academicJournals

Vol. 7(22), pp. 2730-2735, 28 May, 2013
DOI: 10.5897/AJMR12.1660
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation, identification and characterization of a straw degrading *Streptomyces griseorubens* JSD-1

H. W. Feng[1,3], Y. E. Zhi[1,3], W. W. Shi[2,3], L. Mao[1,3] and P. Zhou[1,2,3]*

[1]School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China.
[2]School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China.
[3]Key Laboratory of Urban Agriculture (South) of Ministry of Agriculture, Shanghai 200240, China.

Accepted 25 March, 2013

To reutilize rice straw immediately during the agricultural production process, an actinomycete named JSD-1 was isolated from soil and rotten straw using multiple selective culture media. It was identified as *Streptomyces griseorubens* through polyphasic taxonomy consisting of morphological and physiological characterization combined with the result of 16S rDNA gene sequence and phylogenetic analysis. It also released a series of enzymes, including cellulase, pectinase, xylanase and ligninase simultaneously. According to the experiment of weight loss, JSD-1 decomposed about 76% of the straw in 30 days. Besides, it had the ability to resist certain plant diseases, such as *Sclerotinia rot* of melon and so on. It was obviously that JSD-1 could degrade rice straw effectively and had great potential of being applied to the utilization of straw resource.

Key words: Rice straw, Utilization, *Streptomyces griseorubens*, Polyphasic taxonomy, enzymes, weight loss, diseases.

INTRODUCTION

According to incomplete statistics, over ten billion tons of straw had been produced recent years all over the world and almost one billion tons of straw was produced in China per year. Rice straw, which accounted for 31.6% totally, had the largest amount among crop straw (Song and Yang, 2005). As the structure of rice straw is mainly cellulose and hemicellulose encrusted with lignin and covered by pectin, the degradation and utilization of rice straw were relatively difficult compared with straw of other crop (Parr et al., 1992; Gao et al., 2002). Mass of rice straw were usually eliminated through burning, which might cause great fire and bring serious threats to environment. Nearly 90% straw had been used improperly, which meant great resource waste and environmental pollution.

Another approach of dealing with rice straw was mixing it into the soil. As the existence of soil microorganisms, it was easier for the lignocellulose components to transform into compost. However, it usually took a long time to decompose and impoverished the soil easily. It had been found that degradation of lignocellulose was mainly attributed to bacteria and fungi and the importance of actinomycetes in this process was greatly underestimated (Li, 1996; Sudeep, 2001; Souichiro et al., 2004).

Actinomycetes were a kind of prokaryotes with hyphal morphology which was fit for the penetration and degradation of insoluble substrates (Tuncer et al., 1999). Just as bacteria and fungi, it also could produce and release multiple enzymes involved in the degradation of lingocellulose (Ball and McCarthy, 1989). The enzymes involved included cellulases, laccase, lignin peroxidase and manganese peroxidase (Wang and Wang, 2003). The streptomycetes had been the main focus point as the lignocellulose degrading ability had been found in some *Streptomycete sp.* (Spear et al., 1993).

Meanwhile, their apparent widespread ability to generate

*Corresponding author. E-mail: peizhousjtu@163.com. Tel: +86-21-34205762. Fax: +86-21-34205762.

soluble lignocellulose of straw was also confirmed (Ball et al., 1990; Mason et al., 2001). All these could contribute to the reutilization of agricultural wastes.

For these reasons, it was necessary to search for more efficient actinomycetes for the biodegradation of straw. This study was mainly about the isolation, identification and characterization of a *Streptomycete sp.*, aiming to accelerate the reutilization of straw.

MATERIALS AND METHODS

Media

The enrichment medium contained CMC-Na (10.0 g), K_2HPO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), $FeSO_4 \cdot 7H_2O$ 0.1g, $MnSO_4$ 1.0×10^{-4}g, peptone 10.0g, yeast extract 5.0g, natural pH (per liter).

Nutrient salt agar medium containes KH_2PO_4 1.0g, $MgSO_4 \cdot 7H_2O$ 0.3g, $FeCl_2$ 0.01g, $CaCl_2 \cdot 2H_2O$ (0.1 g), $NaNO_3$ (2.5 g), NaCl (0.1 g), agar (20.0 g), pH 7.0 to 7.2 (per liter).

Cellulose-degrading culture medium contained CMC-Na (2.0 g), $(NH_4)_2SO_4$ (2.0 g), $MgSO_4$ (0.5 g), KH_2PO_4 (1.0 g), NaCl (0.5 g), agar (20.0 g), pH 7.0 (per liter).

Cellulose congo-red agar medium contained K_2HPO_4 (0.5 g), $MgSO_4$ (0.25 g), CMC-Na (1.88 g), yeast extract (1.0 g), congo-red (0.4 g), agar (14.0 g), gelatin (2.0 g), pH 7.0 (per liter).

The xylanse detection medium contained yeast extract (1.0 g), xylan (5.0 g), agar (15.0 g), $(NH_4)_2SO_4$ (2.0 g), KH_2PO_4 (4.0 g), $Na_2HPO_4 \cdot 12H_2O$ (15.4 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), pH 7.0 (per liter).

The pectinase detection medium contained pectin (0.7 g), yeast extract (1.0 g), agar (1.5 g), natural pH (per 100 ml).

Isolation of JSD-1

JSD-1 was isolated from soil and rotten straw collected under stooks stacked for several years in natural condition of Pujiang town, southwest of Shanghai, China. About 1 g sample (fresh weight) was added to 100 ml enrichment medium and shaken at 150 rpm. About 10 ml enriched liquid was transferred to another 100 ml enrichment medium 5 days later, which repeated four times totally. The final liquid was well kept and used to isolate as follows.

The final liquid was inoculated onto filter paper medium plates (prepared by placing a piece of appropriate size of filter paper over nutrient salt agar medium in plate) with 10-fold gradient diluted samples. After 7 days of incubation at 30°C, colonies could be observed on the filter paper medium plates. Then larger colonies were picked, purified and subcultured on plates of cellulose-degrading medium.

Little mycelia were picked out from the purified plates and inoculated to cellulose congo-red agar plates. After 5 days of incubition at 30°C, hydrolysis zones of different sizes were observed clearly on plates (Hendricks et al., 1995). Carboxymethyl cellulase activities were estimated roughly by their ratios of diameter of hydrolysis zones and colonies (Xu and Yang, 2010), and those with high ratio values were selected and stored on slants at 4°C for the further detection of activities of other enzymes.

The determination of straw degrading enzymes

A little mycelia of the selected strains was picked out onto the other detection plates. Xylanase activity and Pectinase activity were detected with the method of Shimizu and Kunoh (2000) and Mnamiyamat et al. (2003). Ligninase activity was estimated with the method of Zhang et al. (2005).

The actual activities of straw degrading enzymes including carboxymethyl cellulase (CMCase), filter paper cellulase (FPase), xylanase, pectinase, laccase (Lac), lignin peroxidase (Lip) and manganese peroxidase (Mnp), were all measured under certain conditions. The enzyme liquid was taken from fermentation liquid of straw degrading process at the 5^{th} day. All enzymes activities were defined and calculated according to the methods of Ni et al. (2008) and Xu and Yang, (2010).

Identification of JSD-1

Morphological and physiological characteristics of JSD-1

Morphological and physiological characteristics of JSD-1 were identified by China Center of Industrial Culture Collection (CICC), Beijing, China.

16S rDNA gene sequence and phylogenetic analysis of JSD-1

DNA was extracted with DNA isolation kit, and the 16S rDNA gene was amplified by PCR using the set of primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′). Amplification was as follows: initial denaturation at 95°C for 5 min followed by 24 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 90 s and final elongation at 72°C for 10 min. PCR Purification Kit was finally used for PCR product purifications. Sequencing was performed by the commercial services of Shanghai Major Bio Technology Co (Shanghai, China). DNA sequence of the cloned 16S rDNA fragments was compared using NCBI. The phylogenetic tree was constructed using the Neighbour Joining method in the software Mega 4.1 (Tamura et al., 2007). 1000 times similarity repeated calculation was performed and only the value of Bootstrap above 50% was displayed on phylogenetic tree node in the figure.

The biodegradation of straw

Rice straw was cut into fragments of about 0.5 cm. 5 g straw fragments was added into 150 ml conical flask with 30 ml nutrient salt medium and autoclaved (15 min at 121°C). Conidia of a inoculating loop cultured in seed medium for 48 h was prepared for rice straw degradation experiment. The inoculation volume was 10% (v/v) and the whole fermentation process was kept at 30°C for 30 days at 150 rpm. The rate of weight loss was measured through the difference between sample weight of each period and the original weight of straw.

Biocontrol potentials of JSD-1

For the certification resistance ability of JSD-1 against plant diseases, series of standoff plates were performed. A plate was inoculated with two different microorganisms simultaneously; one was JSD-1 and the other one was a certain disease microorganism. After 5 days of incubation at 30°C, the inhibition zone was observed between two inoculums to estimate the resistance of JSD-1 to the microorganisms.

RESULTS

Isolation and characterization of JSD-1

After the enrichment, the population of cellulose- degrading microorganisms had dramatically increased. By the selection of filter paper agar plates and the other selective

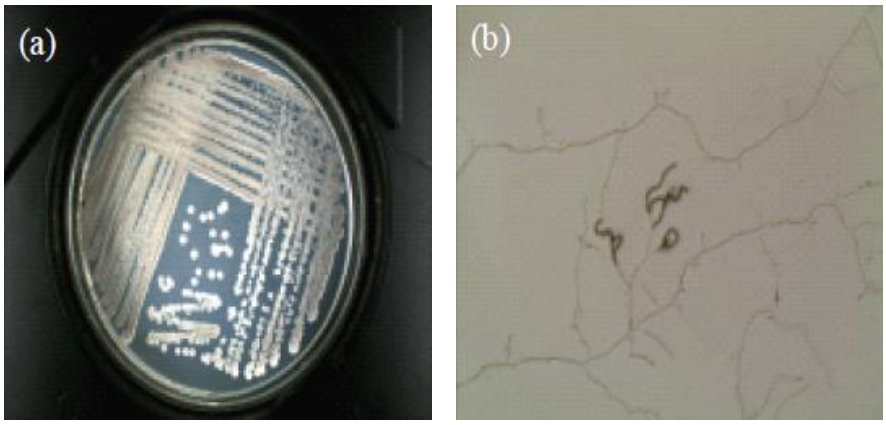

Figure 1. a. Morphology pictures on Gause 1 cultural medium. **b.** Image through microscope (40×).

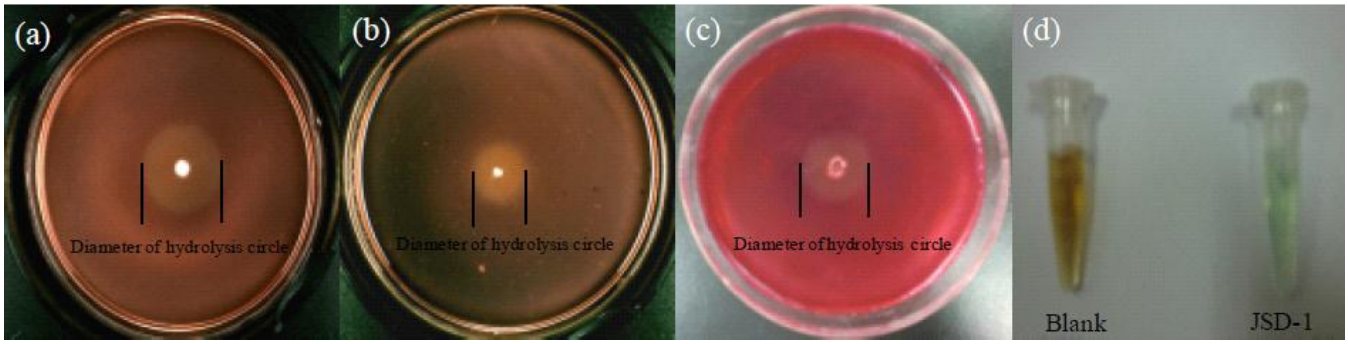

Figure 2. (a) A colony and clear halo formed by JSD-1 on cellulose Congo-red agar plate. **(b)** Plate assay for demonstration of the xylanase activity. **(c)** Plate assay for demonstration of the pectinase activity. **(d)** Liquid assay for demonstration of the peroxidase activity.

Table 1. The activity of straw degrading enzymes.

Isolate	CMCase (U/ml)	FPase (U/ml)	Xylanase (U/ml)	Pectinase (U/ml)	Lac (U/L)	Lip (U/L)	Mnp (U/L)
JSD-1	59.19	31.68	4.27	3.66	50.00	16.12	13.23

medium, only one streptomycete strain was isolated finally. The morphology of JSD-1 on Gause 1 medium plate was shown in Figure 1a. The morphology of JSD-1 through optical microscope (40×) is shown in Figure 1b. It was shown clearly, after 8 days of inoculation, that long spores chains with 1-3 loose, open and spiral loops were formed on ISP2 medium at 30°C.

The determination of straw degrading enzymes

The activities of cellulase, xylanase, pectinase and ligninase were all detected. From Figure 2, different sizes of hydrolysis zones were shown on the plates. Therefore, the activities of xylanase, cellulase and ligninase were clearly presented as a result of the hydrolysis of carbon substrate. Moreover, all enzymes activities are listed on Table 1 in detail, but the values were not measured under optimum conditions.

Identification of JSD-1

According to Bergry's Manual of Systematic Bacteriology, JSD-1 was identified as *Streptomyces griseorubens* through morphological and physiological characterization combined with the result of 16S rRNA gene sequence and phylogenetic analysis.

The characteristics of strain JSD-1 is shown in Tables 2 and 3. It was an aerobic Gram-positive actinomycete which grew well on various organic or inorganic medium (Table 2). The color of aerial mycelium was white and the

Table 2. Cultural characteristics of JSD-1.

Medium	Amount of growth	Color			Production of soluble pigment
		Aerial mycelium	Spores	Vegetative mycelium	
Gause 1 cultural medium	Abundant	White	White	Yellow-brown	None
Yeast extract-malt extract agar (ISP medium 2)	Abundant	Light yellow-brown	White	Brown	None
Oatmeal agar (ISP medium 3)	Abundant	Gray	White	Gray-brown	None
Inorganic salts-starch agar (ISP medium 4)	Abundant	Light yellow-brown	White	Yellow-brown	None
Inorganic salts-starch agar (ISP medium 4)	Abundant	Light yellow-brown		Yellow-brown	None
Glycerol-asparagine agar (ISP medium 5)	Abundant	Gray-white	White	Gray-brown	None

Table 3. Biochemical and physiological characteristics of JSD-1.

Substrate	Result	Substrate	Result	Substrate	Result
L-Arabinose	+	D-Mannose	+	Erythritol starch	+
D-Glucose	+	Glycerol	+	Glycogen	+
D-Xylose	+	Lactose	+	Sorbitol	+
Sucrose	–	Sodium gluconate	+	Cellobiose	+
Rhamnose	+	Ribose	+	L-Sorbose	–
Raffinose	–	Galactose	+	Maltose	+
D-Fructose	+	Sodium succinate	+	Starch hydrolysis	+
Inositol	+	Dulcitol	+	Gelatin liquefaction	–
Mannitol	+	Sodium malonate	+	Milk coagulation	+
Sodium malate	+	Sodium acetate	+	H_2S	+
Sodium propionate	+	Trehalose	+	Cellulose hydrolysis	+
L-Asparagine	+	Sodium tartrate	+	Tyrosinase	+
Melibiose	+	Sodium oxalate	–	Nitrate reduction	+

+, positive; –, negative.

reverse side of the colony was yellow-brown on Gause 1 cultural medium. No fragmentation of the substrate mycelium was observed and no soluble pigments were formed. Most of the carbohydrates tests of JSD-1 were positive except raffinose, L-sorbose, sodium oxalate, sucrose (Table 3). Besides, it did not have the ability to liquefy gelatin either.

Upon comparison of the partial 16S rDNA gene sequence (1401bp) obtained from JSD-1 (Accession NO. KC736485) with sequences from the genbank database, the highest degree of identity was obtained from 16S rDNA gene sequence of B-3981 (Accession No. AB294554), 12889 (Accession No. 12889) and 12780 (Accession No. AB184139), with sequence identities of 99.6, 99.5, 99.4% respectively according to BLAST analysis. All the three strain were *Streptomyces sp.*. It also had sequence identities of above 97% with some other type strains in *Streptomyces sp.*. The phylogenetic tree was constructed based on the 16S rDNA gene sequence by Neighbor-Joining method using MEGA 4.1. The results again suggest that strain JSD-1 belonged to the *Streptomyces griseorubens* group (Figure 3). Therefore, the strain was designated as *Streptomyces griseorubens* JSD-1.

The biodegradation of straw

Figure 4 shows the changes of the straw weight during 30 days' fermentation. The results indicate that the degradation of straw was improved significantly with the inoculation of JSD-1 at the beginning of fermentation. It also indicated that the treatment with JSD-1 composed about 76% of the straw compared to 6% of blank control; besides, JSD-1 had a relatively high initial rate of degradation and degraded almost 24% of straw in the first 5 days as a result of large production and release of enzymes.

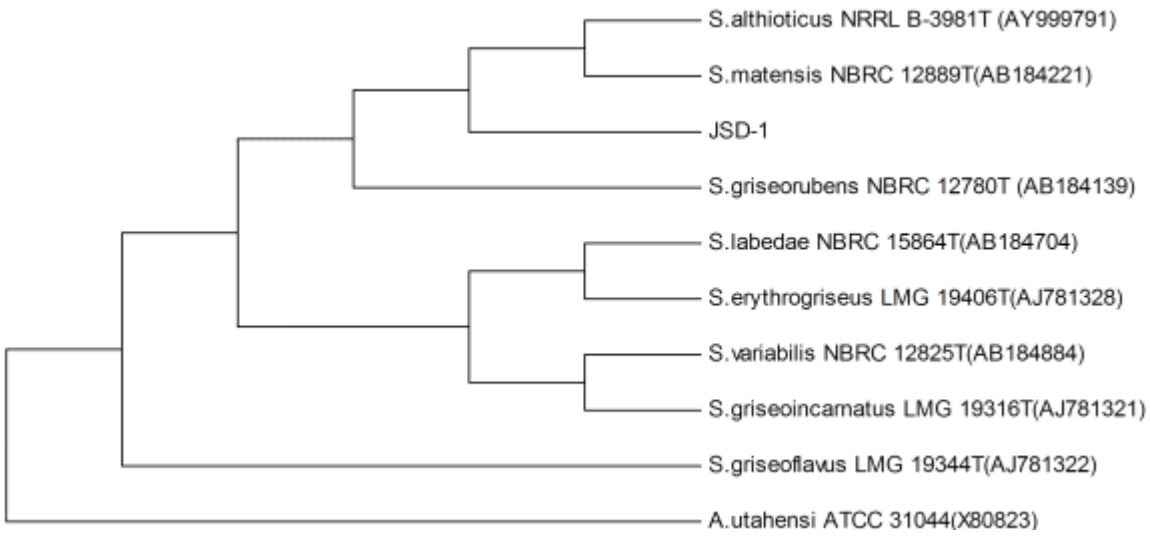

Figure 3. Phylogenetic tree based on 16S rDNA sequence using neighbour-joining method. T represents type strain.

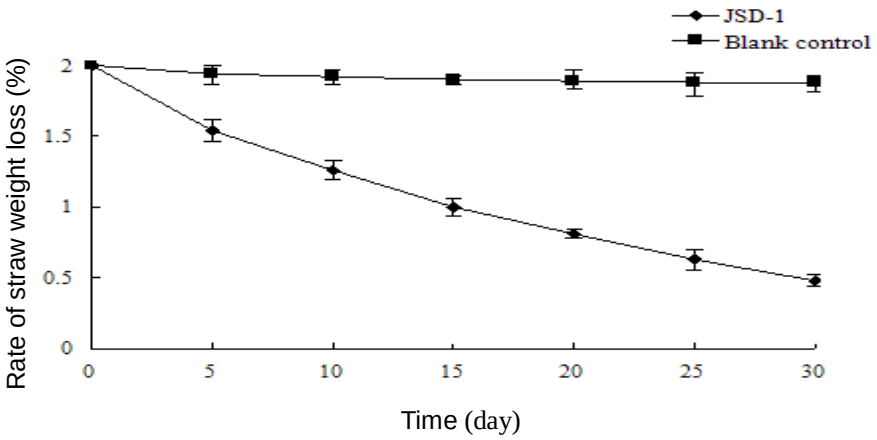

Figure 4. Rate of straw weight loss.

Biocontrol potentials of JSD-1

JSD-1 also had the ability to resist some plant diseases existed in soil widely, such as *Sclerotinia Rot*, *Saro-cladium oryzae*, *Fusarium oxysporum* and some other diseases, especially those caused by bacteria. It is clearly shown in Figure 5, that the growth of *Sclerotinia Rot* was significantly inhabited as it was close to JSD-1, which indicated JSD-1 had the ability to resist certain kinds of plant diseases.

DISCUSSION

Another straw degrading *S. griseorubens* C-5 had been reported before (Xu and Yang, 2010). Both Streptomy-cetes had strong abilities to degrade rice straw and release cellulase, xylanase, pectinase, and ligninase.

However, JSD-1 had higher capability of producing cellulase by comparison. The activity of carboxymethyl cellulase and filter paperlyase could be as high as 59.19 and 31.68U/ml respectively (Feng et al., 2012) and it degraded the rice straw more completely during 30 days liquid fermentation compared with C-5. What is more, the specific value of enzymes involved in the process of lignin degradation, especially Lac, Lip and Mnp, were all listed. Previous reports mainly focused on cellulase and ignored the ligninase.

Besides, JSD-1 had great differences in morphological and physiological characteristics with C-5. The two strains had different characteristics of using carbon sub-strate and sequences of 16S rDNA gene. With the study of these information combined with the result of 16S rDNA gene sequence and phylogenetic analysis, we could know the exact phylogenic position better. Besides,

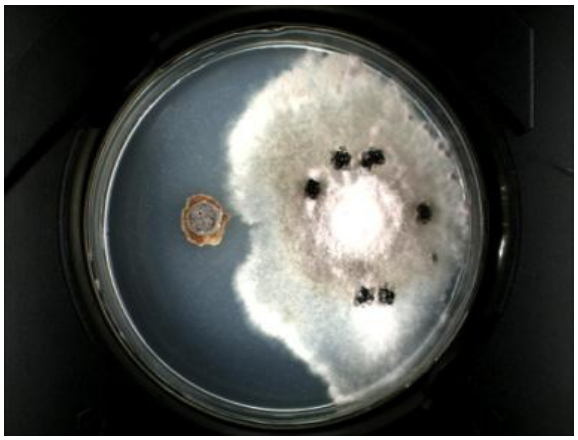

Figure 5. Standoff plate of JSD-1 versus *Sclerotinia Rot.*

it had been proven that JSD-1 had the ability to resist microorganisms of certain plant diseases, so it had great potential in biocontrol.

The impressive characteristics of JSD-1 above should be further discussed and used in different approaches, such as bio-resource management, biological pesticide and land reclamation. So far, JSD-1 had been successfully used in the composting of rice and asparagus straw. Research on the application of mass production is ongoing.

ACKNOWLEDGEMENTS

The work was supported by the National High Technology Research and Development Program (“863” Program) of China (2012AA101405), the National Natural Science Foundation of China (31071860), the Special Fund for Agro-scientific Research in the Public Interest of China (200903056), STCSM (10DZ1960103) and STCSM (10DZ1960102) and the National Key Technology R&D Program (2010BAK69B18)..

REFERENCES

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

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

Feng HW, Zhou P, Mao L, Shi WW, Zhi YE (2012). Screening on a cellulose-decomposing microbe and optimizing its conditions for cellulose production. J. Shanghai Jiaotong University (Agriculture Sciences). In press (in Chinese with English abstract).

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).

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

Li XZ, Xu DG, Gao PJ, Rin GY, Jin FX (1996). CMC-Ligquefying enzyme with low molecular weight from newly isolated *streptomyces*. Microbiol. 23(5): 277–281.

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.

Mnamiyamat H, Shmizum M, Kunoh H, Furumai T, Igarashi Y, Onaka H, Yoshida R (2003). Multiplication of isolates R-5 of Streptomyces galbus on rhododendron leaves and its production of cellwall-degrading enzymes. J. Gen. Plant Pathol. 69: 65-70.

Ni QL, Wang DQ, Qi TY (2008). Discussion on methods of ligninolytic enzymes activity mensuration. J. Guangxi Agric. 23(2): 54-57 (in Chinese with English abstract).

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.

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

Song B, Yang J (2005). Screening of a cellulose-decomposing actinomyces strain and its enzyme producing conditions. J. Microbiol. 25(5): 36–39.

Souichiro K, Shin H, Zong JC, Masaharu I, Yasuo I (2004). Effective cellulose degradation by a mixed-culture system composed of a cellulolytic *Clostridium* and aerobic non-cellulolytic bacteria. FEMS Microbiol. Ecol. 51: 133-142.

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.

Sudeep PG, Absar A, Mala BR (2001). Studies on carboxymethyl cellulase produced by an alkalothermophilic actinomycete. Bioresour. Technol. 77: 171–175.

Tamura K, Dudley J, Nei M, Kumar S (2007). Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24(8): 1596–1599.

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.

Wang HL, Wang ZY (2003). Three important enzymes for lignin degradation. J. Biol. 20(5): 9–11.

Xu J, Yang Q (2010). Isolation and characterization of rice straw degrading *Streptomyces griseorubens* C-5. Biodegr., 21: 107–166.