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The microbial upgrading of straw for agricultural use

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Straw, particularly when left on the soil surface, can retard cereal establishment and reduce crop yield and therefore, in Britain, it is often burned. Treatment with a microbial consortium attempts to eliminate the negative value of straw while using it as a fertilizer, soil conditioner and biocontrol agent. Cellulolytic fungi, preferably *Trichoderma* species which are parasitic on some plant pathogenic fungi, convert the cellulosic components of straw to sugars. These sugars become available for growth of an anaerobic N_2-fixing bacterium, *Clostridium butyricum*. Part of the N fixed in microbial biomass is recycled to the fungus to stimulate further cellulolysis and ultimately a product rich in N and with no phytotoxicity results. A third organism, *Enterobacter cloacae*, that produces copious extracellular polysaccharide is co-inoculated as an active member of a microbial community. The O_2 consumption by *E. cloacae* and its associated gum aids respiratory protection of the anaerobe to form a consortium that is relatively insensitive to O_2. The gum has additional value as a soil conditioner.

Introduction

Straw is one of the major agricultural wastes. For example, in England and Wales about 15 Mt are produced and about one half is burnt in the field. Farmers burn straw because poor establishment of crops and yield reductions occur when it is present in soil; the magnitude of this effect is highly variable but on a particular heavy clay soil in a wet autumn the crop yield of a direct-drilled wheat crop was reduced by up to 56% (Lynch *et al.* 1981). Straw can introduce mechanical difficulties in drilling but even if these are overcome, biological problems remain. In Britain and the U.S.A. the principal problem appears to be that bacteria use the cellulose and hemicellulose components of straw (75–80% by mass) as substrates in fermentative metabolism and produce phytotoxic acetic, propionic and butyric acids (Lynch 1977; Tang & Waiss 1978). However, it has also been demonstrated recently that pseudomonad bacteria colonizing straw can penetrate the root cortex and stunt growth (Elliott & Lynch 1984*a*), but the generality of this effect is unclear at present. Traditionally it has been considered that the wide C:N ratio of straw (about 100:1) in relation to the much smaller ratio of degrading micro-organisms (about 10:1) causes crop nitrogen deficiency as available soil nitrogen is immobilized into microbial biomass. This certainly occurs during establishment of the crop but nitrogen is not lost from the system and is eventually recycled.

Recognizing that straw has a negative value to the farmer at present, our aim has been to devise and evaluate a strategy aimed at the microbial utilization of straw to give it a positive value in agriculture. The key to the latter goal is a kinetic one in that phytotoxic metabolites are only produced during the early stages of straw decomposition (Harper & Lynch 1981). If decomposition could be accelerated before drilling the crop, the negative value would be largely eliminated.

Cellulolytic N_2-fixing microbial communities

In nature, N_2 fixation is expensive in energetic (ATP) terms, and in free-living bacteria is generally considered to be limited by available carbohydrate (Postgate & Hill 1979). For the polymeric sugars of lignocelluloses to be channelled to this process both cellulolysis and hemicellulolysis are necessary. There is evidence that this process can occur during the decomposition of rice straw (Yoneyama *et al.* 1977) and barley straw (Rice & Paul 1972), but the decomposer micro-organisms were not identified. With the exception of the specialized environment of marine shipworms (Carpenter & Culliney 1975; Waterbury *et al.* 1983), no organism possessing both cellulolytic (cellulase) and N_2-fixing (nitrogenase) functions has yet been isolated from nature or genetically engineered. We therefore assume that in nature mixed microbial communities of at least two members must bring about these functions. If such communities could be isolated, identified and reconstructed and maintained at elevated population levels, there would be potential for a novel biotechnological use of micro-organisms in agriculture.

Dinitrogen fixation is particularly intense in decomposing straw (or cellulose) at aerobic–anaerobic interfaces (Buresh *et al.* 1980; Harper & Lynch 1984). This, therefore, seemed an appropriate niche from which to attempt isolation of N_2-fixing cellulolytic communities. When wheat straw was inoculated in a mineral salts medium with a soil inoculum, the only bacteria which could be isolated on N-free media containing glucose or cellobiose under anaerobic conditions were identified exclusively as *Clostridium butyricum* (Harper & Lynch 1984). In aerobic conditions no bacteria were isolated on N-free media, but when ammonium chloride was added much larger bacterial populations were obtained and most isolates (more than 90 %) were *Pseudomonas* spp. Predominant amongst other isolates were strains of *Enterobacter cloacae* which did not fix N_2 but produced copious quantities of extracellular polysaccharide. No cellulolytic bacteria were found in these isolations.

To isolate cellulolytic fungi, an agar containing the combined cellulose and hemicelluloses ('holocellulose') of wheat straw was used (Harper & Lynch 1984). The ability to produce diffusible cellulase activity was assayed by transferring isolates to an agar containing ball-milled cellulose only and measuring the depth of clearing. The fungi isolated included species of *Acremonium*, *Botryotrichum*, *Chaetomium*, *Fusarium* (five species), *Geotrichum*, *Penicillium* (ten species), *Sordaria* and *Trichoderma*. Maximum depth of clearing of the agar was achieved by *Trichoderma* spp. (table 1). It therefore seemed that *T. harzianum* would be a suitable candidate organism to associate with *C. butyricum* in bringing about associative cellulolysis–N_2 fixation.

In a model system the two organisms were grown under axenic conditions with pure cellulose as the sole source of carbon and energy (Veal & Lynch 1984). By using co-cultures of *T. harzianum* and *C. butyricum*, nitrogen gains of 7.87 mg fixed per gram of carbon lost were achieved as measured by elemental analysis and confirmed by acetylene reduction tests. Neither acetylene reduction nor N gains were found with pure cultures of *T. harzianum* or *C. butyricum*. By using ^{15}N-labelled cells of *C. butyricum*, it was shown that part of the N_2 fixed by *C. butyricum* was available to *T. harzianum* and presumably was used for cellulase production because substrate decomposition was stimulated. Small amounts of $(NH_4)_2SO_4$ (0.1 mg ml^{-1}) supported good fungal growth before establishment of the N_2-fixer. Air was not excluded from the experiments and therefore the anaerobe co-existed with the aerobe by the latter acting as a sink for O_2, providing 'respiratory protection'. It was also demonstrated that in the absence of oxygen the fungus could be replaced by pure cellulase.

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Table 1. Cellulolytic acivity of some fungal isolates on sterilized straw and cellulose agar[a]

	mass loss from straw/(mg g^{-1})	clearing of cellulose agar/mm
Acremonium persicinum (IMI 284720)	305	4
Botryotrichum piluliferum (IMI 284721)	145	0
Penicillium corylophilum (IMI 284722)	69	0
P. hordei (IMI 284723)	125	5
P. janthinellum (IMI 284724)	240	6
Sordaria alcina (IMI 267236)	267	5
Trichoderma harzianum (IMI 284726)	250	11
T. harzianum (IMI 275950)	238	10
T. longibrachiatum (IMI 284728)	236	15
T. viride (QM 9414)	75	0

[a] Both assays were of 4 weeks duration.

In an initial study, testing the application of these principles to growth on straw contained in columns under non-axenic conditions, *C. butyricum* was co-inoculated with another cellulolytic fungus, *Penicillium corylophilum* (Lynch & Harper 1983). Inoculation stimulated the decomposition rate constant of straw (Harper & Lynch 1981) from 0.0096 d^{-1} to 0.0139 d^{-1}. The nitrogen gain by the inoculated straw during decomposition was 5 mg N per gram of straw. When applied to a cereal crop yielding 7 t ha^{-1} of grain this would amount to a gain equivalent to 35 kg N ha^{-1}. The N already present in the straw was 3.1 mg N g^{-1}. Therefore, the total N in the degraded straw would be equivalent to 57 kg N ha^{-1}, or about one third of the normal fertilizer application to arable crops. Microbial N appears to become available to the crop (Ito & Watanabe 1981; Lethbridge & Davidson 1983).

The screening procedure had indicated that other fungi might be preferable to *P. corylophilum* as the cellulolytic partner and therefore in a second series of trials some of these were screened individually and in co-cultures for their potential in straw decomposition and N_2 fixation. *T. harzianum* and *Sordaria alcina* were both more satisfactory than *P. corylophilum* in stimulating decomposition (table 1). In some of the treatments *Enterobacter cloacae*, which had been isolated in the original studies, was also added to the community. This strain, although not able to fix N_2, produced a copious extracellular gum and it was considered that it might aid the respiratory protection of the N_2-fixing anaerobe. Neither bacterium appeared to stimulate straw decomposition independently but their addition in combination to the fungus was effective in stimulating N gain (table 2). Again a more satisfactory association was provided with *T. harzianum* and *S. alcina* than with *P. corylophilum*. The total N gains were not as large as had been obtained with *P. corylophilum* and *C. butyricum* in the initial experiment, but the conditions and duration of the assay were different. Clearly the goal is to optimize the process and to determine the process-dependent variables. Already gains of 12 mg N g^{-1} straw have been obtained with the *T. harzianum–C. butyricum–E. cloacae* consortium.

The optimum concentration of each component of the inoculum required for the community to function most efficiently has yet to be determined. The bacterial inoculants function very satisfactorily when applied at 10^6 colony-forming units per gram of straw, although they have been equally successful when the inoculum size has been as low as 10^4. The lower limit is partly dependent on the natural populations of the inoculants present in soil. Indeed in some situations it is possible that one component of the inoculum could be left out but it would be short-sighted

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TABLE 2. EFFECT OF INOCULANTS ON THE DECOMPOSITION OF STRAW AND N_2 FIXATION

	percentage mass loss	N gain / mg g^{-1}
uninoculated	8.1	1.24
Clostridium butyricum	8.6	1.04
Enterobacter cloacae	8.5	1.27
Penicillium corylophilum	9.9	1.40
Sordaria alcina	18.5	1.13
Trichoderma harzianum	16.6	1.34
C. butyricum + *E. cloacae*	12.4	1.30
C. butyricum + *E. cloacae* + *P. corylophilum*	11.7	1.53
C. butyricum + *E. cloacae* + *S. alcina*	16.8	1.94
C. butyricum + *E. cloacae* + *T. harzianum*	17.3	2.02
l.s.d. ($p = 0.05$)	3.3	0.43

to take such a risk for general application. *C. butyricum* was never detected unless *E. cloacae* was inoculated but the converse was not true.

When straw was treated with the microbial consortium for 56 days and then added to pots of soil, wheat seedlings established at the same rate as those growing in the absence of straw. This contrasted with the effect of undecomposed straw, which reduced the shoot dry weight of plants (determined 24 days after sowing) by 60%. This demonstrates the elimination of the negative effects of straw by the decomposition but it would have been too early in the decay process for seedlings to take advantage of the fixed N_2 from the consortium.

MICROBIAL POLYSACCHARIDES AS SOIL CONDITIONERS

It is recognized that microbial polysaccharides stabilize soil structure and prevent erosion (Cheshire 1979). In an initial study to demonstrate the usefulness of straw to provide a substrate for this function, no advantage could be found of inoculation over the natural organisms present (Lynch & Elliott 1983). The production of polysaccharide was greater when the N content of straw was small (Elliott & Lynch 1984*b*). Polysaccharide extracted by hot water from decomposed straw was composed mainly of galactose, glucose and mannose with smaller quantities of arabinose, xylose, rhamnose, fucose and ribose, indicating a mainly microbial origin (Chapman & Lynch 1984). More recent studies (S. J. Chapman & J. M. Lynch, unpublished results) have demonstrated an advantage of inoculation with the microbial community. Notably *E. cloacae* is a critical component. Its usefulness in aggregation depends on the specific strain used and on the cellulolytic fungus with which it associates.

BIOCONTROL VALUE OF MICROBIAL INOCULANTS

Amongst the biocontrol agents that have been evaluated in the laboratory and in the field over many years, *Trichoderma* spp. have been amongst the most successful (Henis 1984). *T. harzianum* is effective in controlling *Rhizoctonia solani* and *Sclerotium rolfsii*. Recently it has been demonstrated that *E. cloacae* is effective in controlling the damping-off disease of lettuce caused by *Pythium* spp. (Hadar *et al.* 1983). It is therefore fortunate that both these antagonists are members of the straw-degrading microbial consortium.

In an initial *in vitro* test, the cellulolytic fungi isolated as potential consortium members were

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screened against the pathogens *Fusarium culmorum* and *Pythium ultimum*. The pathogen was placed on one side of a Petri dish containing malt agar and the antagonist on the other side. After five days, the proportion of the plates covered by the pathogen was assessed. Of the eleven cellulolytic fungi tested, only *T. harzianum* completely suppressed the growth of *F. culmorum* and *P. ultimum*. *S. alcina* was similarly active against *P. ultimum* but was only mildly active against *F. culmorum*. Further tests showed *T. harzianum* to be active against the following pathogens: *Botrytis cinerea*, *Fusarium avenaceum*, *F. oxysporum* sp. *dianthi*, *F. oxysporum* sp. *narcissi*, *F. solani*, *Phomopsis sclerotioides*, *Pyrenochaeta lycopersici* and *Sclerotinia sclerotiorum*. Because bacteria do not generally spread on plates in the same manner as fungi, *E. cloacae* was not demonstrated as an effective antagonist in these tests. However, it was included in a subsequent *in vivo* screen where lettuce plants (*Lactuca sativa*) were grown in sand moistened with nutrient solution in the presence of *S. sclerotiorum*, which causes a butt rot. Both *T. harzianum* and *E. cloacae* showed activity in preventing seedling death.

Conclusion

The description of the multi-functional properties of the cellulolytic N_2-fixing microbial consortium is a summary of laboratory observations that will need field evaluation to determine whether they will be appropriate for agricultural technology in the 1990s. The efficiency of substrate consumption by various N_2-fixing bacteria is usually around 12 mg N fixed per gram of carbon energy source consumed, but figures up to 36 mg N fixed per gram of carbon energy source consumed have been measured for *Azotobacter chroococcum* (Postgate & Hill 1979) Although care must always be taken in extrapolating from pure culture systems on defined laboratory media, it is clear that the present maximal N gains of 12 mg N fixed per gram of original straw (*ca.* 24 mg N fixed per gram of straw consumed) is probably fairly close to an optimal figure. Such a gain applied to an agricultural system would contribute appreciably to satisfying the needs of an arable crop. We are encouraged by recent observations that straw appears to stimulate N_2 fixation under field conditions (Roper 1983) although the magnitude of the N gain is not given. However, N gain to the crop may not be the major economic feature of the consortium unless fertilizer prices rise dramatically in the 1990s. Stimulation of straw breakdown by channelling the N back to the cellulolytic fungus, which would probably function with relatively small N gain, is potentially the most economic aspect of the treatment.

The soil-conditioning value of the consortium would be likely to lead to long-term benefits agronomically and in terms of the preservation of soil as a natural resource. Such long-term benefits, however, become difficult to cost economically. The crop protection potential is also difficult to cost because there are few methods available for chemical control of root diseases. Indeed it is even difficult to assess the overall crop losses that result, particularly for minor pathogens such as *Pythium* spp. Any such treatment would presumably be applied prophylactically. The present laboratory results show a potentially useful target for soil biotechnology (Lynch 1983). Following these results, field trials, fermentation scale-up, product formulation and toxicological testing will be necessary before a product could be provided for the farmer but if present goals are achieved, this could be realized within five years. There is also scope for the utilization of the treated straw in horticulture, analogous to the current usage of composted bark substrates (Hoitink *et al.* 1982).

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References

Buresh, R. J., Casselman, M. E. & Patrick, W. H. 1980 *Adv. Agron.* **33**, 149–192.
Carpenter, E. J. & Culliney, J. I. 1975 *Science, Wash.* **221**, 1401–1403.
Chapman, S. J. & Lynch, J. M. 1984 *J. appl. Bact.* **56**, 337–342.
Cheshire, M. V. 1979 *Nature and origins of carbohydrates in soils.* (216 pages.) London: Academic Press.
Elliott, L. F. & Lynch, J. M. 1984*a* *Soil Biol. Biochem.* **16**, 69–71.
Elliott, L. F. & Lynch, J. M. 1984*b* *Pl. Soil* **78**, 335–343.
Henis, Y. 1984 In *Current perspectives in microbial ecology* (ed. M. J. Klug & C. A. Reddy), pp. 353–361. Washington D. C.: American Society for Microbiology.
Hadar, Y., Harman, G. E., Taylor, A. G. & Norton, J. M. 1983 *Phytopathology* **73**, 1322–1325.
Harper, S. H. T. & Lynch, J. M. 1981 *J. Soil Sci.* **32**, 627–637.
Harper, S. H. T. & Lynch, J. M. 1984 *J. appl. Bact.* **57**, 131–137.
Hoitink, H. A. J., Nelson, E. B. & Gordon, D. T. 1982 *Ohio report on research and development, no.* 67, pp. 7–10.
Ito, O. & Watanabe, I. 1981 *Soil Sci. Pl. Nutr.* **27**, 169–176.
Lethbridge, G. & Davidson, M. S. 1983 *Soil Biol. Biochem.* **15**, 375–376.
Lynch, J. M. 1977 *J. appl. Bact.* **42**, 81–87.
Lynch, J. M. 1983 *Soil biotechnology. Microbiological factors in crop productivity.* (191 pages.) Oxford: Blackwell Scientific.
Lynch, J. M. & Elliott, L. F. 1983 *Appl. environ. Microbiol.* **45**, 1348–1401.
Lynch, J. M. & Harper, S. H. T. 1983 *J. gen. Microbiol.* **129**, 251–253.
Lynch, J. M., Ellis F. B., Harper, S. H. T. & Christian, D. G. 1981 *Agric. Environ.* **5**, 321–324.
Postgate, J. R. & Hill, S. 1979 In *Microbial ecology. A conceptual approach* (ed. J. M. Lynch & N. J. Poole), pp. 191–213. Oxford: Blackwell Scientific Publications.
Rice, W. A. & Paul, E. A. 1972 *Can. J. Microbiol.* **18**, 715–723.
Roper, M. M. 1983 *Aust. J. Agric. Res.* **34**, 725–739.
Tang, C. S. & Waiss, A. C. 1978 *J. chem. Ecol.* **4**, 225–232.
Veal, D. A. & Lynch, J. M. 1984 *Nature, Lond.* **310**, 695–697.
Waterbury, J. B., Calloway, C. B. & Turner, R. D. 1983 *Science, Wash.* **221**, 1401–1403.
Yoneyama, T., Lee, K. K. & Yoshida, T. 1977 *Soil Sci. Pl. Nutr.* **23**, 287–295.

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