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Original

Potentialities of the microbial consortium *Curvularia kusanoi -Trichoderma pleuroticola* as a biological pretreatment for the degradation of fibrous sources

Maryen Alberto Vázquez[1*]; Elaine Cristina Valiño Cabrera[1]; Livio Torta[2]; Armando Laudicina[2]; María Teresa Sardina[2]; Giulia Mirabile[2].

[1]Instituto de Ciencia Animal (ICA), San José de Las Lajas, Mayabeque, Cuba.
[2]Università degli Studi di Palermo, Dipartmento di Scienze Agrarie, Alimentari e Forestali, Italy.
*Correspondence: mvazquez@ica.co.cu

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ABSTRACT

Objective. To evaluate the potentiality of the microbial consortium *Curvularia kusanoi* L7- *Trichoderma pleuroticola* as biological pretreatment of high fiber sources destined for animal production. **Materials and methods**. The Strains used where *Curvularia kusanoi* L7 and *Trichoderma pleuroticola*. The degradative potential was evaluated through the production kinetics of cellulolytic (endo-1,4-β-glucanase and exo-1,4-β-glucanase) and ligninolytics enzymes (laccase and peroxidase) in solid submerged fermentation of bran wheat and sugarcane bagasse. The growth of the co culture in plates was analyzed. The effect of the consortium on the carbon mineralization of raw wheat straw was determined and the degree of fiber degradation was evaluated by infrared spectroscopy (IR). **Resulted.** Both strains showed high cellulolytic production. Only *C. kusanoi* L7 showed ligninolytic activity, with a maximum laccase activity of 1400 U/L. No antagonism was found between the strains and the results of carbon mineralization and evaluation of their final products by IR indicate the effectiveness of the consortium to degrade the cell wall more efficiently than each of the strains individually. **Conclusions.** It is concluded that the microbial consortium *C. kusanoi* L7-*T. pleuroticola* has great potential for structural modification of fibrous sources destined for animal feeding.

Keywords: Degradation; enzymes; fiber; cell wall; fungi (*Source: CAB*).

RESUMEN

Objetivo. Evaluar la potencialidad del consorcio microbiano *Curvularia kusanoi* L7- *Trichoderma pleuroticola* como método biológico de pretratamiento de alimentos altos en fibra destinados a la producción animal. **Materiales y métodos**. Se utilizaron las cepas *Curvularia kusanoi* L7 y *Trichoderma pleuroticola*. Se evaluó el potencial degradativo a través de las cinéticas de producción de las enzimas celulolítica (endo-1,4-β- glucanasa y exo-1,4-β- glucanasa) y ligninolíticas (lacasa y

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peroxidasa) en fermentación sólido sumergido de salvado de trigo y de bagazo de caña de azúcar. Se analizó el crecimiento del cocultivo en placas. Se determinó el efecto del consorcio sobre la mineralización del carbono de la paja de trigo cruda y se evaluó el grado de degradación de la fibra por espectroscopía infrarroja (IR). **Resultados**. Ambas cepas mostraron alta producción celulolítica . Solo *C. kusanoi* L7 mostró actividad ligninolítica, con actividad lacasa máxima de 1400 U/L. No se encontró antagonismo entre las cepas y los resultados de mineralización del carbono y evaluación de sus productos finales mediante IR, indican la efectividad del consorcio para degradar la pared celular de forma más eficiente que cada una de las cepas de manera individual. **Conclusiones.** Se concluye que el consorcio microbiano *C. kusanoi* L7-*T.pleuroticola* presenta grandes potencialidades para emplearse en la modificación estructural de fuentes fibrosas destinadas a la alimentación animal.

Palabras clave: Degradación; enzimas; fibra; pared celular; hongos (*Fuente: CAB*).

INTRODUCTION

The research of unconventional sources of food for animals constitutes a core issue of current international affairs. In this sense, different strategies are developed where the revaluation of lignocellulosic biomass shows particular interest (1). However, despite the great potential that this resource represents for animal production, the structural complexity of the plant wall and the low digestibility of many of these sources, its field of use decreases, especially in the feeding of monogastric species. In these cases, the fiber pretreatment processes stand out as effective solutions that allow improving its nutritional quality (2).

The scientific literature describes several pretreatment methods that manage to modify the macroscopic and microscopic structure of the plant fiber (3). According to their nature, these methods can be classified into physical, chemical and biological pretreatments, the latter being more developed due to their numerous advantages. This type of pretreatment has lower energy intake, does not use aggressive chemical products, operates in conditions close to the environment and also allows the reduction of inhibitors and toxic chemicals in the reaction medium (4).

The fiber degradation by biological treatment occurs due to the action of certain microorganisms such as fungi and bacteria. These are capable of degrading the complex structure of the plant cell wall, through the expression of highly specific enzymes such as cellulases and enzymes that modify lignin. To increase the effectiveness of pretreatments, it can be used microorganisms with high lignocellulolytic capacity such as white rot fungi, or different variants of microbial consortia to achieve a much more exhaustive degradation of the plant cell wall (4). According to Singh et al (5), these microbial consortia or co-cultures are an effective solution in the biotransformation of complex substrates.

The *Curvularia* and *Trichoderma* genera are widely described as microorganisms with high lignocellulolytic capacity, hence this research aims to: Evaluate the potential of the *Curvularia kusanoi* L7-*Trichoderma pleuroticola* microbial consortium as a biological pretreatment of fibrous foods destined for animal production.

MATERIALS AND METHODS

Microorganisms: The studies were carried out with the non-pathogenic strains *Curvularia kusanoi* L7 and *Trichoderma pleuroticola*, with sequence numbers registered in GenBank: KY795957.1 and MK992922, respectively. Belonging to the Bank of Microorganisms of Microbiology Laboratory from Institute of Animal Science, Cuba. Both strains were isolated from the surface of the lemon tree.

Individual characterization of the degradative potential of the strains

Growth on tannic acid. From the pure cultures of 5 days of growth in potato dextrose agar (PDA), 1 cm^2 of each culture was taken and planted separately in agarized medium with tannic acid as the only carbon source as described by Nobles (6). Plates were incubated at 30°C in complete darkness for 7 days. All experiments were performed in triplicate and the potency index (PI) of the strains was evaluated according to the following formula:

$$PI = \frac{(z-C)}{C} * 100$$

Where:
z- Diameter of the tannic acid degradation halo
c- Diameter of microbial growth

Individual quantitative determination of the enzymatic potential in solid submerged fermentation of wheat bran and solid submerged fermentation of sugarcane bagasse. To evaluate the enzymatic potential of each strains, the production kinetics of the main enzymes of the cellulolytic complex and the lignin modifying enzymes were analyzed in submerged solid fermentation of wheat bran and then in submerged solid fermentation of sugarcane bagasse. In both cases, 1 cm^2 of the 7-day-old mycelium grown in PDA from pure cultures was taken as inoculum. From the pure cultures of 7 days of growth in PDA, the microorganisms were inoculated in flasks containing 3 g of Allbran-Kellogg's wheat bran-based cereal (Table 1) and 100 mL of sodium citrate buffer (50 mM, pH 6.0) and in flasks containing 4 g of sugarcane bagasse and 100 mL of citrate buffer (50 mM, pH 6.0). All were incubated in an orbital shaker (120 rpm for 7 days at 30°C). Fermentation samples were taken every 24 hours and analyzes were performed in triplicate. The content of each flask was filtered, centrifuged (4ºC, 10,000 rpm) and the crude extract was stored in Corning tubes at -20°C for further study (7).

Table 1. Nutritional composition (%) according to the commercial product Allbran-Kellogg's Cereal.

Composition	Content(%)
Proteins	13
Fats	3
Carbohydrates	40
Sugars	17
Dietary fiber	26.5
Sodium	0.5

Evaluation of cellulolytic activity. The cellulolytic activity were determined in the enzymatic extracts obtained from the fermentation, the capacity of endo-1,4-β-glucanase (CMCase) was evaluated on carboxymethylcellulose 2.2% (w/v) in 50 mM sodium citrate buffer, pH 6, and the activity of exo-1,4-β-glucanase (PFase) was quantified on Whatman No. 1 filter paper (50 mg) in 0.6 M sodium acetate buffer, pH 6.0. In both cases, the reactions were incubated at 50ºC for 30 min and the content of reducing sugars released during the process was quantified (8). Endo- and exoglucanase enzyme activities were expressed in International Units per milliliter (IU/mL) (9).

Laccase and lignin peroxidase activity. To evaluate laccase activity, 100 µL of syringaldazine (5 mM) and 800 µL of citrate buffer (50 mM, pH 4.5) were used. This mixture was previously incubated at 30ºC and then 100 µL of the enzyme extract were added to reach the final volume of 1 mL. Substrate oxidation was analyzed in a UV-Vis spectrophotometer at 530 nm. One unit of laccase activity (U) is equivalent to the amount of enzyme that converts 1.0 µmol of syringaldazine per minute (10).

Lignin-peroxidase activity was determined by H_2O_2-dependent oxidative dimerization of 2,4-dichlorophenol (2,4-DCF) at 25°C. For this, 200 µL of sample, 5 mM 2,4-DCF, 3.2 mM 4-aminoantipyrine, 10 mM H_2O_2 in 20 mM potassium phosphate, pH 7.0, were used. The reaction was monitored for 5 minutes after the change in absorbance at 510 nm (ε=1.85x104 $M^{-1}cm^{-1}$). One unit of enzyme activity was defined as the amount of enzyme that catalyzes the increase of one unit of absorbance per minute (11). The enzyme activity determinations used the reaction mixture without the enzyme extract as a negative control.

Statistical analysis. The data of each kinetics were analyzed by simple analysis of variance, to evaluate the effect of fermentation time on enzyme production. The study of the data was carried out with the statistical package InfoStat (12).

Microbial confrontation tests. To determine the ability of the *C. kusanoi* L7 strain to grow in co-culture with the *T. pleuroticola* strain, PDA was used. The plate was divided in half and a microorganism was seeded on each side to show signs of overlap or antagonism between the cultures. All plates were incubated at 30°C in complete darkness for 10 days (13).

Carbon mineralization of raw wheat straw. To evaluate the fibrolytic capacity of each of the strains vs. their co-culture, the carbon mineralization of raw wheat straw was determined by gas spectroscopy. A total of 3 g of the substrate were placed in 200 mL glass bottles closed with pierceable rubber stoppers, 3 mL of minimal salt medium were added (Lee's

modification of the minimal medium: KH2PO4 (2.0 g/L), $MgSO_4$ (0.3 g/ L) $(NH_4)2SO_4$ (1.4 g/L), $CaCl_2$ (0.3 g/L), Urea (0.3 g/L), proteose peptone (0.75 g/L)) and the corresponding inoculum of each of the strains (1 cm^2 from 7 day old mycelium grown on PDA). Four treatments (control with uninoculated wheat straw, wheat straw inoculated with *C. kusanoi* L7, wheat straw inoculated with *T. pleuroticola* and wheat straw inoculated with the co-culture) and six replicates each were evaluated. The CO_2 production was monitored for one month, taking samples of the gas produced by the fermentation every 72 hours and injecting it into the gas chromatograph (Trace GC, Thermo Electron) equipped with a thermal conductivity detector.

Chromatographic conditions. A run time of 2 min was used at a Split flow rate of 45 mL/ min. The CO_2 calibration curve was constructed using the STG, UK brand reference standard calibration gas (99 ppm CO_2, 5 ppm CH4, and 10 ppm N_2O). To carry out the dilutions of the CO_2 standard, a propylene bag and compressed air as solvent were used. To perform the injection of the samples, a 5 mL syringe for gases was used. All readings were done in duplicate.

Evaluation of the level of degradation of wheat straw fiber by Infrared Spectroscopy. Fiber degradation of raw wheat straw after carbon mineralization study was evaluated by Fourier Transform Attenuated Total Reflection Infrared Spectroscopy (ATR-FT-IR) (15). The residues of this assay were evaluated in a Pelkin Elmer equipment with MCT/A detector. The scans were from 4000 to 400 cm^{-1}.

RESULTS

Qualitative and quantitative determination of the enzymatic potential of the strains under study. The growth of *C. kusanoi* L7 and *T. pleuroticola* in Nobles medium, showed the ability of the strains to degrade tannic acid. Regarding the quantitative determination of its enzymatic potential, the submerged solid fermentation studies of the substrates wheat bran and sugarcane bagasse, showed a high fibrolytic capacity, supported by similar values to strains already reported with great degradative capacity. The results by studies are showed below.

Submerged solid fermentation of wheat bran. The degradation of wheat bran in submerged solid fermentation by the strains *C. kusanoi* L7 and *T. pleuroticola* are showed in tables 2 and 3.

Regarding the ligninolytic activity in this substrate (wheat bran), only the *C. kusanoi* L7 strain showed the presence of laccase and peroxidase. Its kinetics of laccase and peroxidase enzymatic production is shown in figure 1.

Table 2. CMCase (endo-1,4-β-glucanase) cellulolytic activity of C. kusanoi L7 and T. pleuroticola in submerged solid wheat bran medium.

Strains/hours	Endo-1,4-β-glucanase enzyme activity (IU/mL)							SE and Signf.
	24	48	72	96	120	144	168	
C. kusanoi L7	0.535^{e}	0.197^{c}	0.981^{d}	0.185^{c}	0.081^{b}	0.035^{a}	0.029^{a}	±0.022 p=0,0003
T. pleuroticola	0.032^{b}	0.061^{ab}	0.064^{ab}	0.082^{b}	0.126^{c}	0.014^{a}	0.002^{a}	±0.001 p=0.023

^{a,b,c,d,e} Means with different letters in each row differ at $p<0.05$ [14]

Table 3. Cellulolytic PFase activity (exo-1,4-β-glucanase) of C. kusanoi L7 and T. pleuroticola in submerged solid medium of wheat bran.

Strains/hours	Endo-1,4-β-glucanase enzyme activity (IU/mL)							SE and Signf.
	24	48	72	96	120	144	168	
C. kusanoi L7	0.340^{c}	0.191^{b}	0.151^{b}	0.082^{a}	0.080^{a}	0.084^{a}	0.052^{a}	±0.011 p=0.045
T. pleuroticola	0.001^{a}	0.006^{b}	0.036^{b}	0.031^{b}	0.107^{c}	0.043^{b}	0.002^{ab}	±0.001 p=0.032

^{a,b,c,d} Means with different letters in each row differ at $p<0.05$ [14]

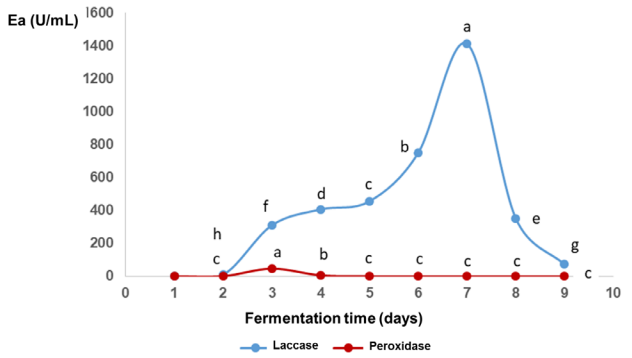

Figure 1. Kinetics of laccase (SE ±3.52; p<0.0001) and peroxidase (SE ±1.18; p<0.0001) production of the *C. kusanoi* L7 strain in submerged solid wheat bran medium. Ea: enzyme activity. Simple analysis of variance for each enzyme. Statistical package InfoStat (12)

Submerged solid fermentation of sugarcane bagasse. As in the case of submerged solid fermentation of wheat bran, the cellulolytic activity of the strains against sugarcane bagasse is also considerable. The results are showed in tables 4 and 5.

Regarding the production of ligninolytic enzymes, there was little laccase activity (Figure 2) and no activity for peroxidase enzymes.

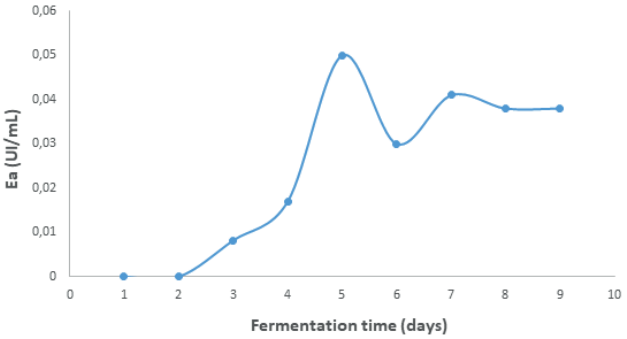

Figure 2. Laccase production of the *C. kusanoi* L7 strain in submerged solid fermentation of sugarcane bagasse. Ea: enzyme activity. (SE ±0.03; p=0.1861)

Table 4 CMCase (endo-1,4-β-glucanase) cellulolytic activity of *C. kusanoi* L7 and *T. pleuroticola* in submerged solid fermentation of sugarcane bagasse.

Strains/hours	Endo-1,4-β-glucanase enzyme activity (IU/mL)							SE and Signf.
	24	48	72	96	120	144	168	
C. kusanoi L7	2.73^{g}	2.06^{e}	2.36^{f}	1.85^{d}	1.14^{c}	1.04^{b}	0.93^{a}	±0.22 p=0.0004
T. pleuroticola	2.08^{d}	2.96^{g}	2.82^{f}	2.25^{e}	1.73^{c}	1.26^{b}	1.04^{a}	±0.31 p=0.0007

^{a,b,c,d,e} Means with different letters in each row differ at p<0.05 (14) ***p<0.001

Table 5. Cellulolytic PFase activity (exo-1,4-β-glucanase) of *C. kusanoi* L7 and *T. pleuroticola* in submerged solid fermentation of sugarcane bagasse

Strains/hours	Exo-1,4-β-glucanase enzyme activity (IU/mL)							SE and Signf.
	24	48	72	96	120	144	168	
C. kusanoi L7	0.261^{b}	0.802^{d}	0.511^{c}	0.007^{a}	0.007^{a}	0.003^{a}	0.0006^{a}	±0.36 p=0.0021
T. pleuroticola	0.750^{d}	1.691^{f}	1.180^{e}	0.472^{c}	0.056^{b}	0.006^{a}	0,0008^{a}	±0.52 p=0.0033

^{a,b,c,d,e,f} Means with different letters in each row differ at p<0.05 (14)

Evaluation of growth in co-culture. The comparisons at 5 and 10 days show the growth of *T. pleuroticola* on *C. kusanoi* L7, so it is inferred that there is no production of toxic or antagonistic metabolites for the growth of these species as observed in figure 3. It should also be noted that the *C. kusanoi* L7 strain is a dermatiaceous ascomycete characterized by rapid growth with abundant aerial mycelium, while *T. pleuroticola* shows slower mycelial growth with abundant sporulation. However, after 10 days, a joint growth of both strains was observed, occupying the entire surface of the plate.

Carbon mineralization of raw wheat straw. Figure 4 shows the carbon mineralization by the action of the lignocellulolytic fungus *C. kusanoi* L7, the cellulolytic fungus *T. pleuroticola* and their co-culture.

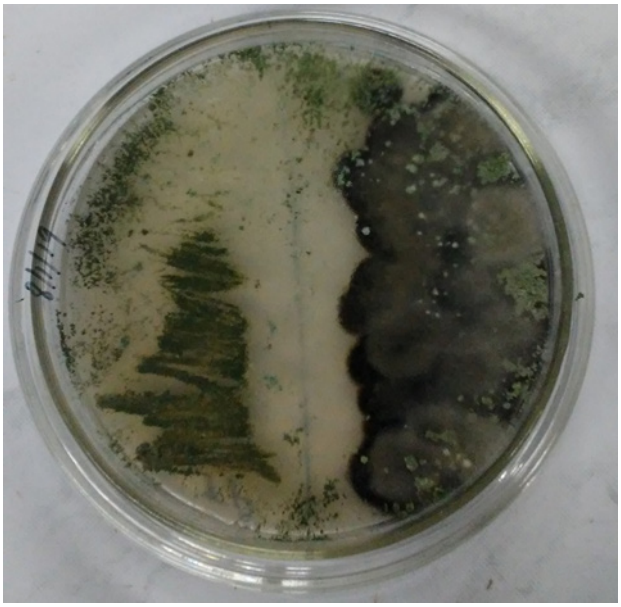

Figure 3. Microbial confrontation tests *C. kusanoi* L7 (on the right of the plate) - *T. pleuroticola* (on the left) after 5 days of growth in PDA medium at 30ºC.

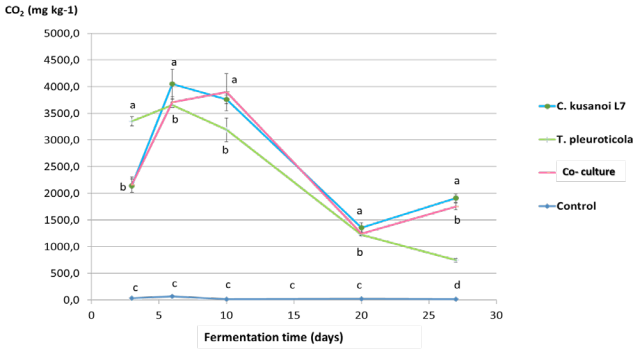

Figure 4. Carbon mineralization of raw wheat straw by strains *C. kusanoi* L7, *T. pleuroticola* and their co-culture. a,b,c,d: Different letters in the same fermentation time show significant differences p<0.05 (14). The significance was the same in all cases (p<0.0001). Day 3: SE±46.71, day 6: SE±73 .41, day 10: SE±93.49, day 20: SE±24.47, day 27: SE±23.95.

Carbon mineralization has a particular performance that fundamentally depends on the type of microorganism and the characteristics of the substrate. However, in the current study, both strains showed a similar performance during the first 6 days of growth. This period can be associated with the process of colonization of the substrate. It is necessary to point out that the *T. pleuroticola* strain colonizes the substrate more quickly, an aspect that is defined by the characteristics of this genus. From the tenth day of fermentation, a drastic decrease in CO_2 production was observed, which may be related to the decrease in the most easily accessible nutrients and the beginning of the degradation of more complex structures that are part of the cell wall. After 20 days of fermentation, the carbon mineralization of *T. pleuroticola* was lower than in the other treatments (p<0.0001). There were no differences in the results between the kinetics of *C. kusanoi* L7 and those of its co-culture with *T. pleuroticola*.

When comparing the infrared spectra (ATR-FT-IR) obtained from the carbon mineralization study of raw wheat straw by the fungi *C. kusanoi* L7, *T. pleuroticola* and their co-culture at the end of the study (27 days), it was observed that the treatments with *C. kusanoi* L7 and its co-culture with *T. pleuroticola* showed a higher decrease in the signals associated with lignin (Figure 5). Despite this being a semi-quantitative determination, the decrease in the intensity of the signals associated with lignin is clearly evidenced, an aspect that suggests the structural modification of this compound by enzymatic action.

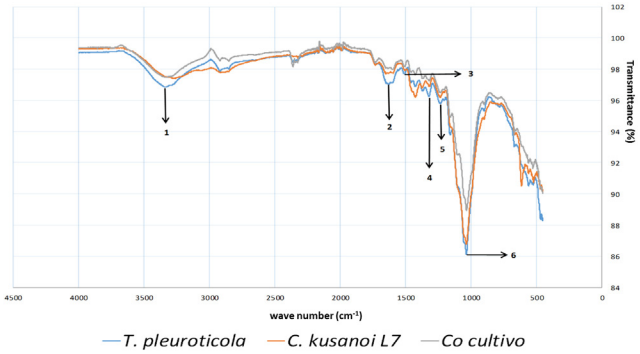

Figure 5. ATR-FT-IR spectrum of the samples resulting from the study of carbon mineralization of raw wheat straw by the strains *C. kusanoi* L7, *T. pleuroticola* and their co-culture in the zone of 4000 to 400 cm^{-1}. Numbers 1-6 correspond to the characteristic signals of lignin.

The signals that are numbered in Figure 5 (1. Band in the interval from 3400 to 3200 cm^{-1}, associated with the vibration of phenolic and aliphatic OH groups, 2. Band at 1610 cm^{-1} corresponding to the presence of aromatic Carbon-Carbon double bonds, 3. Band at 1510 cm^{-1} corresponding to the vibrations of the Carbon-Carbon bonds of lignin (aromatic and phenolic units), 4. Band at 1420 cm^{-1} corresponding to the phenylpropane skeleton of lignin, 5. Band at 1210 cm^{-1} associated with

the C–O bonds of the guaiacyl ring and 6. Band at 1035 cm^{-1} corresponding to the O-CH3 bonds of the guaiacyl and syringyl units of lignin), are characteristic bands of functional groupings present in the lignin molecule. The decrease in the intensity of these signals indicates their structural modification. This result corroborates previous approaches, where in treatments with *C. kusanoi* L7, the expression of laccase enzymes modifies the structure of lignin and allows a more exhaustive degradation of the plant wall.

DISCUSSION

The qualitative study of both strains showed growth capacity in a complex medium such as the noble medium, where the microorganisms must grow at the expense of the degradation of tannic acid as the only carbon source. According to Reid (16), this test shows the possible secretion of oxidase enzymes that allow the degradation of structures similar to lignin.

The kinetics of enzymatic production of both strains (Tables 2, 3, 4 and 5) both in wheat bran and in sugarcane bagasse reflected how these fungi are capable of producing the main enzymes of the cellulolytic complex. It is also important to point out that in both cases, the maximum activity is reached during the first days of fermentation. According to Valiño et al (17), candidate strains for enzyme production fermentation processes are those capable of expressing their maximum enzyme capacity during the first hours of fermentation, key aspects to reduce working time and optimize the enzyme production process. It is known that within the fungi with the highest cellulolytic capacity are the genera *Trichoderma*, *Aspergillus* and *Penicillium* (18). According to Vázquez et al (18), the cellulase production of the strains under study can be considered high, however, they are lower than what is reported for *T. viride* (M5-2) in sugarcane bagasse, which showed high cellulolytic activity after 24 hours and reached its maximum expression at 72 hours, reaching values for exoglucanase of 1.84 IU/g DM and 7.26 IU/g DM for endoglucanase as published by Valiño et al (17).

Regarding the production of enzymes that modify lignin, it is necessary to point out how *C. kusanoi* despite being an ascomycete fungus, its production of laccase enzymes are in the range of those reported for several species of basidiomycetes producers by excellence of this type of enzymes such as white rot fungi (19). The expression of laccase enzymes in the genus *Curvularia* is not well known. There are few reports in the literature, although some authors such as Bello et al (20) found values of laccase activity in *Curvularia lunata* similar to those obtained in this study. The findings of this research are a measure of the high potential that this genus may have in the degradation of recalcitrant compounds such as lignin. Another significant aspect is the pattern that this strain has in the kinetics of laccase enzyme production, like most of the lignolytic strains reported as producers of oxide reductases (21), *C. kusanoi* L7 also reaches its maximum laccase activity after 7 days of fermentation.

The *C. kusanoi* L7 strain not only showed high values of a ligninolytic activity, but also high production of cellulases, so that promising results can also be expected in the bioconversion processes of lignocellulosic residues.

The possibility of *C. kusanoi* L7 and *T. pleuroticola* to grow in co-culture on plates without presenting antagonistic traits and then efficiently mineralize the carbon from wheat straw in co-culture, shows a possible microbial consortium with great potential that should be tested against to other substrates since the high degradative capacity of these strains individually allows to predict that this consortium is effective in fiber degradation. The combination between the enzymes that degrade cellulose and those that degrade lignin is essential to achieve a more complete degradation of fibrous substrates (22). According to Singh et al (5), co-cultures of lignocellulolytic fungi are an effective solution to achieve a better and more complete degradation of the plant cell wall. For these reasons, the present result constitutes an interesting variant for biodegradation studies of fibrous sources destined for animal feeding. Despite the positive results discussed in the literature on the use of co-cultures for the degradation of different fibrous sources, there are very few studies in this regard in livestock sector. Despite this, Ghorai et al (23) and Medina et al (24) successfully reported the use of co-cultures of basidiomycete fungi in the biodegradation of different fibrous sources for ruminant feeding.

The use of co-cultures is an attractive option to improve the nutritional quality of alternative fibrous foods. Its use benefits above all, the

degradation of sources with high fiber content, improving the bioavailability of nutrients and allowing revaluation of this type of sources (25). The use of fungal consortia can lead to an increase in enzyme production and even contribute to the generation of more efficient degradative processes (26). Several microorganisms in situations of stress or nutritional limitations are capable of having beneficial relations with each other (27). It is known that in fungal co-cultures where there is variation of nutrients in the medium, the synergism of both microorganisms can be stimulated (26). In this regard, there are several studies where high enzymatic productions are found when using microbial consortia (28,29,30). Yang et al (30) showed a five-fold higher production of manganese peroxidase in the co-culture *Trametes sp*.-*Chaetomiun sp*. regarding the enzymatic production of each microorganism in a particular way. This study also showed in the co-culture, a significant increase (96%) in the percentage of biodegradation of triphenylmethane dyes.

Although the use of co-cultures has not spread considerably in the agricultural sector, its application at an industrial level is extensive, highlighting its use from obtaining biofuels to residues treatment and bioremediation. Undoubtedly, the techniques that correctly use microbial consortia are potential tools that improve the degradation processes of fiber and complex compounds with similar structures. Its implementation significantly contributes to the development of these sectors.

The biodegradation process of lignocellulosic substrates can be considered as a difficult phenomenon. Its degree of difficulty is closely related to the characteristics of the fiber and its content of lignin and structural carbohydrates. For these reasons, it can be stated that the CO_2 production of a microorganism is affected by the quality of the organic material that degrades (31).

Raw wheat straw is a substrate that exhibits, on average, 73% NDF, which is distributed as 38% cellulose, 25% hemicellulose, and 10% lignin (31). These aspects result in the microorganism needing the expression of certain enzymes that catalyze the degradation of these components, which results in a slower degradation rate. The researchers on this subject, agree that the values of carbon mineralization depend both on the type of substrate and on the characteristics of the microorganisms that degrade it (32).

When comparing the mineralization dynamics of the strains under study, the differences between the treatments with *C. kusanoi* and its co-culture with respect to the treatment with *T. pleuroticola*, exemplify how the microorganisms that are capable of producing a broader battery of lignocellulolytic enzymes, mineralize complex substrates high in fiber to a greater extent.

The FTIR evaluation of the final products of mineralization reaffirms the previous results. In the case of treatment with *T. pleuroticola*, the results of both studies show that there is no typical degradation of lignin. If it is desired to use this microorganism in plant fiber modification processes, it would be more convenient to combine it with ligninolytic microorganisms to achieve a more complete degradation, as was observed in the case of its co-culture with *C. kusanoi* L7.

The structural modification that was observed for the co-culture shows not only the degradation of functional groupings present in the structure of cellulose and hemicellulose, but also the decrease in the intensity of signals that are directly associated with the lignin structure. For these reasons, the microbial consortium for the biological pretreatment of fibrous sources needs an effective combination of cellulolytic and ligninolytic activity. These enzymatic interactions between strains are also reinforced by biological interactions that are established between species. Similar results are described for the ascomycete-basidiomycete combination evaluated by Dwivedi et al (33) with the *Penicillium oxalicum* and *Pleurotus ostreatus* strains.

In conclusion, the microbial consortium *C. kusanoi* L7-*T.pleuroticola* has great potential to be evaluated in the structural modification of fibrous sources of different composition. Hence, this preliminary study suggests this new variant of co-cultivation as a starting point for new researches that allows its efficient use in the use of high-fiber residues destined for animal feeding. In this regard, it is known the need to evaluate alternative raw materials that allow to replace imports, protect the environment and guarantee a source of animal food that does not compete with human food. Therefore, these results will allow, in long term, to develop more efficient, sustainable and ecological agricultural production.

Conflict of interests

The authors declare that there is no conflict of interest with the results of the research and the publication of this manuscript.

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