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DECOMPOSITION AND HUMIFICATION OF PLANT RESIDUES BY SOME SOIL FUNGI

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SUMMARY: Decomposition and humification of powdered plant material of Leptochloa fusca L. Kunth and Sesbania aculeata Pers. by eight soil fungi was studied in pure culture. Maximum decomposition was caused by Sporotrichum pruinosum, and maximum humification by Stachybotrys atra. Significant differences were observed in some chemical and optical properties of humic compounds produced by these fungi.

INTRODUCTION

Organic amendment is a recognized method for improving soil productivity and has been recommended for the amelioration of salt-affected soils (Yadav and Agarwal, 1961; Sandhu and Malik, 1975). Such soil are, however, poor in microbial population, resulting in a low rate of organic matter decomposition. Inoculation of such soils with appropriate microbes or addition of decomposed plant residues may be necessary for the desired effects.

The present investigation was aimed at studying the role of fungi in decomposition and humification of plant residues. The fungi used were previously reported highly salt-tolerant and efficient cellulose decomposers (Malik et al., 1982). Some of these species e.g. D. rostrata, Papulospora sp. and S. atra, form dark coloured phenolic polymers called "melanins" which are either incorporated into their mycelia or secreted into the growth media. Melanins have been reported to participate in soil humus formation (Haider et al., 1974). The substrates used for their growth were L. fusca (Lf) and S. aculeata (Sa); the former is a highly salt-tolerant grass while the latter is a moderately salt-tolerant forage legume. Both the grass and the legume have been proposed by Sandhu and Malik (1975) as primary and secondary colonizers, respectively, of salt-affected soils. These substrates were chosen for the study as they can be obtained in bulk quantities from such soils for the large scale production of an organic manure.

MATERIALS AND METHODS

Fungi in the study were isolated from salt-affected soils of Pakistan and included: Aspergillus sydowi, A. terreus, Chaetomium globosum, Drechslera rostrata, Papulospora sp., Sporotrichum pruinosum, Stachybotrys atra and Trichoderma piluliferum. Four g. powdered plant material of Lf grass or Sa legume was taken in 50 ml Erlenmeyer flasks, moistened with 12 ml distilled water and autoclaved. The flasks were inoculated with 4 mycelial discs (10 mm) cut from actively growing margins of fungal colonies grown on malt extract agar medium. Each treatment was in triplicate. Inoculated flasks were incubated for 4 weeks at 30°C and shaken by hand at weekly intervals. After incubation the material was dried at 70°C to a constant weight and the loss in weight calculated. Portions fo the air-dried material were extracted with 0.2N NaOH (one hr shaking at room temp.) followed by filtration through Whatman No.1 filter paper. An aliquot of the alkali extract was acidified to pH 2.0 and incubated at 80°C for 30 min. The precipitate (humic acid) was isolated by centrifugation and dissolved in 0.1 N NaOH. Carbon content of the alkali extract (humic acid + fulvic acid) and humic acid were determined by colorimetric method (Malik et al., 1979). The amount of humic compounds was calculated by multiplying C content by 1.724 (Kononova, 1966). Humification productivity i.e. ratio of humus produced to C lost (as CO_2) (Franklova and Novak, 1967) was also calculated. Nitrogen content of humus fractions was estimated by micro-Kjeldahl (Bremner, 1965). Optical density of humic acid was taken at 465 and 665 nm to obtained E_4/E_6 ratio.

Lignin content of the organic material was determined by an acid hydrolysis method (personal communication, K. Haider, FAL, Braunschweig, FRG). Samples used for this purpose were first extracted with 0.2 N NaOH to avoid co-precipitation of humic compounds during acid hydrolysis. Concentrated HCl (12.5 ml) was added to 250 mg of alkali extracted material in 100 ml Erlenmeyer flasks. The flasks were stoppered and after 15 min. 1.25 ml conc. H_2SO_4 was added. The flasks were stoppered again and shaken for 12 hrs on a rotary shaker. After shaking, the volume was made up to 250 ml with boiling water and the flasks kept in boiling water for 10 min. The contents were filtered through a pre-weighed sintered glass crucible and washed repeatedly with hot water until pH of the filtrate was neutral. The residue (lignin) was dried at 105°C to a constant weight.

RESULTS

As judged by loss in weight of the substrate, S. pruinosum caused maximum mineralization of both substrates i.e. 33 and 45% for the grass and the legume, respectively (Table 1). The remaining fungi mineralized only up to 21% of the two substrates. A. terreus a highly efficient decomposer of filtre paper cellulose (Malik et al., 1982), was found to be the least efficient. On the other hand, melanoid fungi namely, D. rostrata, Papulospora sp. and S. atra, with relatively low cellulase activity (Malik et al., 1982) proved better decomposers of both the substrates. Regarding the comparative utilization of the two substrates more weight

loss was observed for the legume. Malik and Azam (1979) have also reported similar differences in the decomposition rate of Lf and Sa. Lignin degradation was more in the Lf grass than in the Sa legume. Maximum degradation of lignin was caused by S. pruinosum. A. sydowi also proved to be a good lignin degrader. The grass Lf showed better humification productivity in all the treatments. Among the fungi tested, A. terreus showed maximum humification productivity, i.e. 0.78 in Lf grass (Table 1).

Table 1. Decomposition and humification of L. fusca (Lf) and S. aculeata (Sa) by some inoculated fungi.

Fungi inoculated	% weight loss		% loss in lignin		Humification productivity	
	Lf	Sa	Lf	Sa	Lf	Sa
A. sydowi	15.4	20.1	21.7	24.5	0.55	0.22
A. terreus	13.1	18.8	13.8	5.1	0.78	0.38
C. globosum	19.6	21.2	22.1	15.5	0.41	0.33
D. rostrata	19.0	18.5	24.0	18.4	0.35	0.35
Papulospora sp.	22.8	25.8	Nil	1.5	0.27	0.21
S. pruinosum	32.7	44.6	40.4	46.1	0.34	0.21
S. atra	26.1	29.1	20.3	9.5	0.43	0.37
T. piluliferum	16.7	20.2	21.4	17.0	0.45	0.28

Table 2 shows the analyses of the decomposed material. S. atra produced maximum humic acid from both Lf grass (6.0%) and Sa legume (3.0%). S. pruinosum and A. terreus also caused good humification of the two substrates but the amount of humic acid was lower. In general, more humic acid was synthesized on Lf than on Sa. The E_4/E_6 ratios of

Table 2. Analyses of humified L. fusca (Lf) and S. aculeata (Sa).

Fungi inoculated	% humic acid		% fulvic acid		% lignin		E_4/E_6 ratio of humic acid	
	Lf	Sa	Lf	Sa	Lf	Sa	Lf	Sa
A. sydowi	2.3	1.3	7.9	4.1	13.7	19.2	9.4	12.0
A. tereus	3.4	1.2	8.4	7.7	19.1	25.7	5.8	8.6
C. globosum	2.9	0.8	7.0	7.9	18.4	22.9	5.8	6.4
D. rostrata	2.8	0.9	5.5	7.0	17.6	20.9	4.3	7.9
Papulospora sp.	2.1	0.5	5.8	5.0	27.2	30.3	8.6	10.2
S. pruinosum	3.6	2.7	12.8	14.0	16.2	19.7	15.2	10.8
S. atra	6.0	3.0	9.1	12.3	21.4	28.4	7.7	5.9
T. piluliferum	1.7	0.5	7.3	6.6	17.8	22.0	7.8	9.6

humic acids (Table 2), which refer to the extent of the maturity of the humic acid molecule (Kononova, 1966), indicated that humic acid produced from Lf was more polymerized than that produced from Sa, as indicated by the generally lower E_4/E_6 ratio. However, fungi differed widely in this respect. Similarly the lignin content of the final product also differed with the type of inoculum and substrate.

The nitrogen content of humic acid (Table 3) also differed with the nature of the substrate and inoculum. Humic acid from the grass Lf contained 1.5-4.3% N and that from the legume Sa contained 2.7-7.5% N depending upon the type of inoculum.

Table 3. Percent N content of humic acid and fulvic acid originating from L. fusca (Lf) and S. aculeata (Sa) as a result of fungal inoculation.

Fungi inoculated	Humic acid		Fulvic acid	
	Lf	Sa	Lf	Sa
A. sydowi	4.3	2.7	2.5	3.2
A. terreus	2.9	4.5	3.6	4.4
C. globosum	3.4	3.3	4.4	3.4
D. rostrata	1.5	3.0	6.0	4.4
Papulospora sp	3.4	7.5	3.5	3.4
S. pruinosum	3.3	3.6	3.8	6.8
S. atra	3.1	3.2	3.2	2.5
T. piluliferum	4.1	5.2	3.4	3.6

Maximum fulvic acid content (14.0%) was produced in Sa inoculated with S. pruinosum. The rest of the fungi, except S. atra, produced relatively less fulvic acid; the N content of the fulvic acids (Table 3) ranged between 2.5-6.0% and 2.5-6.8% for the Lf and Sa treatments, respectively.

DISCUSSION

On both substrates, the high C losses produced by S. pruinosum may be attributed to the high cellulase activity of this fungus (Malik et al., 1982) and its ability to utilize the lignin components, since S. pruinosum was the most active lignin degrader amongst the fungal species tested. A. sydowi was also found to be an efficient lignin degrader, although its lignin degrading ability has not been reported in the literature. Some other species of Aspergillus (e.g. A. fumigatus) have however, been reported to degrade kraft lignin (Drew and Kadam, 1979), as has

S. pulverulentum (Lundquist et al., 1977). Species of Stachybotrys and Chaetomium are known to liberate $^{14}CO_2$ from ^{14}C labelled lignin in intact plant tissues (Haider and Trojanowski, 1975). Delignification of beech wood by C. globosum has been reported (Levi and Preston, 1965). There are few reports of the lignin degrading ability of Trichoderma (Kononova, 1966) and Papulospora (Eslyn et al., 1075). In the present study, Papulospora sp. failed to degrade lignin in either substrate.

As mentioned earlier, the amount of lignin in humified Sa legume was higher than in Lf grass. These higher values were probably due to preferential degradation of Lf lignin and transformation into humus components. The higher values for residual lignin in substrate inoculated with some of the melanoid fungi may be explained by the ability of these fungi to incorporate lignin-derived phenols into melanoid component of their cell walls (Martin and Haider, 1980)

The nature of the substrate influences the humification productivity of the different fungi. However, variation of humification productivity may also be due to differences in oxidase activities of different organisms (Haider et al., 1975). S. atra produced maximum humic acid (6.0% of the end product), and is known to produce phenolic precursors for humic acid synthesis (Haider et al., 1975). However it seems that in addition to its synthetic activities, S. atra also released phenolic units from the substrates (particularly from Lf) which also contributed to the humic acid component. A. terreus also produces phenolic compounds in the growth medium (Malik et al., 1979).

The present sutdy also shows lignin degradation by A. terreus and transformation into humic compounds. S. pruinosum was found to be the most active lignin degrader but its phenol synthesis is not reported in the literature. It is therefore probable that humic acid synthesized in this treatment consists of lignin degradation products. However, the low amount of humic acid produced by this fungus suggests that any lignin derived phenols were metabolized as C source or appeared in the fulvic acid fraction. Although A. sydowi degraded lignin from Lf and Sa and is capable of phenol synthesis (Haider and Martin, 1970), its contribution to humic compounds was very small. Some of the melanoid fungi tested did not show extensive humic acid synthesis although some of them degraded lignin in both substrates. Their low humic acid content may be due to incorporation of phenolic units into their melanoid component (Martin and Haider, 1980). However, addition into soil of material pre-

viously decomposed by melanoid fungi may significnatly add to soil humus (Malik et al., 1982). Maximum fulvic acid production in S. pruinosum treatment may be related to its lignin degrading ability, and a higher fulvic acid content in S. atra, A. sydowi and A. terreus to their phenol-synthesizing as well as lignin-degrading properties.

The studies reported here indicate that S. pruinosum is a desirable species for efficient decomposition of lignocellulosics like Lf grass and Sa legume applied to soil when the objective is high CO_2 evolution. In order to achieve better humification, S. atra may be more suitable. In this case however, the amount of humus produced per unit C loss is low. For best humification productivity therefore A. terreus may be a better choice.

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