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ScienceDirect
Waste Management 27 (2007) 850–853
www.elsevier.com/locate/wasman

Technical Note

Actinomycetes in rice straw decomposition

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Accepted 8 June 2006
Available online 21 August 2006

Abstract

Actinomycetes are well known as lignocellulose decomposers. Forty-two cellulolytic actinomycete isolates have been recovered from soil. Those were identified to the genus level as: *Streptomyces* (26 isolates), *Nocardiopsis* (5 isolates), *Micromonospora* (4 isolates), *Nocardioides* (4 isolates) in addition to three isolates tentatively identified as *Nocardia*, *Kibdelosporangium* and *Saccharomonospora.* These actinomycetes were examined for their cellulolytic activity using filter paper strips and rice straw pieces. Four isolates of the four genera *Kibdelosporangium*, *Micromonospora*, *Streptomyces* and *Nocardioides* were able to efficiently degrade rice straw pieces in minimal medium causing significant weight loss between 50 and 61%. Application of this knowledge may improve the management of waste rice straw.

1. Introduction

Rice cultivation produces large quantities of straw waste, ranging from 2 to about 9 tons/ha globally. Because components of rice straw are mainly cellulose and hemicellulose encrusted by lignin, in addition to only small amounts of protein, it is resistant to microbial decomposition compared to straw from other protein-rich grains such as wheat and barely (Parr et al., 1992). In many countries, massive amounts of the post-harvest rice residues are eliminated through field open-air burning, which represents a threat to public health and poses an environmental pollution problem (Givens, 1996).

Alternatives to burning include straw incorporation into soil, where the actions of microbial enzymes transform the lignocellulose component of the straw into compost. However, direct incorporation into soil is limited by the great bulk of crop straw, slow biodigestion in soil and harbouring of rice-stem diseases (Martin et al., 1978). Actinomycetes are well known for their ability to decompose complex molecules, particularly lignocellulose components, which make them important agents in decomposition processes (Lacey, 1997). Additionally, the apparent widespread ability of actinomycetes to generate soluble lingo-carbohydrate from straw has been confirmed (Ball et al., 1990; Mason et al., 2001). The aim of the present study is to isolate, characterize and examine the potential for specific soil actinomycete isolates to accelerate the decomposition of rice straw. It is hoped that this will be a step towards providing a method of accelerated decomposition of rice straw and humification of its residues in soil using combinations of lignocellulolytic actinomycetes strains with various organic amendments such as cattle manure and municipal sludge.

2. Materials and methods

2.1. Isolation of cellulolytic actinomycetes

Samples of soil were collected from 30-yr-old botanical garden located in Ismailia, Egypt. Soils were loamy-sand and cultivated with evergreen plants. Eight samples were taken at 10–15 cm depth; air dried at room temperature for 7 days and used for isolation of actinomycetes as described by Wollum (1982) on starch casein agar plates (Kuster and Williams, 1964). Isolates were investigated for cellulose decomposition on cellulose medium: cellulose powder 5 g, yeast extract 1 g, K_2HPO_4, 0.7 g; KH_2

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doi:10.1016/j.wasman.2006.06.006

PO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $ZnSO_4$, 0.001 g; agar, 15 g; distilled water, 1000 ml pH 7. Plates were incubated at 28 °C for 10 days; positive results were indicated by clear zone formation (Demain and Davies, 1999). Cellulolytic isolates (a total of 42 morphologically different isolates) were purified and maintained as spore suspensions in 15% glycerol at −20 °C for subsequent investigation (Hopwood et al., 1985). Isolates were investigated for decomposition of filter paper strips in cellulose broth tubes, as described above, with cellulose replaced by filter paper strips.

2.2. Characterization and identification of actinomycetes isolates

Cellulolytic isolates were characterized and identified to the genus level based on the standard morphological and chemotaxonomic methods (Holt et al., 1994). Cultural characteristics and microscopic observations of sporulated mycelia were performed on cultures grown on ISP4 medium (Shirling and Gottlieb, 1966). For chemotaxonomic studies, cultures were grown in TSB baffled flasks on a shaking incubator at 28 °C with 100 rpm for 4–7 days; mycelia were harvested by centrifugation, washed and dried. Determination of the diaminopimelic acid (DAP) isomer and mycolic acids in whole cell hydrolysate was performed as described by Schaal (1985). Whole-cell sugar pattern in dried mycelia was performed according to Staneck and Roberts (1974). Four isolates were selected based on their high cellulolytic activities and were tentatively identified to the species level using appropriate physiological characteristics as recommended by Holt et al. (1994). Physiological criteria included: the ability of the isolates to utilize different carbon and nitrogen sources on ISP 9 and nitrogen utilization basal medium, respectively (Shirling and Gottlieb, 1966); degradation of casein, xanthene and hypoxanthene; growth at different temperatures and at pH 4.3 on modified Bennett's agar medium; and utilization of urea (Williams et al., 1983).

2.3. Decomposition of rice straw under laboratory conditions

Rice straw pieces were prepared from rice cultivar Giza 176, a high yielding commercial cultivar used in Egypt. Straw was cut into 3-cm pieces and weighed after drying overnight at 105 °C. To refresh the isolates, 5 µl of spore suspension of each cellulolytic actinomycete isolate was inoculated into starch casein agar plates and incubated at 28 °C until good growth was obtained (3–5 days). The actively growing mycelia were inoculated into triplicate tubes of cellulose broth, with cellulose replaced by three pre-weighed rice straw pieces in each tube. Two sets of tubes containing the pre-weighed straw pieces were prepared and incubated at 28 °C, one each under static and gentle shake conditions. To examine the effect of low pH on the decomposition rate, a third set of tubes, adjusted at pH 4, was incubated statically. Observations for the three sets were taken at 2-day intervals; any changes in colour or integrity of the straw strips, comparing with uninoculated (control) tubes, were recorded. At 4-week intervals, one straw piece from each of the triplicate tubes was carefully removed, dried and reweighed, as described above, to calculate the percentage of weight loss.

3. Results

Forty-two cellulolytic actinomycete isolates were recovered from soil. Those isolates were identified to the genus level based on morphological and biochemical criteria. Table 1 shows characteristics of the isolated actinomycetes. Genus *Streptomyces* was represented by 26 isolates, five isolates were identified as *Nocardiopsis* while genera *Micromonospora* and *Nocardioides* were represented by four isolates each. The remaining isolates were tentatively identified as *Nocardia*, *Kibdelosporangium* and *Saccharomonospora.* Four of the isolates showed high cellulolytic abilities and were able to completely decompose the filter paper strips by visual observation. Those were further tentatively identified to the species level as: *Micromonospora chalcea*, *Streptomyces roseflavus*, *Nocardiodes fulvus* and *Kibdelosporangium philippinense*. The four highly cellulolytic strains were tested for abilities to decompose rice straw pieces under different incubation conditions (Table 2). At pH 7, all of the isolates decomposed the straw pieces after 3 mo, without significant differences in weight loss under both static and shaking conditions ($p > 0.05$). *M. chalcea* showed the highest rate of decomposition compared to other strains and recorded weight loss as high as 61% at neutral conditions (Fig. 1), with no significant differences between static and shaking conditions. Lowering the pH to 4 accelerated the decomposition rate in the case of *Micromonospora*, *Streptomyces* and *Nocardiodes* strains and a considerable damage in the straw pieces was observed after 2 mo of incubation, although almost the same weight loss under both neutral and shake conditions after extended incubation to 3 mo.

4. Discussion

The present investigation showed that actinomycetes tentatively identified as *M. chalcea*, *S. roseflavus*, *N. fulvus* and *K. philippinens* have strong hydrolytic activities to decompose rice straw strips, utilize it as sole carbon source and cause high weight loss (50–61%) under laboratory conditions. These isolates could represent important straw decomposers and further study is needed to verify their identification. Previous work by Crawford and Sutherland (1979) showed similar results (50%) with phloem discs inoculated with *Streptomyces flavovirens* after 12 weeks of incubation. Although, McCarthy (1987) reported that actinomycetes producing cellulases and hemicellulases exhibit strong preferences for neutral to alkaline pH conditions, in this study the decomposition