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Isolation and characterization of rice straw degrading *Streptomyces griseorubens* C-5

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Abstract To reutilize rice straw generated during the agricultural production process, the actinomycete strain C-5 was isolated from soil that was under the stook for several years in the Heilongjiang province of China by using multiple selective culture media. Strain C-5 was identified as *Streptomyces griseorubens* by China General Microbiological Culture Collection Center (CGMCC) through morphological and physiological characterization combined with the result of 16S rRNA gene sequence and data analysis. This strain has simultaneous cellulase, laccase, peroxidase, xylanase and pectinase activity. The various chemical composition of rice straw were determined during fermentation process. Simultaneously the biodegradation process of rice straw stem was observed by scanning electron microscope (SEM). It is predicted that strain C-5 could decompose rice straw effectively and had promising prospects of being applied in improving the resource utilization of rice straw.

Keywords Biodegradation · Rice straw · Lignocellulose · *Streptomyces griseorubens* · Scanning electron microscope

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Introduction

Rice cultivation produces large quantities of straw waste, ranging from 2 to about 9 tons ha^{-1} globally (Abdulla and El-Shatoury 2007). According to rough statistics, about 175 million tons of rice straw have been produced each year in China, which is the largest amount among crop straw resources and accounted for 31.6% of the total amount of straw. However, compared with straw from other crops, the transformation and utilization ratios of rice straw are the lowest (Gao et al. 2002). Because the components of rice straw are mainly cellulose and hemicellulose encrusted with lignin having in addition, only small amounts of protein, rice straw is more resistant to microbial decomposition compared with straw from other protein-rich grain such as wheat and barely (Parr et al. 1992). In many countries, massive amounts of post-harvest rice residues are eliminated through open air field burning, which presents a threat to public health and poses an environmental pollution problem (Givens 1996).

Alternatives to burning include incorporating the straw into soil, where the actions of microbial enzymes transform the lignocellulose component of the straw into compost. Lignocellulose degradation in natural substrates is largely attributed to fungi, and the importance of actinomycetes in this process may be underestimated (Li 1997). Actinomycetes are prokaryotes which have a hyphal morphology well adapted to the penetration and degradation of

insoluble substrates (Tuncer et al. 1999). Like bacteria and fungi, actinomycetes can also produce multiple enzymes involved in the degradation of lignocellulose (McCarthy 1987; Ball and McCarthy 1989). The enzymes involved in the degradation of lignocellulose include endoxylanases, peroxidase, laccase and cellulases. The products of such enzymatic hydrolysis may be subsequently converted into liquid fuels, single cell protein, solvents and other chemicals by the selective use of specific fermentative microorganisms (Biely 1985). This can also contribute to the elimination of agricultural wastes. Within this group of bacteria, the streptomycetes are of particular interest because cellulose-degrading activity has been found in some streptomycete strains (Enger and Sleeper 1965; Kluepfel et al. 1986; Spear et al. 1993) and their apparent widespread ability to generate soluble ligno-carbohydrates from straw has been confirmed (Ball et al. 1989, 1990; Mason et al. 2001).

For this reason there is still a need for continued search of more efficient lignocellulose-degrading actinomycetes strains for biodegradation. This paper has focused on this approach by isolation and identification of the streptomycete strain and extended to observe microbial colonization and degradation of rice straw tissues by microscopy together with chemical analysis.

Materials and methods

Isolation and cultivation of actinomycete strain C-5

Strain C-5 was isolated from soil collected under stooks which stacking for several years in natural conditions from the reproduction base of rice original variety of the Ministry of Agriculture in Heilongjiang province, People's Republic of China. 1 g (fresh weight) portion of a soil sample was suspended in 2 ml of sterile distilled water, and aliquots of the resulting suspension were inoculated onto cellulose agar plates (which were prepared by placing a piece of appropriate size of filter paper (Whatman No.1) over nutrient salt medium containing 1.5% (w/v) agar in plates) by directly plating 10-fold serially diluted samples. After 7–14 days of incubation at 30°C, several different types of colonies were observed on the filter paper in the plates. Larger colonies of some strains were signed, purified and subcultured on slants of cellobiose agar which contained nutrient salt medium ($MgSO_4 \cdot 7H_2O$ 0.2 g, KNO_3 0.75 g, K_2HPO_4 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.02 g, $CaCl_2 \cdot 2H_2O$ 0.04 g, peptone 2 g, distilled water 1 l) supplemented with 0.2% (w/v) cellobiose and 0.1% (w/v) cellulose (Li 1997).

A little mycelium was picked out from the purified slants and inoculated to cellulose congo-red agar plates. After 5 days of incubation at 30°C, clear hydrolysis zones of different sizes were observed on the cellulose congo-red agar plates (Hendricks et al. 1995). Cx cellulase-producing activities of the isolates were estimated by their carboxymethylcellulose hydrolysis capacity (HC value) on the cellulose congo-red agar, i.e. ratio of diameter of clearing zone and colony (Reese et al. 1950; Hankin and Anagnostakis 1977; Hendricks et al. 1995), and those with high HC values were selected and stored on slants at 4°C for the detection of activity of xylanase, pectinase and laccase and peroxidase. Xylanase activity was detected by the method of Shimizu and Kunoh (2000). Pectinase activity was detected by the method of Saarilahti et al. (1990). Laccase activity plate assay was estimated by the method of Srinivasan et al. (1995). Plate assay of peroxidase activity was estimated by the method of Zhang et al. (2005).

The selected isolate was grown in flasks with liquid fermentation medium (KH_2PO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, NaCl 0.1 g, $CaCl_2 \cdot 6H_2O$ 0.1 g, $FeCl_3$ 0.01 g, $NaNO_3$ 2.5 g, milled rice straw (<2 mm) 20 g, distilled water 1 l, pH 6.8–7.2) for 3–7 days at 30°C. After clarifying the culture fluids by centrifugation cellulase activity (endoglucanase activity and filter paper activity) was assayed. Endoglucanase activity and filter paper activity for cellulose were estimated by the method of Mandels et al. (1975). Enzyme concentration was represented as international unit (IU g^{-1}). One IU is defined as μ mol of product produced ml^{-1} min^{-1} for filtrate. Trichoderma viride AS3.3711 was choosed as the control which purchased from Agricultural Culture Collection of China (ACCC).

Identification of actinomycetes species

Isolate C-5 was identified at China General Microbiological Culture Collection Center (CGMCC), Beijing, China.

Rice straw degradation experiment

Straw was ground using a hammer mill through a 2 mm grid. 10 g of prepared straw was added to 250 ml conical flasks containing 50 ml nutrient salt medium ($MgSO_4 \cdot 7H_2O$ 0.2 g, KNO_3 0.75 g, K_2HPO_4 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.02 g, $CaCl_2 \cdot 2H_2O$ 0.04 g, peptone 2 g, distilled water 1 l) and autoclaved (15 min, 121°C). Then the preparation was 10% (v/v) inoculated (conidia of a inoculating loop were cultured 48 h in seed culture medium and was 10% (v/v) inoculated in the culture medium for rice straw degradation experiment) and incubated at 30°C for 50 days. Samples were taken at 5 days intervals and stored at 4°C for chemical component determination. Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and hemicellulose were determined according to the method of Robertson and Van Soest (1981). Silica was determined according to the method of Van Soest and Robertson (1985).

Rice straw stems were cut into fragments of 0.5 cm length. 5 g of prepared straw was added into 100 ml conical flasks containing 30 ml nutrient salt medium and autoclaved (15 min, 121°C). Then the preparation was 10% (v/v) inoculated (conidia of a inoculating loop were cultured 48 h in seed culture medium and was 10% (v/v) inoculated in the culture medium for rice straw stems degradation experiment) and incubated at 30°C for 50 days. Samples were taken at 5 days intervals and stored at 4°C for observation by scanning electron microscope (SEM). The preparation of undegraded and degraded fragments of straw stems and their SEM examination were according to the method of Powell et al. (2001).

Results

Isolation of strain C-5

Through vast and multiple selective culture medium screening only one streptomycete strain C-5 with multi-lignocellulase simultaneously was isolated. The plates assay with C-5 revealed its cellulase, xylanase, pectinase, laccase and peroxidase activity. Although pectinase activity was not clearly shown in medium plates, cellulase, xylanase, laccase and peroxidase activities were clearly present in their respective media (Fig. 1). Furthermore, the cellulase activity (endoglucase activity and filter paper activity) of C-5 was compared with Trichoderma viride AS3.3711 as the control. The results showed that the endoglucase activity and filter paper activity of C-5 was 46.16 and 16.33 IU ml^{-1}, respectively, which was a little higher than that of Trichoderma viride AS3.3711 which endoglucase activity and filter paper activity was 40.84 and 14.49 IU ml^{-1}, respectively. So we had carried out further analysis on identification and rice straw degradation ability for strain C-5.

Identification of strain C-5

Strain C-5 was identified as *Streptomyces griseorubens* by China General Microbiological Culture Collection Center (CGMCC) through morphological and physiological characterization combined with the result of 16S rRNA gene sequence and data analysis. The Genbank accession number for 16S rRNA gene sequence of strain C-5 is EU 295695.

The characteristics of strain C-5 can be found in Tables 1 and 2. Strain C-5 was an aerobic

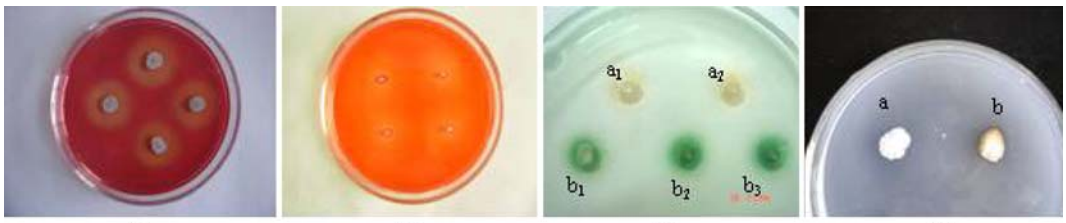

Fig. 1 **A** Colony and clear halos formed by C-5 on cellulose Congo-red agar, **B** Plate assay for demonstration of the xylanase activity of C-5, **C** Plate assay for demonstration of the laccase activity of C-5 (a_1, a_2): Extracellular fluid of C-5 which was boiled for 5 min (control) (b_1, b_2, b_3): Extracellular fluid of C-5, **D** Plate assay for demonstration of the peroxidase activity of C-5 (*a*): C-5 colony without PHB liquid (control) (*b*): C-5 colony with PHB liquid

Table 1 Cultural characteristics of strain C-5

Medium	Amt of growth	Color of		Production of soluble pigment
		Aerial mycelium	Vegetative mycelium	
Czapek's solution agar	Abundant	Gray	Brown	None
Glucose-asparagine agar	Abundant	White	Light yellow	None
Glycerol-asparagine agar (ISP medium 5)	Abundant	Light gray-white	Light white	None
Inorganic salts-starch agar (ISP medium 4)	Abundant	Gray	Gray-brown	None
Yeast extract-malt extract agar (ISP medium 2)	Abundant	Gray	Black-brown	None
Oatmeal agar (ISP medium 3)	Abundant	Gray-white	Light black-purple	None
Gause 1 cultural medium	Abundant	Gray	Brown	None
Sauton's agar	Abundant	Gray	Dark brown	None

Table 2 Growth of strain C-5 on sole carbon source

Carbon source	Result	Carbon source	Result	Carbon source	Result
Inositol	+	Glycerol	+	Lactose	+
Mannitol	+	Sodium gluconate	+	Melezitose	+
Salicine	+	Galactose	+	L-Arabinose	+
D-Raffinose	+	Sodium succinate	+	Dulcitol	+
Rhamnose	+	Sodium malonate	+	Erythritol	+
Starch	+	Sodium citrate	+	Glycogen	+
Sorbitol	+	Cellobiose	+	Ribose	+
L-Sorbose	+	Sodium malate	+	Sodium propionate	+
Sucrose	+	L-Asparagine	+	Sodium acetate	+
Melibiose	+	Sodium tartrate	+	Maltose	+
D-Fructose	+	D-Glucose	+	D-Xylose	+
D-Mannose	+	Trehalose	+	Inulin	+

Gram-positive bacterium. This isolate grew well on various organic or inorganic medium (Table 1). The color of aerial mycelium was from white to gray. The reverse sides of colonies were from gray-brown to dark-brown on inorganic salts-starch agar, Czapek's solution agar, Gause 1 cultural medium, yeast extract-malt extract agar and Sauton's agar but were light yellow on glucose-asparagine agar, light white on glycerol-asparagine agar and light black-purple on oatmeal agar. No fragmentation of the substrate mycelium was observed and no soluble pigments were formed. Most of the carbohydrates tests of C-5 were positive reaction the tyrosinase test and nitrate reduction test were negative reaction (Table 2).

Degradation experiment of rice straw by strain C-5

Table 3 showed the changes in chemical composition of rice straw caused by strain C-5 during 50 days fermentation. The results indicated that various chemical components of rice straw were degraded by different degree during fermentation process. The degradation of ADL and silica were comparatively high, it was 58.47 and 57.62%, respectively; the degradation of ADF was lowest among them, it was 37.87%.

Intact rice straws presented the classical structure of poaceae straws, with an internal cavity in the stem

Table 3 Changes in chemical composition of rice straw during incubation for 50 days with strain C-5, on a dry matter basis (%)

Fermentation days	NDF[a]	ADF[b]	ADL[c]	Cellulose	Hemicellulose	Silica
0	83.85 ± 2.2	48.46 ± 2.4	7.20 ± 1.2	35.89 ± 1.5	35.38 ± 2.3	7.15 ± 0.66
5	79.26 ± 2.4	46.03 ± 2.9	6.86 ± 0.42	33.61 ± 1.5	33.23 ± 2.1	6.75 ± 0.93
10	74.57 ± 1.7	44.58 ± 2.7	6.52 ± 3.0	32.24 ± 1.4	29.99 ± 1.9	6.23 ± 0.89
15	72.26 ± 1.3	43.85 ± 7.2	6.18 ± 2.3	31.73 ± 8.9	28.41 ± 0.86	5.44 ± 1.7
20	65.88 ± 10.4	40.18 ± 6.2	5.95 ± 2.0	28.22 ± 3.4	25.70 ± 1.4	5.26 ± 1.3
25	61.26 ± 3.8	38.18 ± 2.9	5.44 ± 0.53	26.62 ± 4.0	23.08 ± 0.78	4.85 ± 1.1
30	58.25 ± 0.23	37.93 ± 1.5	4.98 ± 0.86	26.62 ± 3.1	20.32 ± 2.7	4.58 ± 1.0
35	56.47 ± 1.2	37.48 ± 1.8	4.24 ± 0.54	26.53 ± 0.98	18.99 ± 2.5	4.13 ± 0.22
40	54.65 ± 0.57	35.98 ± 2.0	3.58 ± 0.41	25.55 ± 0.88	18.67 ± 0.96	3.90 ± 0.48
45	49.64 ± 0.78	31.48 ± 1.4	3.18 ± 0.74	21.36 ± 1.2	18.16 ± 0.76	3.29 ± 0.92
50	47.85 ± 0.55	30.11 ± 0.95	2.99 ± 0.1	20.14 ± 0.66	17.74 ± 1.3	3.03 ± 0.13

Values are means of three replicates

[a] NDF: neutral detergent fibre

[b] ADF: acid detergent fibre

[c] ADL: acid detergent lignin

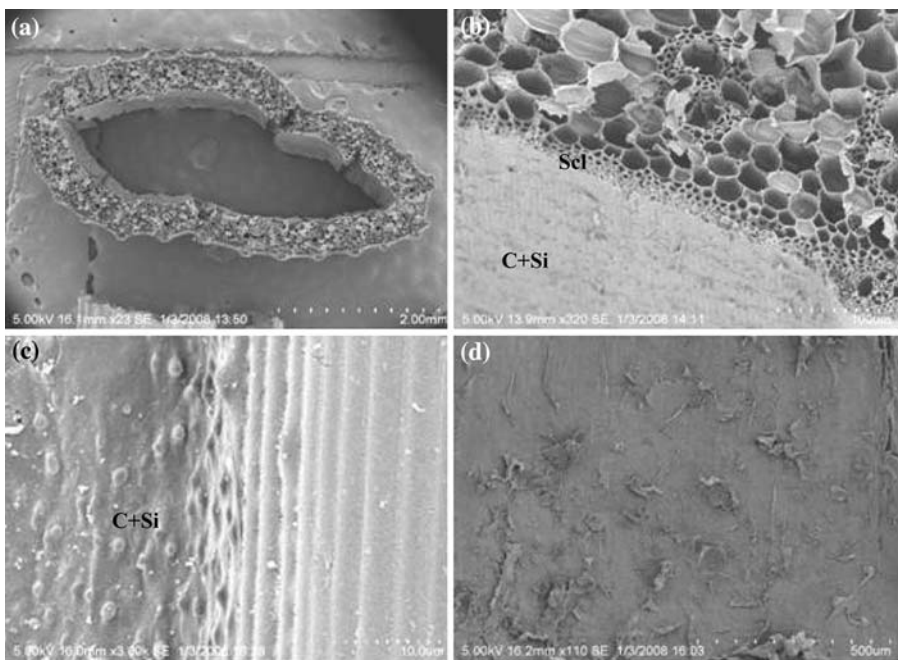

Fig. 2 Scanning electron micrographs of intact rice straw stems. **a** Transverse section of rice stem; *bar*, 2 mm **b** Sub-epidermal sclerenchyma in rice stem with thick-walled cells; *bar*, 0.1 mm **c** Cuticle surface of rice stem with silica layer and bodies; *bar*, 0.01 mm **d** Inner surface of rice stem; *bar*, 0.5 mm. Scl: sclerenchyma; C: cuticle; Si: silica

and vascular bundles distributed over concentric circles in the parenchyma (Fig. 2a). Small vascular bundles were located in the sclerenchyma layer and large vascular bundles in the parenchyma. The specificities of rice straw were (1) the thick-walled cells of the sub-epidermal sclerenchyma layer with a

narrow lumen (Fig. 2b), (2) many aligned two-dimensional excrescences, probably silica bodies, forming with the cuticle a thick layer at the outer surface of the stem (Fig. 2c).

After 5 days incubation, bacterial degradation appeared in the parenchyma and the phloem at the cut ends (Fig. 3a). The inner surface of rice straw began to be degraded (Fig. 3b). The epidermis surface was not degraded obviously (Fig. 3c). At this stage, a few mycelia was already fixed on the internal tissues of rice straw stems.

After 20 days, parenchyma in the rice stem had partially disappeared. Large vascular bundles were separated from the sclerenchyma layer at the fragment extremities (Fig. 4a, b). Inner tissues of rice straws were colonized by mycelial pellets of strain C-5 (Fig. 4c). The silica layer of cuticle surface of rice stem was destroyed and the tissue below it was exposed (Fig. 4d).

After 35 days, most of the large vascular bundles of rice straw were completely separated from the remaining outer tissues (Fig. 5a) and cortical parenchyma was mostly degraded, leaving nude vascular bundles (Fig. 5b). The cuticle surface of rice stem was destroyed more seriously and hypha of C-5 had penetrated the epidermis which had been destroyed (Fig. 5c).

After 50 days incubation, the parenchyma had completely disappeared and the remaining tissues in rice stem were the outer ring of small vascular bundles and sclerenchyma with epidermis (Fig. 6a, b). The rice fragments had lost their structures and badly damaged. Especially the epidermis surface was damaged and the silica layer on the cuticle surface of rice stem was seriously destroyed (Fig. 6c).

Rice straw samples incubated in the same medium after 50 days with exactly the same conditions but without inoculation were presented (Fig. 7). The whole structure of rice straw stems were not obviously destroyed by shaking culture (Fig. 7a, b). The cuticle surface with silica layer and inner surface of rice stem were only slightly damaged (Fig. 7c, d). These results indicated that the degradation ability to rice straw of strain C-5 is very strong and the effect is obvious on rice straw degradation after 50 days incubation.

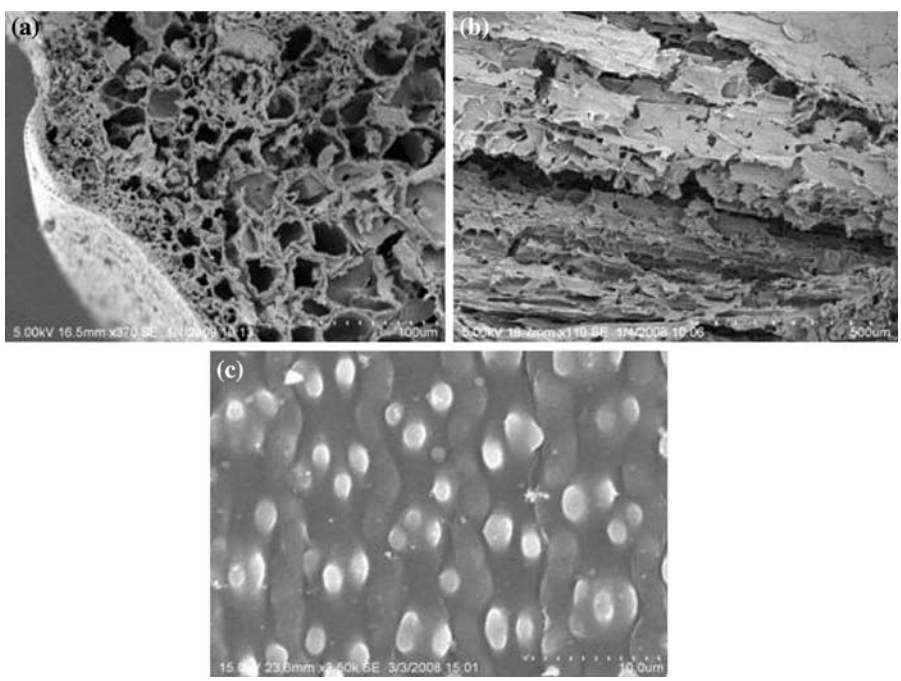

Fig. 3 Scanning electron micrographs of rice straw degradation after 5 days incubation. **a** Upper view of rice stem after incubation; *bar*, 0.1 mm **b** Upper view of inner surface of rice stem after incubation; *bar*, 0.5 mm **c** Cuticle surface of rice stem with silica layer and bodies; *bar*, 0.01 mm

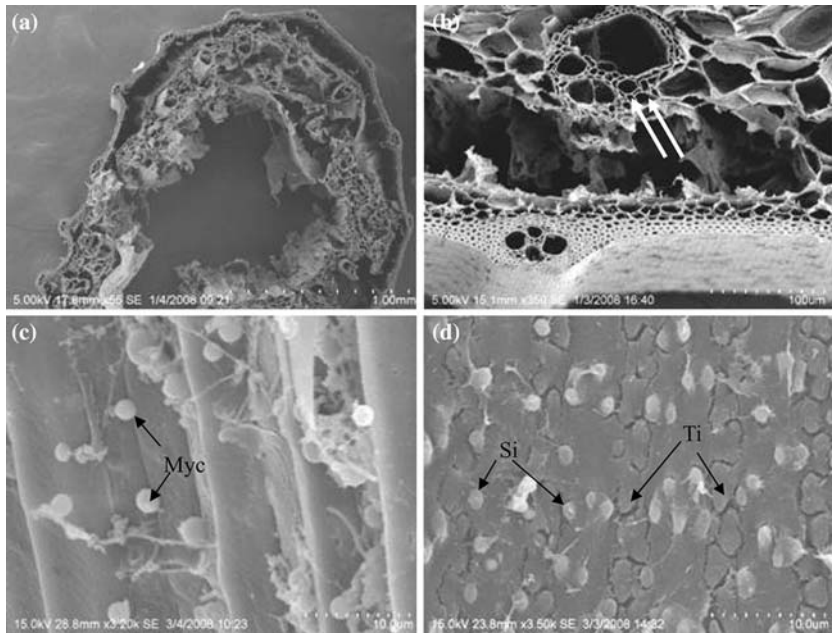

Fig. 4 Scanning electron micrographs of rice straws degradation after 20 days incubation. **a** Cut end view of rice stem; *bar*, 1 mm **b** Cut end view of rice stem; *bar*, 0.1 mm **c** Upper view of inner surface of rice stem; *bar*, 0.01 mm **d** Cuticle surface of rice stem with silica layer and bodies; *bar*, 0.01 mm. Myc: mycelial pellets; Si: silica; Ti: tissues below the silica layer of rice straw. Parenchyma in rice stem was widely degraded, leaving large vascular bundles (*double arrow*) isolated from sclerenchyma layer. Sclerenchyma and vascular bundle fibres were colonized by mycelia in rice straws

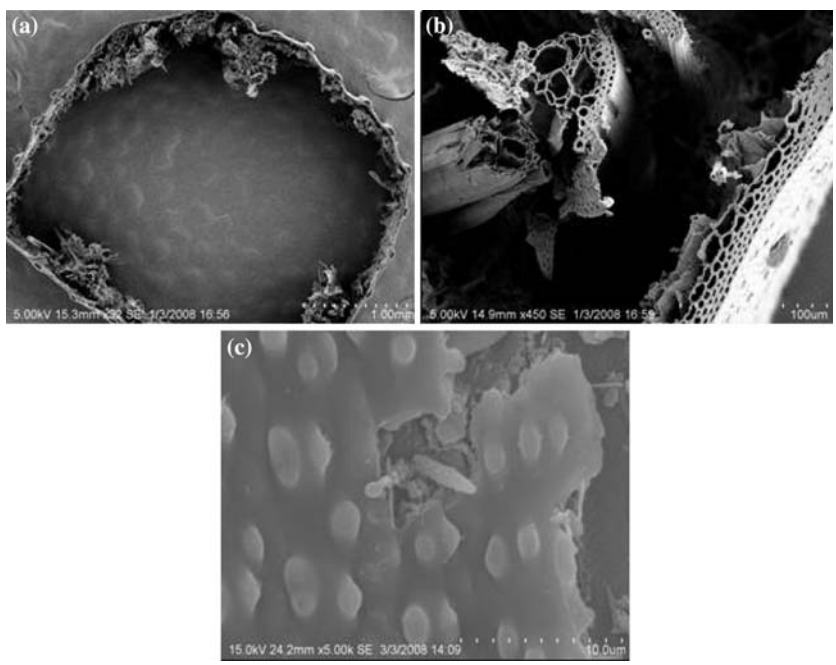

Fig. 5 Scanning electron micrographs of rice straws after 35 days incubation. **a** Cut end view of rice stem; *bar*, 1 mm **b** Cut end view of rice stem; *bar*, 0.1 mm **c** Cuticle surface of rice stem with silica layer and hypha was penetrated the epidermis which had been destroyed; *bar*, 0.01 mm

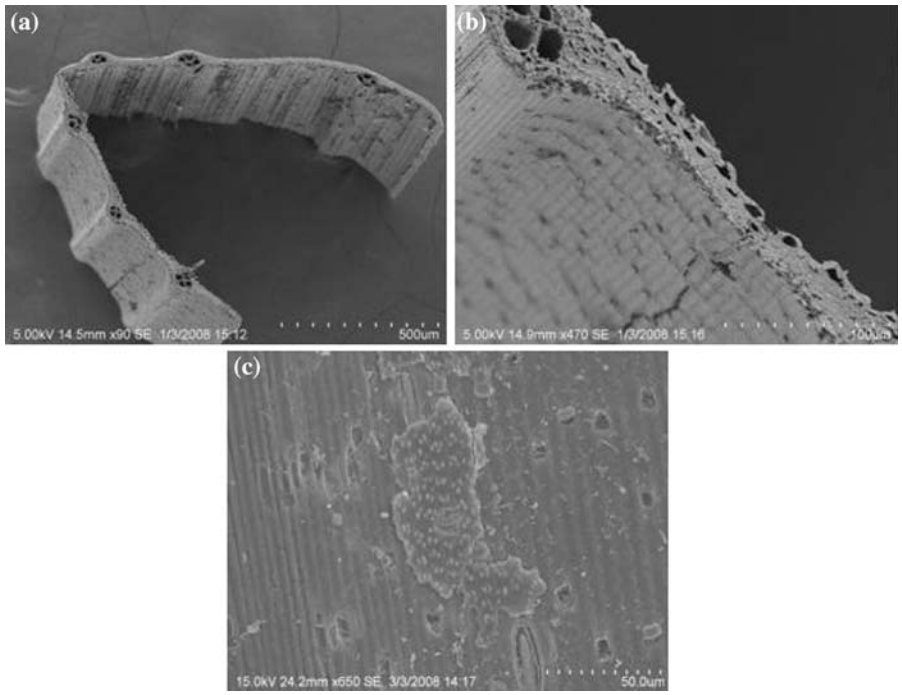

Fig. 6 Scanning electron micrographs of rice straws degradation after 50 days incubation. **a** Outer ring (lignified tissues) of rice stem after incubation; *bar*, 0.5 mm **b** Cut end view of rice stem after incubation; *bar*, 0.1 mm **c** Cuticle surface of rice stem with silica layer; *bar*, 0.05 mm

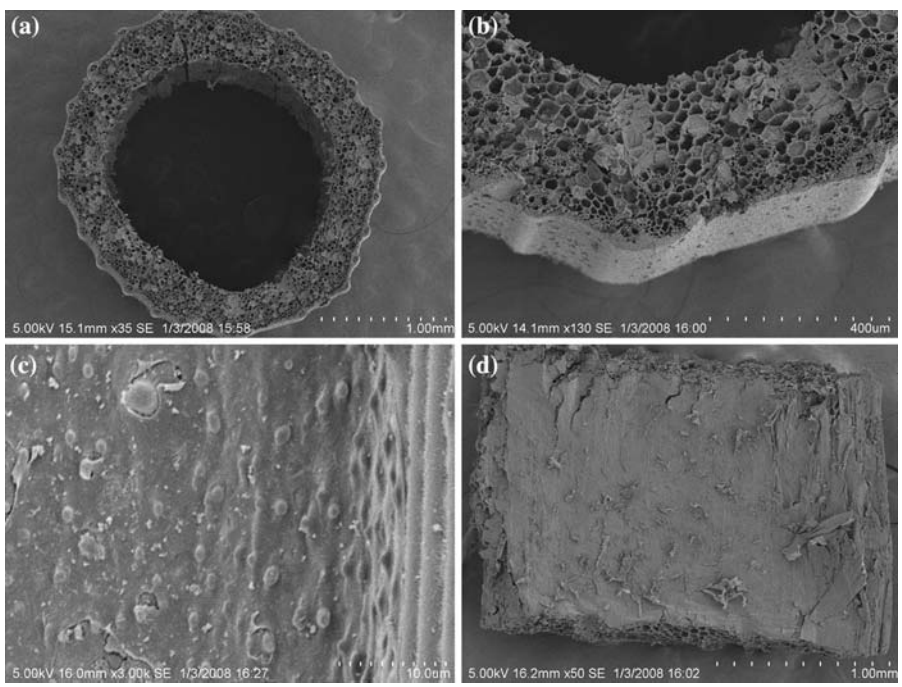

Fig. 7 Scanning electron micrograph of rice stem after 50 days shaking culture without inoculation. **a** Transverse section of rice stem; *bar*, 1 mm **b** Sub-epidermal sclerenchyma in rice stem with thick-walled cells; *bar*, 0.4 mm **c** Cuticle surface of rice stem with silica layer and bodies; *bar*, 0.01 mm **d** Inner surface of rice stem; *bar*, 1 mm

Discussion

In the study of Minamiyama et al. (2003) and Taechowisan et al. (2003), it was shown that *Streptomyces galbus* which was isolated from rhododendron leaves had cell wall degrading enzyme activity including cellulose, xylanase and pectinase activities. *Streptomyces badius* 252, *S. cyaneus* MT813, *S. viridosporus* T7A, *Streptomyces* sp. strain EC1 and *Streptomyces* sp. strain EC22 could solubilize lignocarbohydrate, i.e., producing the acid-precipitated product (APPL) was reported (Ball et al. 1989, 1990). Gottschalk et al. (2008) have reported that *Streptomyces viridosporus* T7A could secrete lignin peroxidase mainly. So far, no streptomycetes could secrete cellulase, laccase, peroxidase, xylanase and pectinase simultaneously like strain C-5 have been reported. So it may be the suggested reason of good effect in rice straw degradation of strain C-5.

At present no clear study about the colonization of actinomycetes on the outer surface of rice straw during biodegradation process was reported. SEM enlighted the degradation process of rice straws by strain C-5 that was not evidenced by chemical measurements.

The scanning electron micrographs presented in this study showed that abundant mycelia appeared on the inner surface of rice straw after 35 days of incubation. At the same time, no mycelia was found on the outer surface of rice straw except several mycelia penetrated the silica layer which had been partially destroyed. Ahoefa et al. (2003) reported that microbial degradation of rice and barley straws in the sheep rumen and the donkey caecum indicated that sclerenchyma and vascular bundle fibres were colonized by anaerobic fungi in both straws, and sporangia could be seen between two vascular bundles, but no mycelia were found on the outer surface of rice straw. But after pretreatment with NaOH or NH_4HCO_3 which may dissolve or crack off the cuticle wax silica layer of rice stem epidermis, spores had appeared on the surface after 12 h of rumen incubation (Wang et al. 2007). So, the special phenomenon in our test of mycelia penetration from silica layer of rice straw was presumable that mycelia penetrated from the inner tissue of rice straw which had been easily degraded to the outer surface which had been partially destroyed, but not from outer surface to inner surface of rice straw. Silica may, therefore, constitute a supplementary physical barrier against microbial colonization, confirming previous observations on rice straw (Kawamura et al. 1973; Harbers et al. 1981; Bae et al. 1997).

Conclusions

The work presented that a newly isolated strain *Streptomyces griseorubens* C-5 which be able to secrete cellulase, laccase, peroxidase, xylanase and pectinase simultaneously and decompose rice straw effectively as observed by SEM. Various chemical composition of rice straw were degraded in different degree during 50 days fermentation process of strain C-5. The finding of rice straw decomposition could be further used in different approaches e.g., bioresources management, biofertilization and land reclamation.

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References

Abdulla HM, El-Shatoury SA (2007) Actinomycetes in rice straw decomposition. Waste Manag 27:850–853

Ahoefa AD, Cornu A, Nozie're P, Besle JM, Dulphy JP, Michel D, Elisabeth G (2003) Microbial degradation of rice and barley straws in the sheep rumen and the donkey caecum. J Sci Food Agric 83:383–394

Bae HD, McAllister TA, Kokko EG, Leggett FL, Yanke LJ, Jakober KD, Ha JK, Shin HT, Cheng KJ (1997) Effect of silica on the colonization of rice straw by ruminal bacteria. Anim Feed Sci Technol 65:165–181

Ball AS, McCarthy AJ (1989) Production and properties of xylanases from actinomycetes. J Appl Bacteriol 66:439–444

Ball AS, Betts WB, Mccarthy AJ (1989) Degradation of lignin-related compounds by actinomycetes. Appl Environ Microbiol 55:1642–1644

Ball AS, Godden B, Helvenstein P, Penninckx MJ, McCarthy AJ (1990) Lignocarbohydrate solubilization from straw by actinomycetes. Appl Environ Microbiol 56:3017–3022

Biely P (1985) Microbial xylanolytic systems. Trends Biotechnol 3:286–290

Enger MD, Sleeper BP (1965) Multiple cellulase system from *Streptomyces antibioticus*. J Bacteriol 89:23–27

Gao XZ, Ma WQ, Ma CB, Zhang FS, Wang YH (2002) Analysis on the current status of utilization of crop straw

in China. J Huazhong Agric Univ 21(3):242–247 (in Chinese with English abstract)

Givens JD (1996) Air quality annual report. Louisiana Department of Environmental Quality, Baton Rouge

Gottschalk LMF, Bon EPS, Nobrega R (2008) Lignin peroxidase from *Streptomyces viridosporus* T7A: enzyme concentration using ultrafiltration. Appl Biochem Biotechnol 147:23–32

Hankin L, Anagnostakis SL (1977) Solid media containing carboxymethylcellulose to detect Cx cellulase activity of microorganisms. J Gen Microbiol 98:109–115

Harbers LH, Raiten DJ, Paulsen GM (1981) The role of plant epidermal silica as a structural inhibitor of rumen microbial digestion in steers. Nutr Rep Int 24:1057–1066

Hendricks CW, Doyle JD, Hugley B (1995) A new solid medium for enumerating cellulose-utilizing bacteria in soil. Appl Environ Microbiol 61:2016–2019

Kawamura O, Senshu T, Horiguchi M, Matsumoto T (1973) Histochemical studies on the rumen digestion of rice straw cell wall and on the chemical determination of its non nutritive residue. Tohoku J Agric Res 24(4):183–191

Kluepfel D, Shareck F, Mondou F, Morosoli R (1986) Characterization of cellulase and xylase activities of *Streptomyces lividans*. Appl Microbiol Biotechnol 24:230–234

Li XZ (1997) *Streptomyces cellulolyticus* sp. nov., a new cellulolytic member of the genus *streptomyces*. Int J Syst Bacteriol 47:443–445

Mandels M, Sternberg D, Andreotti RE (1975) Growth and cellulase production by Trichoderma. In: Bailer M, Enari TM, Linko M (eds) Proceedings of symposium on enzymatic hydrolysis of cellulose. STTRA, Helsinki, pp 81–109

Mason MG, Ball AS, Brandon JR, Silkstone G, Nicholls P, Wilson MT (2001) Extracellular heme peroxidase in actinomycetes: a case of mistaken identity. Appl Environ Microbiol 67:4512–4519

McCarthy AJ (1987) Lignocellulose degrading actinomycetes. FEMS Microbiol Rev 46:145–163

Minamiyama H, Shimizu M, Kunoh H (2003) Mutiplication of isolatesR-5 of *streptomyces galbus* on rhododendron leaves and its production of cell wall-degrading enzymes. J Gen Plant Pathol 69:65–70

Parr JF, Papendick RI, Hornick SB, Meyer RE (1992) Soil quality: attributes and relationships to alternative and sustainable agriculture. Am J Altern Agric 7:5–11

Powell KL, Pedley S, Daniel G, Corfield M (2001) Ultrastructural observations of microbial succession and decay of wood buried at a Bronze Age archaeological site. Int Biodeterior Biodegradation 47:165–173

Reese ET, Siu RGH, Levinson HS (1950) The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis. J Bacteriol 59:485–497

Robertson JB, Van Soest PJ (1981) The detergent system of analysis and its application to human foods. In: Janes WPT, Theander O (eds) The analysis of dietary fiber in food. I Marcell Dekker, NY, pp 123–156

Saarilahti HT, Heino P, Pakkanen R, Kalkkinen N, Palvia I, Palva ET (1990) Structural analysis of the *pheA* gene and characterization of its protein product, endopolygalacturonase, of *Erwinia carotovora* subspecies *carotovora*. Mol Microbiol 4:1037–1044

Shimizu M, Kunoh H (2000) Isolation of thatch-degrading bacteria and their physiological characters. J Jap Soc Turfgrass Sci 29:22–31

Spear L, Gaallagher J, McHale L, McHale AP (1993) Production of cellulase and β-glucosidase activities following growth of *Streptomyces hygroscopicus* on cellulose containing media. Biotechnol Lett 15:1265–1268

Srinivasan C, D'souza TM, Boominathan K, Reddy CA (1995) Demonstration of laccase in the white rot Basidiomycete *Phanerochaete chrysosporium* BKM-F-1767. Appl Environ Microbiol 61:4274–4277

Taechowisan T, John FP, Saisamorn L (2003) Isolation of endophytic actinomycetes from selected plants and their antifungal activity. World J Microbiol Biotechnol 19:381–385

Tuncer M, Ball AS, Rob A, Wilson MT (1999) Optimization of extracellular lignocellulolytic enzyme production by a thermophilic actinomycete *Thermomonospora fusca* BD25. Enzyme Microb Technol 25:38–47

Van Soest PJ, Robertson JB (1985) Analysis of forages and fibrous foods. Cornell University, Ithaca

Wang JK, Liu JX, Li JY, Wu YM, Ye JA (2007) Histological and rumen degradation changes of rice straw stem epidermis as influenced by chemical pretreatment. Anim Feed Sci Tech 136:51–62

Zhang JP, Wang JW, Jiang JM, Yang WD (2005) Comparison on lignin-degrading enzymes of *Ganoderma-lucidum* fungi. Forest Res 18:106–108 (in Chinese with English abstract)