Novel strategy to improve the colonizing ability of *Irpex lacteus* in non-sterile wheat straw for enhanced rumen and enzymatic digestibility

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Abstract

Pretreatment with white rot fungi is a promising method to enhance the digestibility of lignocelluloses; however, sterilization of feedstocks prior to inoculation is one of the costliest steps. To improve the colonizing ability of white rot fungi under non-sterile condition, *Irpex lacteus*, *Pleurotus ostreatus*, and *Phanerochaete chrysosporium* were inoculated in the wheat straw ensiled for 28 days and incubated for 56 days to determine the changes in microbe counts, organic acid content, chemical composition, and rumen and enzymatic digestibility. Results showed that ensiling produced abundant organic acids and suppressed most microbes in wheat straw. Significant growth of *I. lacteus* was observed after 3 days of incubation, and molds were only detectable at day 7 in the group. At the end of incubation, aerobic bacteria and lactic acid bacteria decreased by 18% and 38% in the wheat straw treated with *I. lacteus*, but molds, aerobic bacteria, and lactic acid bacteria thrived in those treated with *P. ostreatus* and *P. chrysosporium*. Even more, *P. ostreatus* and *P. chrysosporium* increased the lignin content of the ensiled wheat straw by 34% and 65%. However, *I. lacteus* selectively degraded lignin by 28% and improved the rumen and enzymatic digestibility by 18% and 34%. The finding indicates that ensiling prior to fermentation with *I. lacteus* is an effective method to control spoilage microbes and to enhance the rumen and enzymatic digestibility of wheat straw.

Keywords Enzymatic hydrolysis · Pretreatment · Rumen digestibility · Wheat straw · White rot fungi

Introduction

Lignocelluloses from agriculture and forestry residues are promising renewable resources for the production of biofuels and ruminant feeds. Because the complex and highly lignified cell walls of lignocelluloses are less accessible to microbial enzymes, effective pretreatment to disrupt such barriers is essential before further utilization (Ding et al. 2019; Thomsen et al. 2016). Thermal and chemical pretreatment methods have proved effective methods to improve enzymatic hydrolysis and rumen digestion of lignocellulose, but these techniques often involve side reactions and produce lignocellulose-derived by-products that can inhibit microbial and enzymatic biocatalysts (Jonsson and Martin 2016; Wan and Li 2012). As an alternative, fungal pretreatment with white rot fungi has received renewed interest for animal feed and cellulosic ethanol or methane production (Kainthola et al. 2019; Kumar et al. 2018; Niu et al. 2018a). However, most fungi do not colonize well under non-sterile conditions, which make sterilization of feedstocks prior to the fungal pretreatment one of the costliest steps and limit its commercialization (Vasco-Correa et al. 2016; Zhao et al. 2014).

In nature, degradation of lignin by white rot fungi often takes place in highly heterogeneous systems, in which they coexist and compete with other non-lignocellulolytic microbes (Johnston et al. 2016; Tejirian and Xu 2010). The symbiosis of white rot fungi and other microbes indicates that there may be some fungi degrading lignin efficiently under particular non-sterile conditions. Recently, multiple research groups have attempted to enhance the competitive advantage of some fungi by increasing the inoculation amount or

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disinfecting the raw materials in hydrated lime solution, but over 30% of inoculum ratio or washing with large amounts of water was necessary for these methods (Song et al. 2013; Vasco-Correa et al. 2016; Zhao et al. 2014). Therefore, developing more efficient strategies to control spoilage microbes and screening new species that is resistant to infection are crucial for the pretreatment of lignocelluloses under non-sterile conditions.

Generally, the spoilage microbes, primarily bacteria and yeasts, grow and propagate much faster than white rot fungi under most neutral and nutrient-rich conditions. Once these spoilage microbes have invaded the fermentation system, they can alter the pH, compete for nutrients with white rot fungi, and degrade the ligninolytic enzymes (Gao et al. 2008). Fortunately, bacteria do not grow well in low-pH and nitrogen-limited media (Yang et al. 2011); yeasts often require easily accessible carbon and nitrogen sources for rapid growth (Broach 2012). On the contrary, most white rot fungi prevail in lignocelluloses under acidic conditions, which is a less efficient carbon source for non-lignocellulolytic microbes (Johnston et al. 2016; Libra et al. 2003). Therefore, acidifying the substrates prior to inoculation, such as in ensiling conditions, might be an effective alternative to replace sterilization in the pretreatment of lignocellulosic biomass.

In the present study, three white rot fungi, *Irpex lacteus*, *Pleurotus ostreatus*, and *Phanerochaete chrysosporium*, were inoculated in the ensiled wheat straw and incubated at 28 °C for 56 days. Changes in pH, organic acid levels, microbial community, and chemical composition during the incubation were compared for assessing their growth and degradation characteristics. Enzymatic hydrolysis and in vitro rumen digestibility of the treated straw were also compared with that of the intact and ensiled straw.

Material and methods

Fungal cultures

The three white rot fungal species used in the present study were purchased from China General Microbiological Culture Collection Center (CGMCC) in Beijing, China. They were maintained on potato dextrose agar (PDA) slants at 4 °C. The fungal strains used were *I. lacteus* CGMCC 5.809, *P. ostreatus* CGMCC 5.374, and *P. chrysosporium* CGMCC 5.829. Before the experiments, the three fungi were grown on PDA plates at 28 °C until the mycelia covered the entire agar plates. Ten 5-mm agar plugs removed from each fungal plate were added to a separate flask containing 200 g of sterilized wheat grains and incubated at 28 °C until all the grains were colonized by mycelia. Thereafter, the three kinds of spawn were stored at 4 °C for further inoculation.

Preparation of substrate and method of incubation

Wheat straw, harvested from an experimental field of the National Experiment Station for Precision Agriculture (40.22° N, 116.20° E, Beijing, China), was chopped into 2–3 cm long pieces using a forage chopper. The prepared straw was adjusted to approximately 55% moisture content and filled into plastic film bags. All the bags were degassed and sealed with a vacuum-packing machine and stored at ambient temperature (26–32 °C). After 28 days of ensiling, the moisture content was adjusted to 70%. After thorough mixing, the samples were weighed into 25×17 cm polyethylene fresh-keeping bags (Miaojie, Wuxi, Jiangsu, China), which has a moderate air permeability and can reduce the water transfer process. Thereafter, 6 g of the wheat grains from each flask was inoculated in 150 g of the ensiled straw. The bags were sealed by tying a knot, and the moisture content was kept around 70% by spraying distilled water on the outer layers of substrates biweekly. All bags containing samples were incubated for 0, 3, 7, 14, 28, 42, or 56 days in an air-conditioned chamber at 28 °C with a relative humidity of 80%. Triplicate samples of each treatment were used for subsequent analyses.

Microbial and chemical analyses

Ten grams (fresh weight) of samples from each treatment was homogenized with 90 mL of sterilized distilled water. Serial dilutions of 10^{-1}–10^{-9} were prepared for microbiological evaluation, and the remaining extracts were filtered for further evaluation. The numbers of lactic acid bacteria (LAB) were determined by plate counting on de Man, Rogosa, and Sharpe (MRS) agar (Difco Laboratories, Detroit, MI, USA) incubated at 37 °C for 48 h under anaerobic conditions. Molds and yeasts were counted on Rose Bengal medium (Aobox, Beijing, China) incubated at 30 °C for 48 h under aerobic conditions. Bacilli and aerobic bacteria were counted on nutrient agar (Nissui, Tokyo, Japan) incubated for 24 h at 37 °C under aerobic conditions. The pH was measured using a glass electrode pH meter (S20K, Mettler Toledo, Greifensee, Switzerland). The organic acid content of the straw was determined by high performance liquid chromatography (HPLC; column: Shodex RS Pak KC-811, Showa Denko K.K., Kawasaki, Japan; detector: DAD, 210 nm, SPD-20A, Shimadzu Co., Ltd., Kyoto, Japan; eluent: 3 m mol/L $HClO_4$, 1.0 mL/min; temperature 50 °C). Ammonia nitrogen (NH_3-N) was determined using the method of phenol–hypochlorite reaction (Broderick and Kang 1980).

Neutral detergent solute (NDS), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were determined according to the method described by Van Soest et al. (1991). The content of hemicellulose was calculated as the difference between NDF and ADF and that of cellulose as the difference between ADF and ADL.

Water-soluble carbohydrate (WSC) content was determined by using the anthrone method (Murphy 1958). Ash content was determined by complete combustion of the samples in a muffle furnace at 550 °C until a constant weight was recorded.

In vitro rumen digestibility

The filter bag method developed by ANKOM Technology Corporation (Fairport, NY, USA) was used to determine the effect of fungal pretreatment on the digestibility of the wheat straw. The incubation media and procedure were same to that described by Zuo et al. (2018) except that rumen fluid was collected from three rumen-fistulated Angus bullocks fed a corn silage and wheat straw-based diet. At the end of incubation (48 h), the bags were rinsed four times with distilled water and dried at 60 °C for 24 h. The dry matter digestibility (DMD) of the samples was calculated and expressed as the percentage of dry matter (% DM).

Enzymatic hydrolysis

Enzymatic hydrolysis of raw, ensiled, and pretreated wheat straw was conducted according to the method of the National Renewable Energy Laboratory (NREL) (Selig et al. 2008). About 2 g (dry basis) of feedstock was supplemented with the enzyme Cellic CTec2 (Sigma-Aldrich, Saint Louis, MO, USA) at 10 FPU/g dry substrate and 0.08 g/L tetracycline hydrochloride (Adamas, Shanghai, China) to prevent microbial contamination. Enzymatic hydrolysis was carried out at pH 4.8 using 50 mM sodium citrate buffer and incubated at 50 °C, 180 rpm for 72 h. Hydrolysates were centrifuged at $12,000\times g$ for 10 min and passed through a 0.2-μm nylon filter. The glucose concentrations were measured by a Dionex Ultimate 3000 ultra-HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) with a Benson BP-100 H^+ carbohydrates column (Benson Polymeric Inc., Reno, NV, USA) and RefractoMax 521 refractive index detector (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

Data of pH, organic acids, microbial, chemical composition, and digestibility were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests and declared when $P < 0.05$ using IBM SPSS 21.0 for Windows (IBM SPSS Inc., Chicago, IL, USA). Regression analysis and graph processing were performed using Origin 2018 for Windows (OriginLab Corporation, Northampton, MA, USA).

Results

Compositions of the intact and ensiled wheat straw

The wheat straw is rich in hemicellulose, cellulose, and ADL contents, however, with low NDS and WSC content (Table 1). After 28 days of ensiling, the content of WSC and NDS of the wheat straw decreased by 89% and 13%, while that of hemicellulose, cellulose, and ADL contents increased by 4%, 7%, and 4%, respectively. Compared with chemical composition, ensiling had stronger effect on the microbial composition of wheat straw. At the end of ensiling, there was only a significant increase in the counts of LAB ($P < 0.05$). The numbers of aerobic bacteria decreased to 6.5 $\log_{10}$ cfu/g fresh matter (FM), while yeasts and molds decreased to a non-detectable level.

Microbial composition and pH

Morphology of the wheat straw treated with different white rot fungi for 56 days is shown in Fig. 1. The straw treated with *I. lacteus* was covered with white mycelium from day 7 to the end of incubation, but those treated with *P. ostreatus* and *P. chrysosporium* were occupied by molds. The experimental data of microbes revealed that the numbers of LAB and aerobic bacteria significantly increased ($P < 0.001$) after 3 days of incubation, with molds incapable of being detected (Fig. 2).

Table 1 Chemical and microbial compositions of the intact and ensiled wheat straw

Item	Intact straw	Ensiled straw
WSC (g/kg DM)	48.5 ± 2.44	5.5 ± 0.23
NDS (g/kg DM)	285 ± 3.7	247 ± 4.5
Hemicellulose (g/kg DM)	267 ± 3.1	278 ± 3.2
Cellulose (g/kg DM)	380 ± 4.6	405 ± 3.9
ADL (g/kg DM)	67.3 ± 1.07	69.7 ± 0.69
Ash (g/kg DM)	50.4 ± 1.09	55.4 ± 1.70
pH	6.69 ± 0.082	5.48 ± 0.055
Lactic acid (g/kg FM)	ND	0.50 ± 0.167
Acetic acid (g/kg FM)	ND	1.35 ± 0.082
Butyric acid (g/kg FM)	ND	6.68 ± 1.293
NH_3-N (g/kg FM)	0.15 ± 0.016	0.26 ± 0.012
LAB ($\log_{10}$ cfu/g FM)	3.4 ± 0.12	6.7 ± 0.30
Aerobic bacteria ($\log_{10}$ cfu/g FM)	6.9 ± 0.21	6.5 ± 0.27
Yeasts ($\log_{10}$ cfu/g FM)	3.8 ± 0.29	ND
Molds ($\log_{10}$ cfu/g FM)	5.5 ± 0.15	ND

The data were expressed as the mean of three replicates ± SD

DM dry matter, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *NDS* neutral detergent solute, *ADL* acid detergent lignin, *WSC* water soluble carbohydrates, *FM* fresh matter, *LAB* lactic acid bacteria, *cfu* colony-forming units, *ND* not detected
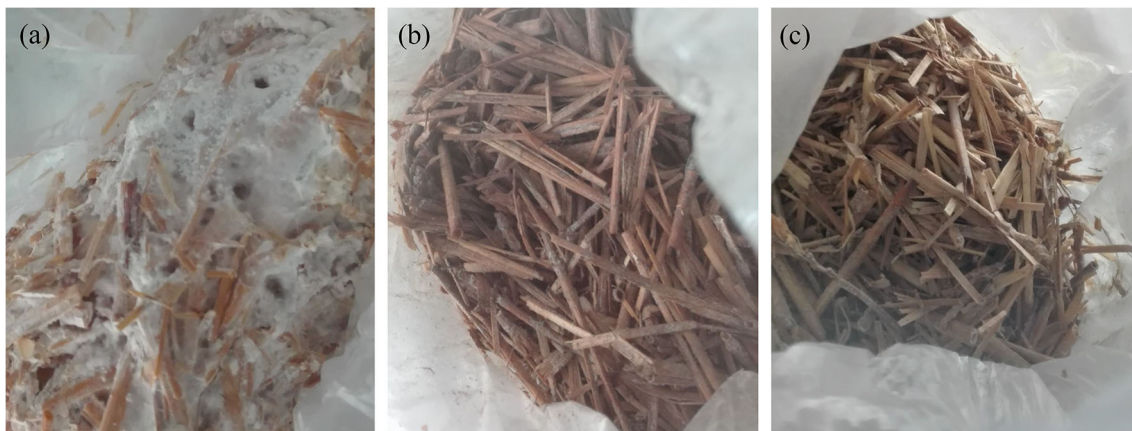
Fig. 1 Wheat straw treated with *Irpex lacteus* (**a**), *Pleurotus ostreatus* (**b**), and *Phanerochaete chrysosporium* (**c**) for 56 days

The number of aerobic bacteria, LAB, and molds in the straw treated with *I. lacteus*, after 7 days of incubation, became significantly lower ($P < 0.001$) than those treated with *P. ostreatus* and *P. chrysosporium*. In the subsequent incubation, molds in the straw treated with *I. lacteus* decreased to non-detectable levels, with the number of aerobic bacteria and LAB decreasing over time. Yeasts were not detected in any of the samples. Correspondingly, the wheat straw treated with

I. lacteus, after 7 days of incubation, showed a significantly lower pH ($P < 0.001$) than those treated with *P. ostreatus* and *P. chrysosporium*.

Organic acid and NH$_3$-N content

The contents of lactic acid, acetic acid, butyric, and NH$_3$-N during 56 days of incubation are shown in Fig. 3. Although
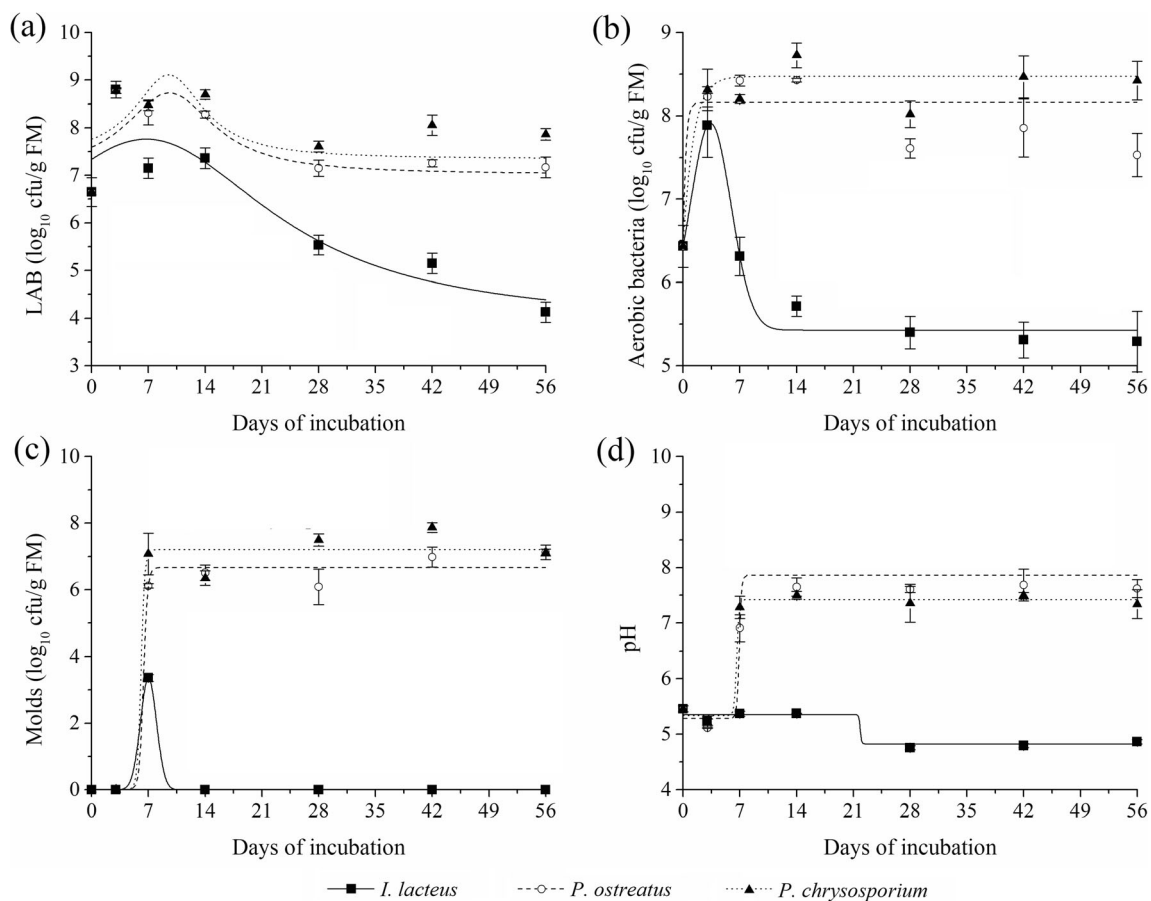

Fig. 2 Changes in microbial composition and pH of wheat straw during the 56 days of incubation with *Irpex lacteus*, *Pleurotus ostreatus*, and *Phanerochaete chrysosporium*. FM, fresh matter. The data were expressed as the mean of three replicates ± SD
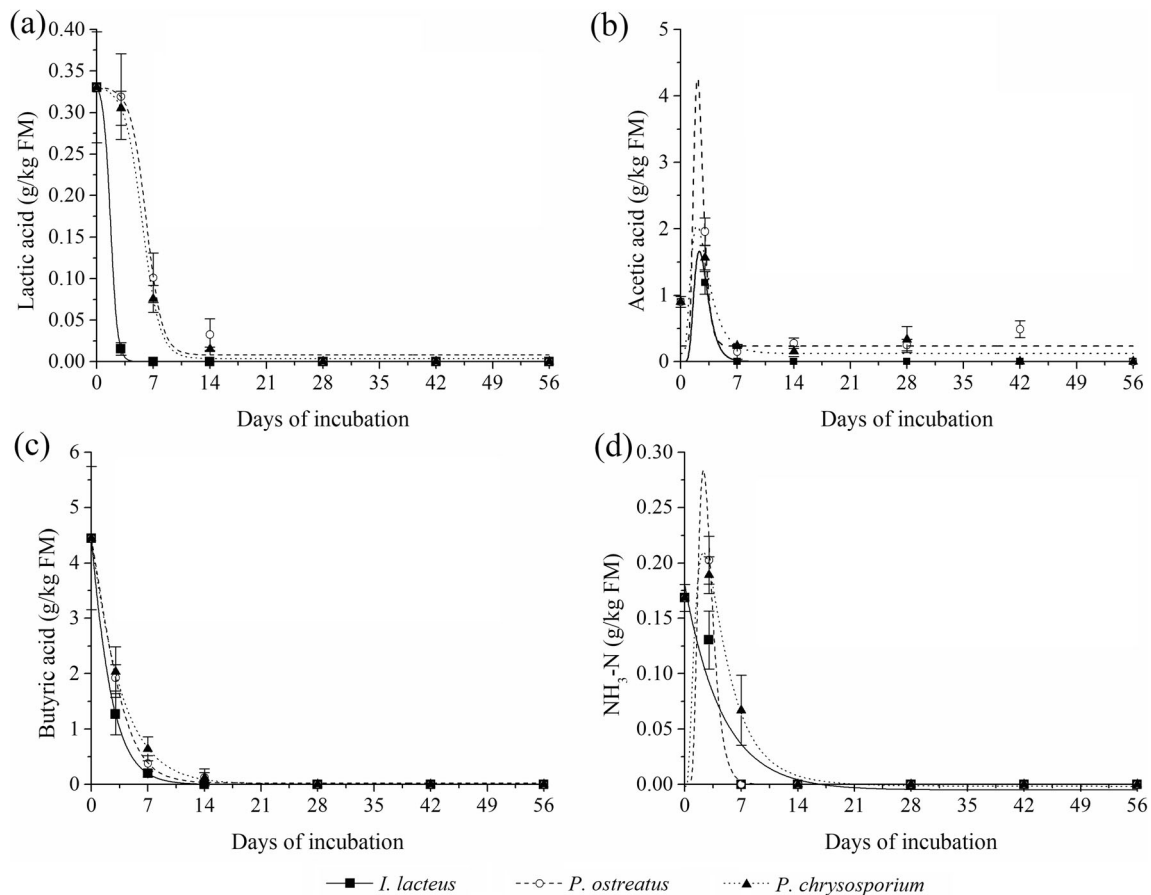
Fig. 3 Changes in organic acids and ammonia nitrogen of wheat straw during the 56 days of incubation with *Irpex lacteus*, *Pleurotus ostreatus*, and *Phanerochaete chrysosporium*. FM, fresh matter. The data were expressed as the mean of three replicates ± SD

the data of each organic acid in the wheat straws treated with *I. lacteus*, *P. ostreatus*, and *P. chrysosporium* had similar kinetic trends, the contents of the former decreased faster than those in the group of *P. ostreatus* and *P. chrysosporium*. After 3 days of incubation, the lactic acid content in the straw treated with *I. lacteus* and butyric acid content in all the treatments decreased significantly ($P < 0.001$), while the acetic acid content increased significantly ($P < 0.05$). The pH of straw in all the treatments showed a significant decrease ($P < 0.01$). However, the contents of lactic acid, acetic acid, and butyric acid in the straw treated with *I. lacteus* were significantly lower ($P < 0.05$) than those treated with *P. ostreatus* and *P. chrysosporium*, the contents of which in all of the treatments decreased significantly after 7 days of incubation ($P < 0.01$). Lactic acid and acetic acid could not be detected in the straw treated with *I. lacteus*, with the butyric acid content significantly lower ($P < 0.05$) than that of the straw treated with *P. chrysosporium*. Contrary to the contents of organic acids, pH of the straw treated with *I. lacteus* was significantly lower ($P < 0.001$) than that in the other treatments after 7 days of incubation. Then, it declined to be lower than 5 in the subsequent incubation, but organic acids could not be detected in all the samples. Similar to organic acids, NH_3-N of the

wheat straw treated with *I. lacteus* was significantly lower ($P < 0.001$) than that of others after 3 days of incubation.

Chemical composition and digestibility

The experimental data of NDS, cellulose, and ADL of each treatment are shown in Fig. 4. All the NDS of wheat straw treated with *I. lacteus*, *P. ostreatus*, and *P. chrysosporium* showed a rising tendency with time, while that of the former was significantly higher than the latter after 28, 42, and 56 days of incubation ($P < 0.05$). The content of both cellulose and hemicellulose could significantly be decreased through the treatment with white rot fungi ($P < 0.05$), but the change of cellulose slowed down after 28 days of incubation. The ADL content of wheat straw treated with *I. lacteus*, similar to NDS and cellulose, also had a different trend with *P. ostreatus*, and *P. chrysosporium*. After 14 days of incubation, the straw treated with *I. lacteus* showed the lowest ADL content ($P < 0.05$), indicating that more lignin was degraded by *I. lacteus*. The ADL content in the straw treated with *P. ostreatus* and *P. chrysosporium*, after 56 days of incubation, was approximately two times higher than that in the straw treated with *I. lacteus*. Corresponding to the high degree of lignification,
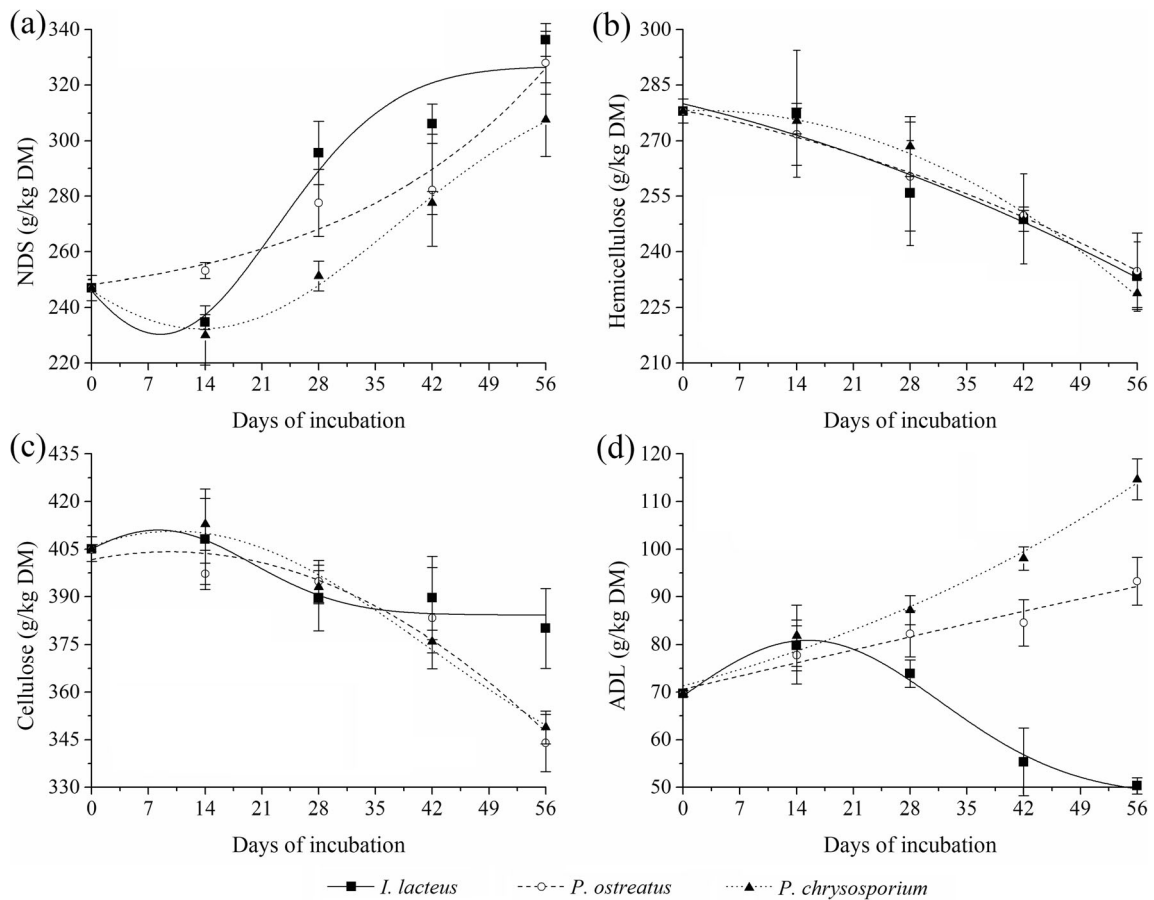
Fig. 4 Changes in chemical composition of wheat straw during the 56 days of incubation with *Irpex lacteus*, *Pleurotus ostreatus*, and *Phanerochaete chrysosporium*. DM dry matter, NDS neutral detergent solute, ADL acid detergent lignin. The data were expressed as the mean of three replicates ± SD

the DMD and enzymatic hydrolysis of intact wheat straw were 49.3% and 19.3% DM, respectively (Fig. 5). After 28 days of ensiling, the enzymatic hydrolysis of ensiled wheat straw was 14.5% DM, which was significantly lower ($P < 0.001$) than that of the intact. Although incubation with *P. ostreatus* and *P. chrysosporium* significantly decreased the DMD and enzymatic hydrolysis ($P < 0.001$), those of wheat straw treated with *I. lacteus* were increased to 59.6% and 25.9% DM.

Discussion

Interaction between microbes and environment

Ensiling is a common method used for preserving moist crops based on natural fermentation of sugars by lactic acid bacteria under anaerobic conditions (Muck 2010). After several weeks of ensiling, the substrates often have low pHs and microbe numbers (Dunière et al. 2013). In the present study, the ensiled

Fig. 5 The in vitro rumen digestibility after 48 h (**a**) and enzymatic hydrolysis after 72 h (**b**) of the intact, ensiled, and treated wheat straw. IL *Irpex lacteus*, PO *Pleurotus ostreatus*, PC *Phanerochaete chrysosporium*. The data were expressed as the mean of three replicates ± SD. Means followed by different letters were significantly different at $P < 0.05$
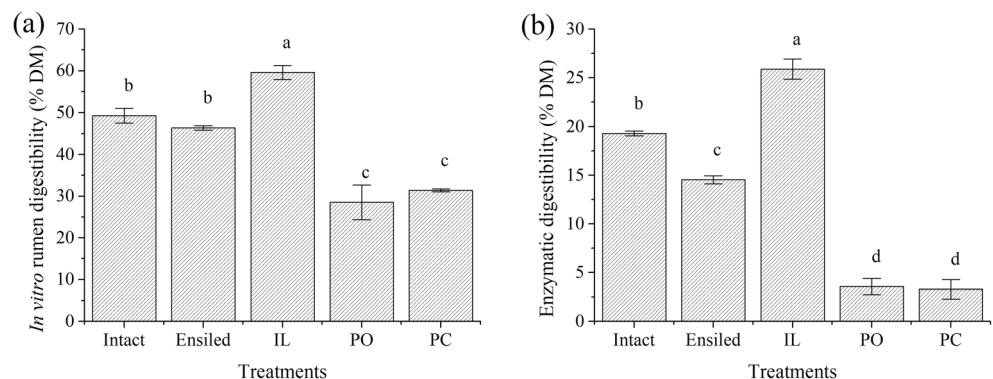
wheat straw had a relatively higher pH than common silages. The poor fermentation quality was also observed by other researchers and attributed to the low WSC content of wheat straw (Yang et al. 2006; Ni et al. 2014). After air exposure, yeasts will be produced firstly in most silages because they grow faster than most other microbes under aerobic and acidic conditions (Muck 2010; Wang et al. 2018). However, natural lignocellulosic substrates serve as a less efficient carbon source for these microbes without lignocellulolytic enzymes (Šlosarčíková et al. 2017), and aerobic bacteria often begin to grow in silages when the pH is approximately 4.5 or higher (Muck 2010). This might help explain why aerobic bacteria dominated the substrates rather than yeasts during the first few days of incubation. In the subsequent incubation, lactic acid and butyric acid decreased to an undetectable level because of that the microbes metabolize organic acids for their growth under aerobic condition (Niu et al. 2018b). Contrary to the changes of organic acids, the pH of the straw treated with *I. lacteus* was kept at a low level and declined to be lower than 5 in the subsequent incubation. It is reported that white rot fungi can produce some organic acids to acidify their substrates and maintain their cultures at low pH, so the value of the pH is a good indicator of whether they gain the upper hand (Lang et al. 1997; Liaud et al. 2014). The low pH caused by *I. lacteus* reflected the fungus grew well in the ensiled wheat straw and secreted some acids for inhibiting the growth of other microbes. Even more, limited nitrogen also exerts strong inhibitory impacts on the growth of molds and plenty of bacteria (Gao et al. 2008; Jourdier et al. 2013; Yang et al. 2011). Thus, the acidified and nitrogen-limited condition might be the main abiotic reasons why *I. lacteus* colonized stably in the ensiled wheat straw.

Although the species of white rot fungi are diverse and the interaction between them and other microbes can be governed by an interplay of various factors, only extremely combative species possess the ability to determine the microflora in their substrates (Johnston et al. 2018). The lower pH and numbers of LAB, aerobic bacteria, and molds revealed that *I. lacteus* is strongly capable of colonizing the ensiled wheat straw compared to *P. ostreatus* and *P. chrysosporium*. In agriculture, antagonisms between white rot fungi and molds, especially *Trichoderma*, are of interest for mushroom cultivation. Molds can compete aggressively with white rot fungi, thus reducing the production of mushrooms (Savoie et al. 2001). It is reported that some white rot fungi can resist *Trichoderma* spp. attack by producing laccases with high quantities (Savoie et al. 2001). Interestingly, Svobodová et al. (2008) found activity of laccase bound to the mycelium of *I. lacteus* in mineral liquid medium began to increase after 7 days of incubation, and peaked after 15 days of incubation. Although the activity of laccase can be affected by many factors, it can be determined that laccase is a crucial enzyme of *I. lacteus*. So the decrease of molds after 14 days of incubation might be

attributed to laccases or other antifungal substances produced by *I. lacteus*. Apart from molds, *I. lacteus* is also strongly capable of competing with soil bacteria, such as *Pseudomonas* and *Sphingomonas* (Borràs et al. 2010; Novotný et al. 2001). Above all, it can be concluded that *I. lacteus* is a highly competitive white rot fungus under non-sterile conditions.

Changes in chemical composition and digestibility

Among the common agricultural by-products, the lignified degree of wheat straw is higher than that of maize and rice straw, so the former often has lower nutritive value than the latter straws (Tuyen et al. 2012, 2013). Although ensiling can convert WSC to organic acids, and inhibit the growth of spoilage microbes, the process has little effects on the structure of cellulose and lignin (Ning et al. 2017). It is reported that most white rot fungi, such as *P. ostreatus* and *P. chrysosporium*, can degrade lignin in biomass effectively and selectively after a long incubation under sterile conditions (Wan and Li 2012). The higher ADL content in the present study also revealed that *P. ostreatus* and *P. chrysosporium* did not colonize well in the ensiled wheat straw. In contrast, the lignin of wheat straw treated with *I. lacteus* was degraded by 28%, but cellulose only decreased 6%. Similar degrading characteristics of *I. lacteus* were also observed in sterile wheat straw and hydrated lime treated corn stover (Niu et al. 2018a; Song et al. 2013). The different changes of cellulose and lignin reflected that *I. lacteus* has limited ability to consume cellulose but high capacity to degrade lignin. Apart from lignin, *I. lacteus* also had a significant effect on the contents of hemicellulose and NDS. Generally, NDS often consist of carbohydrates including starch, sugars, and pectic substances as well as non-carbohydrates, including protein, soluble phenolics, ash, and lipids, and white rot fungi that can liberate some monomers and oligomers from hemicellulose and cellulose (Niu et al. 2018a). The higher hemicellulose loss than that of cellulose in this study revealed that the increased NDS content can be mainly caused by the degradation of hemicellulose.

Typically, the energy content of feedstuff is highly correlated with their DMD (Yan and Agnew 2004), with the improved rumen digestibility of forage capable of increasing their potential as raw materials for biofuel production (Badhan et al. 2014). After 56 days of incubation, *I. lacteus* improved the DMD by 18%, but neither ensiling nor pretreatment with *P. ostreatus* and *P. chrysosporium* exerted positive effects on the digestibility of wheat straw. Similarly, the little or negative effects of ensiling on DMD were also observed in Italian ryegrass, lucerne, and orchardgrass (Yahaya et al. 2001; González et al. 2007). Although *P. chrysosporium* exerts negative influences on rumen digestibility of wheat straw under sterile condition, the DMD can often be slightly improved by *P. ostreatus* (Lynch et al. 2014; Niu et al. 2018a).

Therefore, the lower DMD also indicates that *P. ostreatus* and *P. chrysosporium* cannot colonize well in the ensiled wheat straw.

Glucose, the dominant fermentable sugar from cellulose, serves as the major energy sources of life, which can potentially contribute to animal feeds and biofuel production. Enzymatic hydrolysis indicated the availability of cellulose and saccharification efficiency of the treated wheat straw. Specifically, the sugar yield of wheat straw treated with *I. lacteus*, compared with the intact straw, increased by 34%. Similarly, Salvachúa et al. (2013) reported that *I. lacteus* could improve the glucose yields of wheat straw from 33 to 68% under sterile condition, with the positive results revealing that *I. lacteus* is a promising species in the pretreatment of wheat straw under non-sterile conditions. The contaminating microbes and the poor nutrition might be responsible for the relative low improvement. Thus, future studies should pay more attention to controlling harmful microbes and screening positive additives.

In conclusion, most of the spoilage microbes in wheat straw could be suppressed or killed by the anaerobic and acidic condition during ensiling. The acidic environment and low microbe counts allowed *I. lacteus* to colonize well and degrade lignin efficiently under a favorable environment. Pretreatment with *I. lacteus* for 56 days can efficiently degrade the lignin of ensiled wheat straw and improve the in vitro rumen and enzymatic hydrolysis significantly. Therefore, ensiling is a novel strategy to improve the colonizing ability of *I. lacteus* in non-sterile wheat straw for enhanced rumen and enzymatic digestibility.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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