SHORT COMMUNICATION

Straw as a Substrate for Cooperative Nitrogen Fixation

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Inoculation of wheat straw, contained in glass columns and moistened by continuous recirculation of a solution containing mineral salts, with a cellulolytic fungus, *Penicillium corylophilum*, and a N₂-fixing anaerobe, *Clostridium butyricum*, increased the decomposition rate constant from 0.0096 d⁻¹ to 0.0139 d⁻¹ compared with non-inoculated straw. N₂ fixation during the utilization of the straw resulted in a gain of 11.5 mg N (g straw lost)⁻¹ over a period of 8 weeks at 25 °C.

INTRODUCTION

Nitrogenase function consumes large amounts of ATP and therefore the fixation of dinitrogen in soil is generally considered to be agriculturally significant only in symbiotic associations; inadequate carbohydrate is available for a significant contribution from the free-living N₂-fixers (Postgate & Hill, 1979). Straw is about 75% (w/w) cellulosic (Harper & Lynch, 1981*a, b*) and provides a potential major source of carbon and energy to the soil microflora (Lynch & Panting, 1980), but because its presence can decrease crop yield (Lynch *et al.*, 1980), in the United Kingdom it is usually burnt. We have investigated whether inoculation of straw with a cellulolytic fungus could provide enough simple sugars for co-inoculated non-cellulolytic N₂-fixing bacteria to function in competition with other organisms already present on the straw and thereby increase the N content of the decaying material.

Preliminary studies showed that when straw in columns was inoculated with soil, most of the isolates capable of growth on cellulose as carbon and energy source were *Penicillium* spp.; *P. corylophilum* was the most common. Isolation on to N-free media failed to detect *Azotobacter* spp. as colonizers of straw despite the presence of the bacterium in the soil inoculum. N₂-fixing isolates developed only under anaerobic conditions and were exclusively *Clostridium* spp. We therefore selected *P. corylophilum* and *C. butyricum* for the present studies.

METHODS

Flask study. Wheat (*Triticum aestivum*) straw was chopped into 0.5 cm lengths and subsamples (1 g) weighed into 250 ml conical flasks containing 100 ml N-free mineral salts solution (Dalton & Postgate, 1969) or the mineral salts solution amended with ammonium chloride (2 g l⁻¹). Flasks were autoclaved at 15 lbf in⁻² (104 kPa) for 15 min before inoculation with *Penicillium corylophilum* S1/3 and *Clostridium butyricum* NCIB 7423 [about 10⁶ spores or cells (g straw)⁻¹]. They were incubated for 3 weeks at 20 °C on a rotary shaker (100 r.p.m.). The straw was then harvested on filter paper (Whatman no. 1) but not washed, dried at 60 °C and its chemical composition determined by proximate analysis (Harper & Lynch, 1981*b*). Losses of straw cellulose and hemicellulose were calculated from the loss in total dry weight and change in chemical composition of the remaining straw, after making corrections for initial and final ash content. N content was determined by an automatic N analyser (model 1106, Erba Science, Swindon, Wilts., U.K.).

Column study. Chopped wheat straw (50 g) was packed into glass columns (300 × 50 mm) to occupy a volume of 0.5 l. The columns were sealed with silicone rubber bungs carrying glass tubes for aeration and medium supply at the top and for drainage at the base. The straw was flooded with N-free mineral salts solution for 30 min and then

Table 1. *Decomposition of sterile straw and N gain by Penicillium corylophilum and Clostridium butyricum in the presence or absence of N*

Straw was incubated at 20 °C for 3 weeks in the presence or absence of 50 mg N (g straw)⁻¹.

Treatment	Percentage of polysaccharide decomposed		N gain into straw [mg N (g original straw) ⁻¹]	
	- N	+ N	- N	+ N
Non-inoculated	0	0	0	0
<i>Penicillium corylophilum</i>	2	17	0	3.9
<i>Clostridium butyricum</i>	0	0	0	0
<i>P. corylophilum</i> + <i>C. butyricum</i>	11	15	3.3	4.0
L.S.D. (<i>P</i> = 0.05)	3		1.9	

Table 2. *Decomposition of non-sterile straw contained in glass columns at 25 °C for 8 weeks*

Treatment	Decomposition rate constant, <i>k</i> (d ⁻¹)	No of micro-organisms capable of growth on N-free media		N gain (mg)	
		Aerobes [10 ⁻⁶ × No. (g straw) ⁻¹]	Anaerobes [10 ⁻⁷ × No. (g straw) ⁻¹]	Per g straw lost	Per g original straw
Non-inoculated	0.0096	9	13	8.8	2.8
<i>Penicillium corylophilum</i> + <i>Clostridium butyricum</i>	0.0139	28	24	11.5	5.0
L.S.D. (<i>P</i> = 0.05)	0.0030	16	N.S.	N.S.	0.6

N.S., Not significant.

drained before starting aeration (50 ml min⁻¹) and recycling of the medium with a peristaltic pump (2 ml min⁻¹). The columns were incubated at 25 °C for 8 weeks. At flooding, micro-organisms [about 10⁶ spores or cells (g straw)⁻¹] were added to the columns. Each treatment was replicated twice.

At the completion of the experiment subsamples of straw (about 1 g cut into 1 cm lengths) were shaken for 1 min by hand with glass beads (1 g, 2 mm diameter) in sterile N-free medium. The medium was amended with cysteine (0.5 g l⁻¹) for counts of anaerobes. The straw was allowed to settle and a dilution series then prepared from the supernatant. Aliquots of the diluents were spread on agar containing the N-free mineral salts solution and cellobiose (10 g l⁻¹). Colonies were counted on three replicate plates after incubation for 5 d at 25 °C either aerobically or in an anaerobic incubator (Don Whitley Scientific, Shipley, Yorks., U.K.). The efficiency of release of cells from the straw was checked by resuspension in sterile medium followed by preparation of a further dilution series; these latter counts never exceeded 10% of the original. The subsamples together with the bulk of the straw were dried (100 °C, 16 h) and further subsamples taken for the determination of N content by the automatic N analyser. The decomposition rate constant (*k*) was calculated from the loss of straw weight (Harper & Lynch, 1981*a*) according to the equation $\ln A/A_0 = kt$, where *A* is the weight of straw remaining, *A*₀ is that originally present and *t* is the decomposition time.

RESULTS

Penicillium corylophilum in pure culture decomposed straw polysaccharides (cellulose and hemicellulose) only when nitrogen was added to the straw (Table 1). *Clostridium butyricum* did not grow or fix N₂ in pure culture, but grew and supported cellulolysis by *P. corylophilum* in co-culture, presumably by the provision of a source of fixed N.

Inoculation of the non-sterile straw in columns with *P. corylophilum* and *C. butyricum* resulted in a significant increase in the decomposition rate constant compared with non-inoculated straw (Table 2). Inoculation with these species also resulted in a greater number of organisms on the straw capable of growth on N-free agar at the termination of the experiment, and greater increases may have occurred during it. However, as many of the counted cells would probably have been present originally as spores, increase in bacterial biomass would be the more relevant measurement.

DISCUSSION

In replicated trials, the cellulolytic fungus (*P. corylophilum*) seems to have co-operated with the anaerobic bacterium (*C. butyricum*) to hasten straw degradation and release sufficient sugar to allow the anaerobe to fix N_2 . Although the culture systems were aerated, anaerobic sites are likely to have been created within the straw by the oxygen demand of the aerobes. In simpler degradation systems, we have now found several such associations of cellulolytic fungi with anaerobic N_2 -fixing bacteria and with some aerobic organisms.

Earlier studies with mixed populations of cellulolytic and N_2 -fixing organisms utilizing cellulosic substrates in submerged culture systems have shown a gain of 6–14 mg N (g substrate consumed)⁻¹ (Jensen & Swaby, 1941) and 7–12 mg N (g substrate consumed)⁻¹ (Rice & Paul, 1972), which is comparable to the amounts measured in the present work.

Straw from a cereal crop yielding 7 t grain ha⁻¹ in the United Kingdom usually amounts to about 7 t ha⁻¹. The N gain of 5 mg N (g straw)⁻¹ in this study under conditions sufficiently simple that a farm-scale operation can be envisaged, would thus be equivalent to 35 kg N ha⁻¹. As the straw already contained 3.1 mg N g⁻¹, the total N content of the straw would amount to 57 kg N ha⁻¹, or about half of the annual fertilizer application to arable crops. Such N derived from heterotrophic N_2 -fixation seems to be available to plants at about the same rate as fertilizer N (Ito & Watanabe, 1981). With optimization of the growth conditions and selection of improved microbial strains and associations, the 5 mg N (g straw)⁻¹ could probably be substantially increased.

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