM clay soil without wheat plants. The concentration of ethanol detected in microcosms inoculated with SDF20 (26 $\mu g\ ml^{-1}$) was significantly greater ($p \leq 0.01$) than ethanol from microcosms inoculated with SDF15 (5 $\mu g\ ml^{-1}$) or uninoculated microcosms (9 $\mu g\ ml^{-1}$). Ethanol was not detected in the headspace of microcosms containing LOM clay soil (i.e. no peat added) without plants or in the headspace of microcosms containing plants and either LOM or HOM clay soil types (Holmes, 1995).

2.2. Soil processing and characterization

Low organic matter, sandy loam soil (Gardone series, Aridic Haploxeroll) from the upper 10 cm depth was collected from the Millican Limited Use Area, OR. The soil was prepared by sieving through a 2 mm mesh screen, mixing for 5 min in a large volume soil mixer, and stored in plastic-lined, rubber cans in a greenhouse. Three replicate soil samples were submitted to the Central Analytical Laboratory, Oregon State University (OSU), Corvallis, OR for chemical and physical analysis (Table 1).

2.3. Soil microcosm preparation

The soil used to inoculate 0.97 L glass jar (Ball, Muncie, IN) microcosms was prepared using protocols of Stotzky (1972) and Orchard and Cook (1983).

Table 1
Characteristics of Aridic Haploxeroll, sandy soil collected at Millican, OR

Parameter	pH	P[a]	K[a]	Ca[b]	Mg[b]	NH_4-N[a]	NO_3-N[a]	OM[c]	CEC[b]
Mean[d]	6.2	14.7	358	4.3	1.3	2.35	32.2	1.7	8.64
Std. err.	0.03	0.52	4.33	0.06	0.03	0.14	0.32	0.04	0.30

[a] Concentration in ppm.
[b] Concentration in meq/100 g.
[c] Concentration in percentage.
[d] Mean value for three replicates.

The moisture content of sieved soil was returned to field capacity (−33 kPa) by adding 50% of the water needed at least 72 h before the experiment was to begin. The soil was then placed into plastic bags, mixed daily by kneading and allowed to equilibrate for 2 days. The day of beginning an experiment (i.e. day 0), the remaining water needed to restore field capacity was added to the bags of soil, mixed and allowed to equilibrate for 6 h. The appropriate bacterial inoculum was suspended in sterile tap water to yield a final inoculum density of 1×10^8 colony forming units (CFUs) per gdw^{-1}. On day 0, 200 g of soil was added to each of 90 microcosms. These microcosms were then divided into three separate lots of 30 microcosms each that were: (1) to be left uninoculated, (2) inoculated with SDF15, and (3) inoculated with SDF20 for a total of 90 separate soil microcosms. The uninoculated control microcosms received only the sterile water addition.

Spring wheat seeds (*Triticum aescivum* L.) were obtained from the OSU Wheat Breeding Program at Hyslop Farms near Corvallis, OR. The wheat seeds were surface sterilized in 10% HCl for 5 min, rinsed once with autoclaved tap water, and placed on moistened filter paper for germination at 25°C in the dark for 48 h. One germinated wheat seed was added to one-half of the microcosms in each lot for a total of 45 soil microcosms containing plants.

The total weight of each soil microcosm was recorded on each jar label. Soil moisture lost during incubation was replaced each day with autoclaved tap water by returning the microcosm to its initial weight. All microcosms were incubated under artificial lighting (16 h light; 8 h dark) at 75% relative humidity. On each sample day (day 0, week 1, week 2, week 3, and week 8) three replicate microcosms per treatment were analyzed for plant growth and the presence of key soil biota.

2.4. Bioassays

2.4.1. Plant biomass and soil dry weight

On each sample date, three replicate soil microcosms per treatment were selected using random numbers for destructive sampling. Plants were gently removed from microcosms, roots were cut from shoots at the crown, and roots and shoots were dried in an oven at 110°C for 24 h to determine the dry weight (Gardner, 1965). The soil in each microcosm was thoroughly mixed, and 5 g of soil was removed and dried at 110°C for 24 h (Gardner, 1965). The remaining soil was used to assay for soil biota.

2.4.2. Recovery of K. planticola strains

After removing plants roots from soil, soil suspensions were prepared by mixing the soil in jars, placing 10 g of soil into 90 ml of autoclaved tap water, and shaking for 5 min by hand. Ten-fold soil dilution series of 10^{-1} to 10^{-6} were prepared using autoclaved tap water. Bacterial survivals of SDF15 and SDF20 strains in soil were determined by spread-plating soil from microcosms diluted in autoclaved tap water on MacConkey's medium (Becton Dickinson Microbiological Systems, Cockeysville, MD) (Koch, 1981; Atlas, 1993). The MacConkey's medium was amended with antibiotics (25 μg ml^{-1} kanamycin and 25 μg ml^{-1} chloramphenicol, Sigma, St. Louis, MO) to differentiate the growth of SDF20 from SDF15 and native lactose fermenting bacteria in soil.

2.4.3. Total and active bacterial biomass

The active bacterial biomass in soil was determined using fluorescein diacetate stain (FDA, Sigma) and the soil suspension-thin agar film method (Ingham et al., 1986). The numbers of FDA-stained bacteria (i.e. active bacteria) were determined in five fields per sample using an epifluorescent microscope (Leitz

epifluorescent microscope), at 400× magnification. The active bacterial biomass in soil was determined using the same agar film prepared for measuring fungal biomass as described below.

The total bacterial biomass was determined using fluorescein isothiocyanate stain (FITC, Sigma) as described by Babiuk and Paul (1970). A soil dilution (10^{-2}) was stained for 3 min with 0.5 ml of a FITC solution (20 mg ml^{-1}), filtered through a 0.2 μm pore-size, non-fluorescent (black-stained) Nuclepore filter, and destained with 0.5 M sodium carbonate and 0.01 M pyrophosphate buffers. The filter was mounted on a slide and the numbers of fluorescent bacteria in each of 10 fields per filter were counted using an epifluorescent microscope, at 1000×. The background contamination in buffer solutions was assessed by filtering sterile buffer and counting fluorescent bacteria on the filter. These counts (normally zero) were subtracted from sample counts.

2.4.4. Active and total fungal biomass

The active fungal biomass (fluorescein diacetate, FDA stained) and total fungal biomass were determined by staining 0.5 ml aliquot of a soil dilution (10^{-1}) for 3 min with 0.5 ml of FDA solution in 0.1 M phosphate buffer (pH 6.5). The FDA-soil suspension was mixed with 0.5 ml of 1.5% agar in 0.1 M phosphate buffer (pH 9.5). An aliquot was then placed on a slide with a well of known depth as described by Ingham and Klein (1984). The lengths of fungal hyphae stained with FDA were determined using epifluorescent microscopy, at 250×. The length and diameter of all fungal hyphae were measured for three 18 mm length transects in each agar film using differential interference contrast (DIC) microscopy, at 250× (Lodge and Ingham, 1991). Background contamination was determined by measuring hyphal lengths using sterile phosphate buffer instead of soil suspension.

2.4.5. Most-probable-number of protozoa

The most-probable-number (MPN) of protozoa in soil was determined as described by Darbyshire et al. (1974) and Ingham (1994). A 0.5 ml aliquot of each serial dilution (10^{-1}–10^{-6}) prepared from soil was used to incoulate each of four wells of a 24-well tissue culture plate. Each tissue culture plate was prepared for inoculation by placing 0.5 ml of 10% soil extract agar in each well. The inoculated tissue culture plates were incubated at 22°C in closed plastic bags for 4–7 days. On examination, each well was mixed and one drop placed on a microscope slide and covered with a glass cover slip. The presence of flagellates and amoebae was recorded in one transect of the cover slip using a DIC microscope, at 250×. These results were then converted to the MPN of cells per gram dry weight (gdw) of soil (Darbyshire et al., 1974).

2.4.6. Enumeration and identification of nematodes

To enumerate and identify soil nematodes, subsamples (25 g) of soil were placed in Rapid-Flo milk filters (Filter Fabrics, Goshen, IN). These filters were placed on Baermann extractors, and water added to cover the soil and extract the nematodes (Anderson et al., 1978). This method extracts active adult and juvenile stages of nematode species. After 5 days, nematodes were collected from the bottom of extractors and stored at 4°C until processed.

Each sample was examined in Rodac plates (Becton Dickinson, Cockeysville, MD) with a grid etched on the bottom of the dish to facilitate counting. All nematodes in each sample were counted, and the first 30 nematodes observed were picked, identified to genus with DIC microscopy and separated into trophic groups based on morphological characteristics normally used in taxonomic classification and feeding behaviors, respectively (Bongers, 1988; Yeates et al., 1993).

2.5. Statistical analysis

A split-plot design was used to compare differences in measurements of soil biota within unplanted (without plants) and planted (with plants) soil microcosm treatments. Outlier values were removed from data after reviewing the distribution of raw data values with confidence intervals (99%). Residuals of the means were plotted against predicted values to examine constant error variance among data values. Residual plots indicated that a log transformations of the raw data for bioassays were necessary before testing for statistical significance by analysis of variance (SAS Institute, Cary, NC). The least significant difference ($p \leq 0.01$, Fisher protected) was calculated for bioassays using the interaction of microcosm type and bacterial treatment as the error term.

3. Results and discussion

3.1. Recovery of introduced bacteria

Survival of the two *Klebsiella* strains decreased from 10^8 CFU gdw^{-1} to approximately 100 CFU gdw^{-1} of soil over the 8 weeks incubation period but did not fall below detection (Fig. 1). This indicated that the genetically engineered and parent strains were able to persist in experimental conditions that were similar to many field conditions. Other lactose-fermenting bacteria were not recovered from the uninoculated soil treatments (i.e. controls), indicating that *Klebsiella* bacteria recovered from soil represented only introduced bacteria. The number of introduced *Klebsiella* declined to populations densities more similar to what *K. planticola* may exist in natural environments near the limit of detection for recovery using spread plating methods.

3.2. Active and total bacterial biomass

After 2 weeks of incubation, active bacterial biomass in unplanted, uninoculated, and SDF15 treatments remained significantly higher than in the SDF20 (Fig. 2(a)) suggesting that the genetically engineered bacteria resulted in decreased bacterial activity. Active bacterial biomass was not different in planted treatments, indicating that wheat plants improved bacterial activities in the SDF20 treatment to levels comparable to uninoculated and SDF15 treatments (Fig. 2(b)). The total bacterial biomass within unplanted and planted treatments was not significantly different (Fig. 3(a) Fig. 3(b)). After week 3, the increase of total bacterial biomass in all treatments represented the naturally occurring bacteria but not introduced *Klebsiella* bacteria whose survivals were decreasing.

3.3. Active fungal biomass, total fungal biomass and protozoa

Active fungal biomass within unplanted and planted treatments were not significantly different during each sample date (unplanted, $p=0.073$; planted, $p=0.013$), also total fungal biomass within unplanted and planted treatments were not significantly different during each sample date (unplanted, $p=0.055$; planted, $p=0.025$). However, some transient fluctuations in active and total fungal biomass occurred in all treatments (Table 2). The numbers of flagellates and amoebae within unplanted and planted treatments decreased significantly ($p \leq 0.01$) from day 0 to week 1 but remained low throughout the remainder of the experiment (Table 3). Flagellates decreased 10-fold (10^5–10^4) while amoebae decreased more than 100-fold (10^4–10^2) during the experiment.

3.4. Soil nematodes

The total numbers of nematodes within unplanted treatments were not significantly different during each sample date (Fig. 4(a)). In comparison, the nematode community in planted treatments responded to bacterial additions after 2 weeks of incubation (Fig. 4(b)).

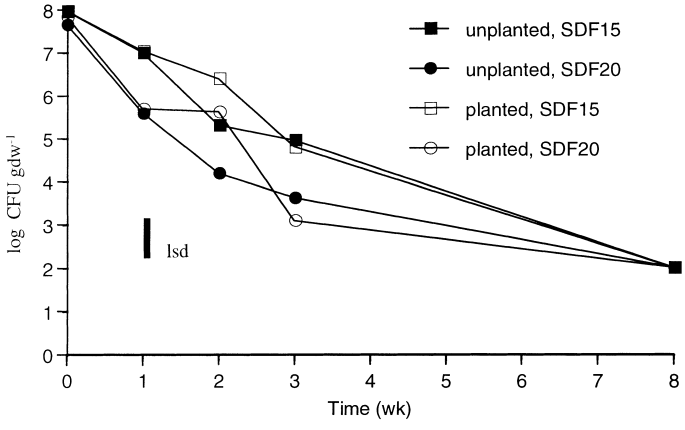

Fig. 1. Survival rates of *Klebsiella planticola* strains in soil. The least significant difference is indicated by the bar ($p \leq 0.01$).

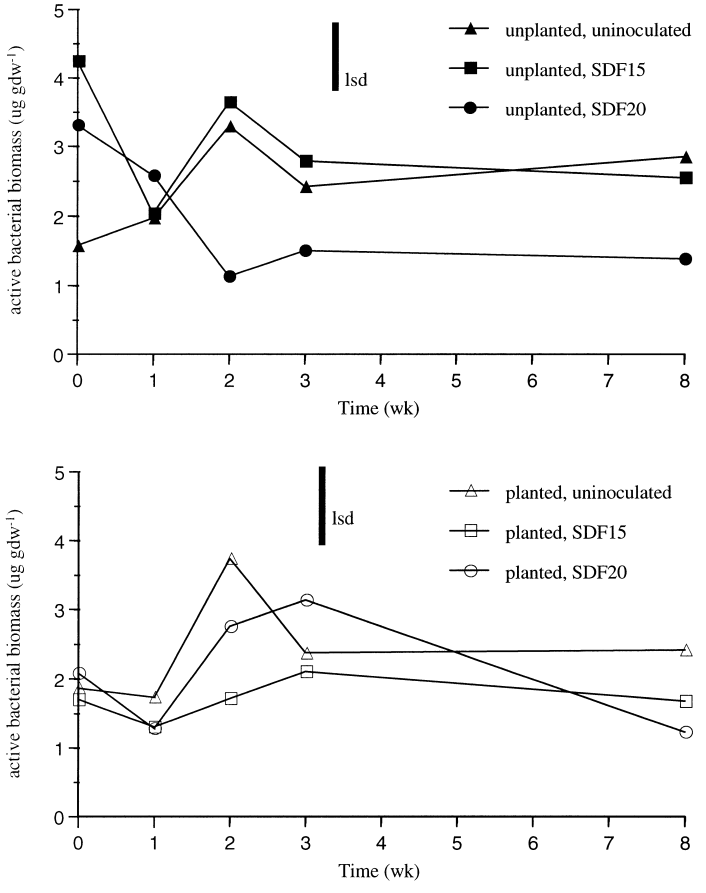

Fig. 2. (a): Active bacterial biomass in unplanted soil treatments. The least significant difference is indicated by the bar ($p \leq 0.01$). (b): Active bacterial biomass in planted soil treatments. The least significant difference is indicated by the bar ($p \leq 0.01$).

Nematodes in soil inoculated with SDF20 (0.6 nematodes gdw^{-1}) increased significantly compared to uninoculated soil (0.1 nematodes gdw^{-1}) and soil inoculated with SDF15 (0.3 nematodes gdw^{-1}). The increases of nematodes observed in soil inoculated with SDF20 consisted of fungal feeders (*Aphelenchus* sp.) during mid experiment, then returned to bacterial feeding (*Acrobeloides* sp.) genera at the end of the experiment (Table 4). Nematodes in soil inoculated with SDF15 were primarily bacterial feeders (*Acrobeloides*). After week 3, the number of bacterial feeding nematodes in soil inoculated with SDF15 continued to increase (0.5 nematodes gdw^{-1}), while the number of nematodes in soil inoculated with SDF20 decreased significantly (0.3 nematodes gdw^{-1}). At the end of the experiment, the numbers of nematodes in soil inoculated with SDF15 were significantly higher than in the uninoculated soil.

3.5. Wheat plant growth

The ratio of root biomass to shoot biomass of wheat plants was used to assess changes in plant growth associated with bacterial treatments. After 1 week, the root to shoot ratios of plants in soil inoculated with SDF15 or SDF20 were significantly greater than that of plants in uninoculated soil (Fig. 5). At this time, plants in soil inoculated with SDF15 or SDF20 produced 34-fold and 24-fold respectively, more root than shoot biomass. In comparison, plants in the uninoculated soil treatment produced only 8-fold more root biomass than shoot biomass. After 2 weeks, root to

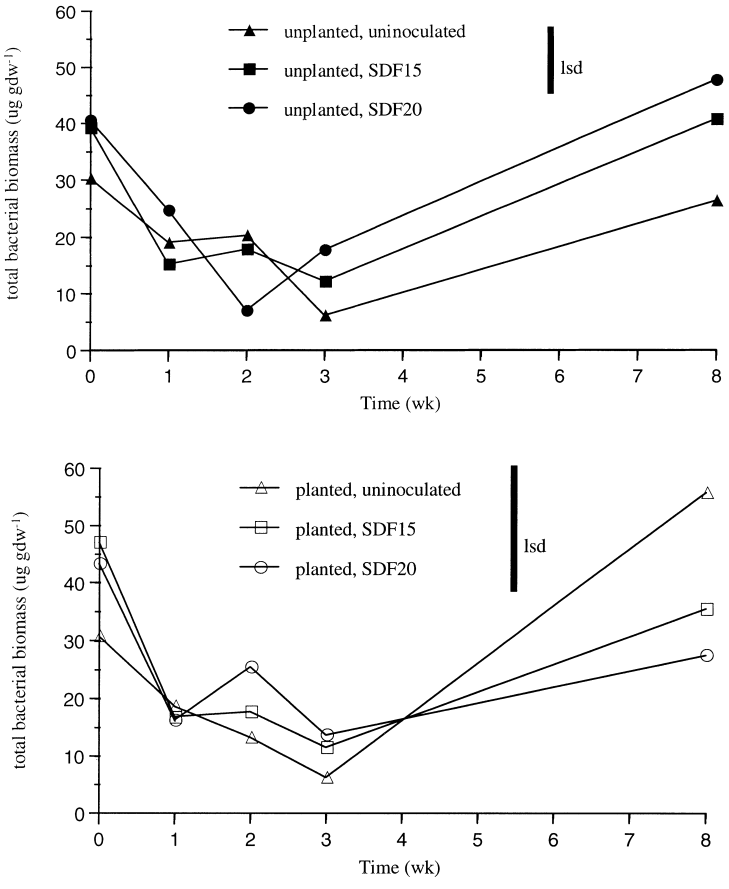

Fig. 3. (a): Total bacterial biomass in unplanted soil treatments. The least significant difference is indicated by the bar ($p \leq 0.01$). (b): Total bacterial biomass in planted soil treatments. The least significant difference is indicated by the bar ($p \leq 0.01$).

shoot ratios of plants in SDF15 or SDF20 treatments were similar to plants in the uninoculated soil treatment. However, at the end of the experiment, plants in soil inoculated with SDF20 were chlorotic and wilting, while plants in uninoculated soil and soil inoculated with SDF15 were flowering. The root to shoot ratio of plants in soil inoculated with SDF20 was significantly greater than the root to shoot ratios of plants in uninoculated soil and soil inoculated with SDF15.

4. Conclusions

Our results demonstrated that nematode community composition and plant growth were affected after the introduction of *K. planticola* SDF20 to soil and that effects can be associated with GEM additions. We suspect substrates from plant roots were crucial for observing some effects on these organisms, as evidenced by the fact that no significant effects were detected after the addition of SDF20 and SDF15 to soil without plants. Moreover, the results of this study demonstrated the importance of using experimental test systems that incorporate biological interactions and include direct measurements of soil biota to assess the effects of GEMs introduced to soil (Doyle et al., 1995).

The exponential decrease of introduced bacteria in unplanted soil was similar to previous studies of GEMs in test systems without plants (Short et al., 1991; Rattray et al., 1992; Doyle and Stotzky, 1993). In this study, however, the survivals of *K. planticola* strains were measurable after 8 weeks of incubation

Table 2
Treatment means (n=3) for active and total fungal biomass measurements in micrograms per gram dry weight of soil. The means were not significantly different ($p \leq 0.01$) between bacterial treatments for each sample date

		Sample dates					
Assay	Treatment[a]	day 0	wk 1	wk 2	wk 3	wk 8	lsd[b]
Active fungal biomass	un-p uninoculated	1.13	1.43	0.26	6.63	0.99	2.131
	un-p SDF15	1.85	2.07	3.05	1.56	0.33	
	un-p SDF20	2.22	3.35	0.25	1.20	1.25	
	p uninoculated	3.38	1.13	0	0.97	0.37	2.164
	p SDF15	2.94	3.05	0.03	1.94	1.61	
	p SDF20	4.44	0.33	0.03	3.34	0.12	
Total fungal biomass	un-p uninoculated	7.17	7.12	6.53	16.73	5.37	5.122
	un-p SDF15	7.27	9.28	6.87	18.16	4.86	
	un-p SDF20	9.05	7.95	4.03	9.17	5.68	
	p uninoculated	9.02	3.58	1.46	8.27	6.85	3.727
	p SDF15	7.43	7.56	6.37	8.61	3.36	
	p SDF20	9.61	4.59	5.30	10.64	2.86	

[a] un-p: Unplanted microcosm; p: Planted microcosm.
[b] Least significant difference.

Table 3
Treatment means (n=3) for the number of protozoa per gram dry weight of soil. The means were not significantly different ($p \leq 0.01$) between bacterial treatments on each sample date

		Sample dates				
Assay	Treatment[a]	day 0	wk 1	wk 3	wk 8	lsd[b]
Flagellates	un-p uninoculated	315 726	3611	95 684	32 125	103 742
	un-p SDF15	369 037	623	58 777	22 865	
	un-p SDF20	228 290	65 520	193 640	32 216	
	p uninoculated	379 313	334 952	47 855	177 914	nc
	p SDF15	201 791	44 578	182 268	203 176	
	p SDF20	318 831	208 396	12 950	20 806	
Amoebae	un-p uninoculated	55 000	56	42	184	48 488
	un-p SDF15	70 996	362	1231	118	
	un-p SDF20	55 000	857	422	275	
	p uninoculated	45 985	6709	112	227	28 832
	p SDF15	61 161	3099	1883	738	
	p SDF20	55 000	640	163	362	

[a] un-p: Unplanted microcosm; p: Planted microcosm.
[b] Least significant difference; nc: Not calculated because f-value too small.

(Fig. 1), unlike other GEMs that were undetectable 3 weeks after inoculation into soil (Doyle and Stotzky, 1993). The ability of SDF20 to persist in soil was expected, because this GEM was constructed from a bacterium occurring naturally in soil (Killham, 1994). In comparison, genetically engineered *Escherichia coli* strains that survived poorly in soil (Doyle and Stotzky, 1993)

The addition of *K. planticola* SDF15 and SDF20 to soil with plants significantly altered the nematode community composition. This alteration was not observed in uninoculated soil with plants (Fig. 4(b)) nor soil treatments without plants (Fig. 4(a)). It is probable that, increases in the number of nematodes following the introduction of SDF20 to soil with plants resulted in a transient increase in plant growth because

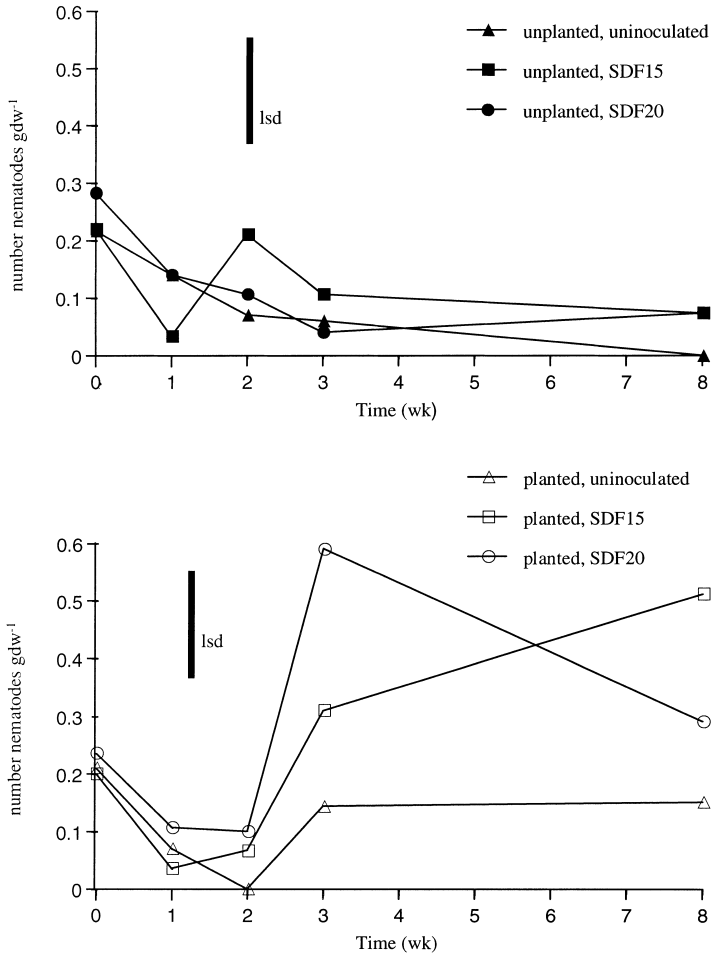

Fig. 4. (a): Number of nematodes in unplanted soil treatments. The least significant difference is indicated by the bar ($p \leq 0.01$). (b): Number of nematodes in planted soil treatments. The least significant difference is indicated by the bar ($p \leq 0.01$).

the grazing activity of nematodes on soil microbiota can increase mineralization rates and the availability of nitrogen in soil (Ingham et al., 1986; Hunt et al., 1987; Freckman, 1988). On the other hand, the increase in nematodes associated with the addition of SDF20 could decrease the number of beneficial microbiota in the rhizosphere, leading to a decrease of nitrogen retention in soil, reduced plant growth in nutrient limiting conditions, and an increase of plant disease (Ingham, 1985). It is also possible that the bacterial feeding nematodes were responsible for decreases in survival of introduced bacteria.

The decline in wheat plant growth may demonstrate the potential for this GEM to affect important soil biota and result in ecological effects. After an increase in fungal feeding nematodes during a week period (Fig. 4(b)), shoot growth stopped in the soil treatments inoculated with SDF20 and the plants began to die (Fig. 5). Although, the mechanism was not clear by which the addition of SDF20 contributed to the decline in plant growth, the decline could be attributed to the significant increase in the numbers of fungal feeding nematodes because an increase in the numbers of bacterial feeding nematodes in soil inoculated with SDF15 did not result in a decline of plant growth. The alternative explanation is that, SDF20 produced ethanol within the root system, although ethanol was not detected in the headspace of the microcosms with

Table 4
Percentage of nematode genera extracted and trophic groups from soil microcosms per sample date

Nematodes genera	Trophic[a]	day 0	wk 1	wk 2	wk 3	wk 5
Acrobeloides	BF	18.7		15.7	23.7	64
Cephalobus	BF	50		5.3		
Acrobeles	BF	6.2	33.3		5.3	
Tylenchus	FF	3.1				
Aphelenchus	FF	21.8	50	63.1	44.7	32
Thonus	PF			10.5		
Trichodorus	PF			5.3		
Ditylenchus	FF					4
Aphelenchoides	FF				26.3	
Eudorylaimus	FF		16.7			
% Bacterial-feeding		74.9	33.3	21	29	64
% Fungal-feeding		24.9	66.7	63.1	71	36
% Plant-feeding				15.8		

[a] BF: Bacterial-feeding; FF: Fungal-feeding; PF: Plant-feeding.

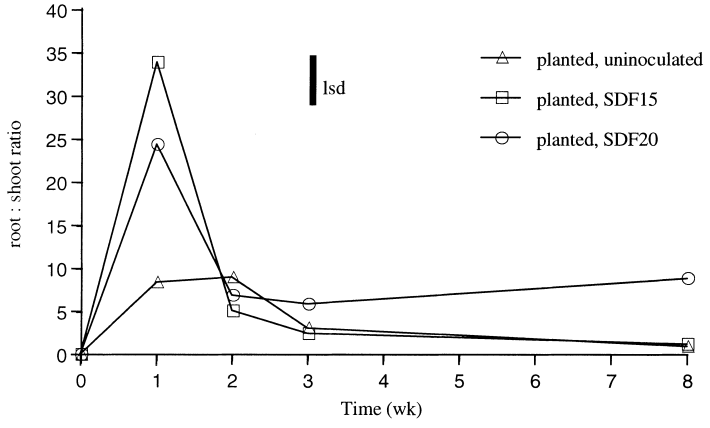

Fig. 5. Ratio of root biomass to shoot biomass of plants in planted soil treatments. The least significant difference is indicated by the bar ($p \leq 0.01$).

plants using similar experimental conditions (Holmes, 1995).

The results from this study support some views expressed by Doyle et al. (1995) that alterations in microbial processes after the introduction of GEMs needs to be evaluated across numerous trophic groups of organisms. Ecological effects induced by the introduction of SDF20 were assessed by monitoring microbial components of the soil biota and plant health. The addition of SDF20 to soil with and without plants yielded different ecological effects as mediated by soil biota and we stress the need to assess biotic interactions as indicators of ecological effects. If similar alterations in soil biota occur in field environments after the introduction of SDF20, plant growth and nutrient cycling processes would most likely be affected. Soil microcosms with plants that include biological interactions and direct assays of soil biota were important for evaluation of ecological effects. Measurements of other soil biota such as plant symbionts, microarthropods, and earthworms need to be included in the monitoring process to more completely assess ecological effects associated with the introduction of GEMs like SDF20 to soil.

As much as 20% of photosynthates produced by some crop plants are released into the soil by exuda-

tion and related processes (Lynch, 1990). Plant roots also add organic carbon to soil as mucilaginous secretions from the root cap and sloughing of root cells during growth. Gilbert et al. (1993) demonstrated that the presence of plant roots increased the survival of a biological control agent, *Bacillus cereus* UW85 introduced to soil. Consequently, the presence of plants was expected to enhance the survival of introduced bacteria in this study and perhaps provide sufficient substrate for enhanced ethanol production by the GEM. Unfortunately, we were unable to detect ethanol in the air headspace of soil microcosms, when plants were present suggesting that other mechanisms in which survival and gene expression of SDF20 in soil would result in ecological effects. For example, ethanol produced by *K. planticola* SDF20 could stimulate the growth of ethanol utilizing organisms that are beneficial or cause plant disease. However, if ethanol production by the GEM is enhanced in the presence of plants, there is a probability for even greater effects on the soil biota as suggested by Jones (1989).

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