SCIENTIA AGRICOLIS VITA
Revista de Investigación Científica de la Facultad de Agronomía - UANL

Article

Utilization of agro-industrial wastes to obtain thermostable plant cell wall degrading enzymes using a co-culture of basidiomycetes.

Iosvany López-Sandin [1], Guadalupe Gutiérrez-Soto[1]*, Joel Horacio Elizondo Luevano[1], Roberto Parra Saldívar [1], Monserrat Franco Flores[1], Diana Castillo Martínez[1], Denisse Melissa Garza Hernandez[2]

[1] Facultad de Agronomía, Campus Ciencias Agropecuarias; Universidad Autónoma de Nuevo León; C.P. 66050, General Escobedo, Nuevo León; iosvany.lopezs@uanl.edu.mx (ILS), ggutierrez0402@gmail.com (GGS); joel.elizondolv@uanl.edu.mx (JHEL); ibqrps@gmail.com (RPS),
[2] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nuevo León, cisco Villa S/N, Col. Ex Hacienda el Canadá, C.P. 66050, General Escobedo, Nuevo León, México, dgarzahr@uanl.edu.mx (DMGH).
* Correspondenciaggutierrez0402@gmail.com

Abstract: The enzyme production of CMCases, avicellases, xylanases, amylases, and laccase in a co-culture of *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2 was evaluated in submerged culture media based on agro-industrial wastes. The results showed a synergy in the production of several enzymes, including CMCases, avicellases, xylanases, amylases, and laccases, when different combinations of substrates and supplements were used. Although only one condition for maximum enzyme production was expected to be found, the highest titers of amylases (445 Ul^{-1}) and β-D-glycosidase (1249 Ul^{-1}) were quantified in treatment 4. The highest titers of CMCases (1983 Ul^{-1}) were found in the Tx 5 medium, while the highest levels of avicellases were detected in the Tx1 medium (890 Ul^{-1}). The best xylanase production (837 Ul^{-1}) was found in the Tx8 treatment. Regarding laccase production, the best medium was Tx9 with 142 Ul^{-1}. All hydrolases showed a maximum activity at 70 °C, which they retained at 90 °C. These results allow us to conclude that the co-culture of *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2 is an alternative to obtain low-cost enzymatic adjuvants with promising applications in various industries.

Keywords: circular bioeconomy; citrus peel; valorization

1. Introduction

Basidiomycete co-cultures have emerged as a promising strategy for valorizing agricultural waste, as evidenced by various studies (Sarris et al., 2020). Under these growth conditions, the degradation and metabolization of substrates are enhanced by the joint metabolic activity of different microorganisms within the same culture (Bader et al., 2010). Unlike monocultures, co-cultures of fungi of different species are considered an alternative to enhance the production of metabolites and enzymes, and it is estimated that they can increase the yield and efficiency of obtaining enzymes (Brijwani et al., 2010).

The joint cultivation of two species of fungi in an environment with limited nutrients and specific environmental conditions favors their interaction, which can be antagonistic or synergistic depending on their nature. In antagonism, fungi produce metabolites that inhibit the other species' growth in the co-culture. In contrast, synergism is characterized by a positive interaction in which both fungi benefit from each other (Zhang et al., 2020b).

The evidence on the effectiveness of co-cultures underscores their simplicity and efficiency, as they do not necessitate complex genetic manipulations or the use of expensive inducing chemical reagents (Reyes et al., 2020). Moreover, the use of various fungal species in a single culture allows the obtaining of enzymatic extracts rich in several types of enzymes, such as cellulases, xylanases and lignin-modifying enzymes (da Silva et al., 2022).

Mixed culture fermentations also allow the use of inexpensive and impure substrates, a cost-effective approach. The inexpensive substrate, which is frequently a mixture of various lignocellulosic materials, is chosen in industrial practice. In biomass processing, a mixed culture that degrades both cellulose and starch and sugar is more efficient. The combination of cellulolytic fungi and starch- and sugar-consuming yeasts results in a more effective process, producing more products in less time (Harrison et al., 1978).

Using different fungal species in co-cultures can enhance the activity of various lignocellulosic enzymes, resulting in higher production. Gutiérrez et al. (1999) demonstrated that combining Trichoderma and Aspergillus species in-

creases the speed and degree of hydrolysis of the substrates on which they are grown, thus improving cellulolytic capacity. Fungi such as Pleurotus ostreatus have been shown to have good synergism with other basidiomycete fungi, increasing the production of la-cas in co-culture (Velma & Madamwar, 2002; Baldrian, 2004; Dwivedi et al., 2011). Based on the above, the present research evaluates the effect of co-cultivating *Trametes maxima* CI 1 and *Pycnoporus sanguineu*s CS2 on enzyme production using agro-industrial waste and the effect of temperature on enzyme activity

The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance. The current state of the research field should be carefully reviewed and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the principal conclusions. As far as possible, please keep the introduction comprehensible to scientists outside your field of research. References should be numbered in order of appearance and indicated by a numeral or numerals in square brackets—e.g., [1] or [2,3], or [4–6]. See the end of the document for further details on references.

2. Materials and Methods

2.1. Reagents.

All reagents and substrates used in this research were carefully selected and purchased from reputable suppliers. The reagents were from Sigma-Aldrich (USA), known for their high-quality products, and the culture media used was from MC de Lab (Mexico), a trusted source in the field.

2.2. Biological material and inoculum.

The fungal strains, *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2, chosen for their unique properties, were used in this study. These strains are preserved by periodic reseeding in potato dextrose agar (PDA) medium in the Natural Sciences laboratory of the Faculty of Agronomy.

To prepare the inoculum, a crucial step in the study, the strains were grown in 100 ml of potato dextrose broth medium at 28 °C for seven days. The sowing was done with two 0.5 cm diameter discs from a 5-day-old culture. In the case of co-culture treatments, one disc of each strain was added. The cultures were homogenized using an Oster® blender (Mexico) for three 30-second cycles.

2.2. Plant material and production medium.

The agro-industrial waste used in this study was carefully selected from businesses and industries in the metropolitan area of Monterrey. This waste was chosen for its potential as a sustainable resource. The treatments were established in a 60 mM potassium phosphate buffer solution at pH 6.0, supplemented with a 100x trace element solution. The agro-industrial substrates or combinations were added to the buffer solution, and the media were sterilized at 121 °C for 30 minutes for three consecutive days.

Table 1. Substrates used in mono and co-culture.

Treatment	Substrate(s)
Tx1	Wheat straw (0.5%)
Tx2	Wheat Straw (0.5%) + Orange Peel (5%)
Tx3	Orange Peel (5%)
Tx4	Wheat Straw (0.5%) + Grapefruit Peel (5%)
Tx5	Grapefruit peel (5%)
Tx6	Wheat Straw (0.5%) + Tangerine Peel (5%)
Tx7	Tangerine Peel (5%)
Tx8	Wheat Straw (0.5%) + Peanut Shell (5%)
Tx9	Peanut Shell (5%)

2.2. Enzyme activities

2.2.1. Laccase activity was determined following the method of Abadulla et al. (2000), using 2,6-dimethoxyphenol (DMP) as substrate. The reaction mixture consisted of 2 mM DMP in 200 mM sodium acetate adjusted to pH 3.5. The absorbance was measured at 468 nm in a Shimadzu 1800 UV-Vis spectrophotometer (Japan).

2.3.2. Carbohydrase activity

Carbohydrolase activity was determined using a comprehensive approach, employing the reducing sugar method reported by Miller (1959). The reaction mixture consisted of 500 µl of 50 mM sodium citrate buffer adjusted to pH 5.0, 300 µl of the substrate corresponding to 1% (w/v), and 200 µl of the sample. A range of substrates including starch, Avicel®, carboxymethylcellulose (CMC), D-(+)-cellobiose, xylan, and pectin were used to determine the enzymatic activities of amylases, avicelases, carboxymethylcellulases (CMCases), b-D-glucosidases, xylanases and pectinases, respectively. The reaction mixture was incubated at 60 °C for 15 minutes.

To calculate the enzyme units, 100 µl of the reaction mixture was taken at time 0 and at the end of the incubation. These aliquots were placed in tubes with 100 µl of the dinitrosalicylic acid (DNS) reagent to determine reducing sugars. The mixtures were boiled for 5 minutes and then placed in an ice bath for 5 minutes. 1 ml of double-distilled water was added before the spectrophotometric reading at 517 nm. The calibration curves were glucose, xylose, and D-galacturonic acid, ranging from 0 to 1 mg. One enzyme unit (U) was required to release 1 µmol of glucose, xylan, or D-galacturonic acid per minute. All determinations were performed in triplicate at 25 °C.

2.3 Effect of temperature on enzyme activity.

To evaluate the effect of temperature on the activity of carbohydrolases, the reducing sugar's reaction described in section 2.2 was used. This reaction was incubated at different temperatures (25, 50, 60, 60, 60, 70 and 80 °C) and all assays were performed in triplicate. The supernatants used were selected taking into account the highest number of enzymatic activities present.

2.4 Statistical analysis

The ANOVA test, a comprehensive statistical tool, was used to evaluate statistical differences in enzyme production at day 18th of growth, with a confidence level of 5%, using the SPSS Statistics statistical package. The test for comparison of means was applied using the Tukey method with a probability of 5%, ensuring a thorough analysis of the data.

3. Results

3.1. Enzyme production

Figure 1 shows the enzyme production curves for the co-culture of *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2 and the monoculture of *Trametes maxima* CU1. On day 18th, the co-culture exhibited the higher amylolytic activity in the Tx1, Tx2, Tx3, Tx4, Tx5 media, reaching values close to 400 U/l. In contrast, the monoculture of *Trametes maxima* CU1 reached its maximum production in the Tx4 medium on day 20, with levels below 300 U/l (Figures 1A and 1B). Regarding cellulase activity, viceless showed their highest titers in co-culture in the Tx1 medium on day 18, while monoculture reached its maximum in the Tx4 medium on day 20 (Figures 1C and 1D). CMCases showed the highest activity in co-culture in the Tx1 medium, while the Tx2 medium was the best for monoculture (Figures 1E and 1F). B-D-glucosidase showed similar behavior to the other cellulases, with co-culture reaching its maximum on day 18 and monoculture on day 20 in the Tx4 medium (Figure 1G and 1H). Xylanases in co-culture were detected in Tx6 and Tx8 media on days 10 and 20, respectively. The monoculture exhibited the highest activity in Tx1, Tx3, Tx4, and T9 media on days 16, 20, and 24, respectively (Figure 1I and 1J). Tx4 and Tx3 media showed the highest pectinase activities in co-culture and monoculture at days 18 and 20, respectively (Figure 1K and 1L).

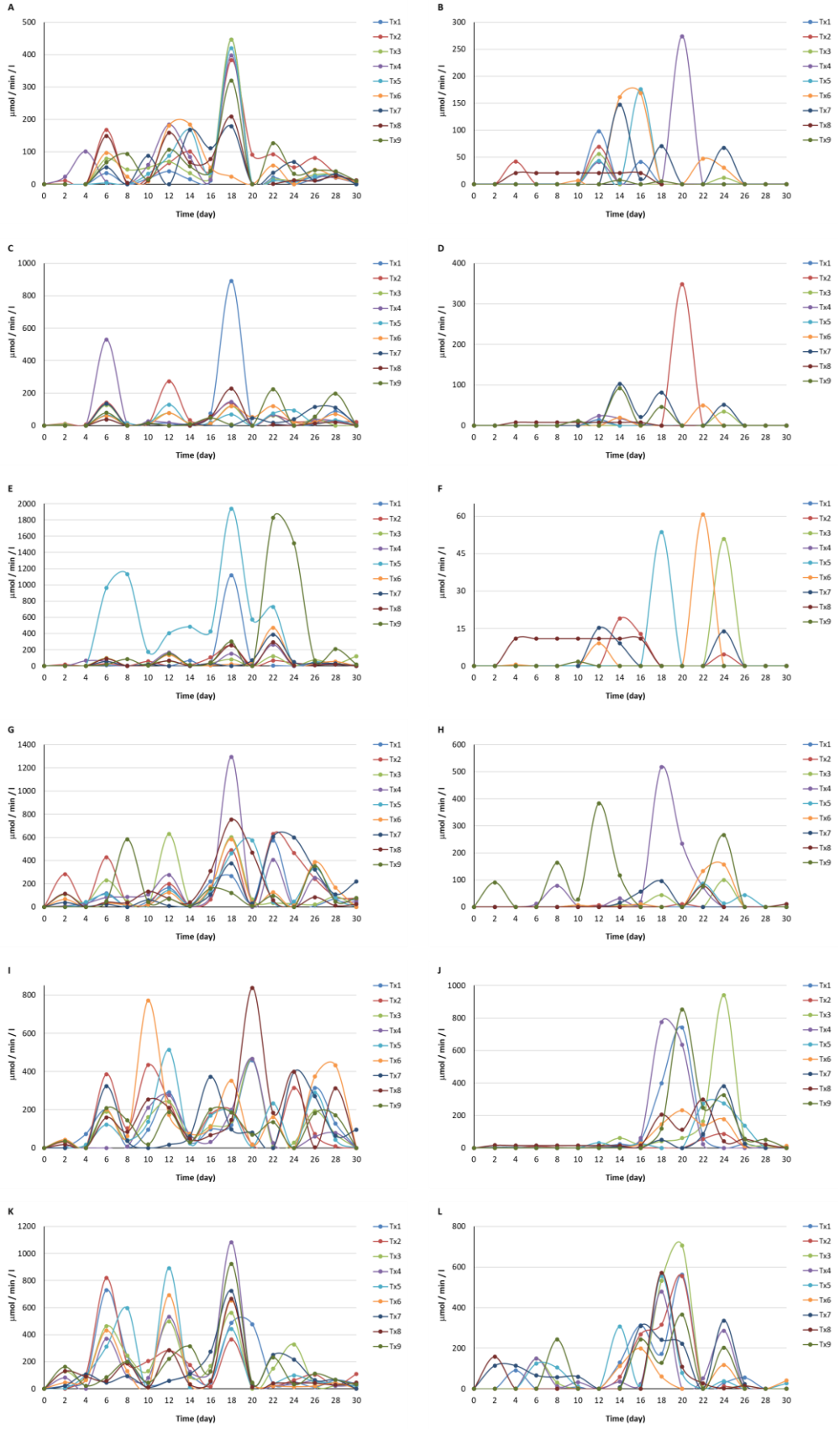

Figure 1. Enzyme production in agro-industrial wastes. Where A) corresponds to amylases, C) Avicellases, E) CM cases, G) β-D-glucosidases, I) xylanases and K) pectinases under co-culture conditions, while B) corresponds to amylases, D) Avicellases, F) CM Casas, H) ⍟-D-glucosidases, J) xylanases and L) pectinases from *Trametes maxima* CU1 in monoculture. Assays were performed in triplicate and the standard deviation was less than 5% between replicates.

3.2. Comparison between co-culture and monoculture

In general, the co-culture of *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2 presented higher levels of enzymatic activity, at 18 days of growth, while the monoculture reached its production peaks at day 20th for most activities. However, no medium was observed where all enzymatic activities were expressed under monoculture and co-culture conditions. Furthermore, the kinetic behavior was different in all media for each activity. When analyzing the maximum levels of the enzymes determined in the co-culture and the monoculture, significant differences were observed between both treatments in the different culture media. In the case of amylase, the Tx1, Tx3, and Tx5 media showed titers higher than 400 U/l in the co-culture, while the monoculture did not exceed 300 U/l (Figure 1A). This difference in enzyme production represents a significant increase in the co-culture compared to the monoculture. The highest cellulase activity in the co-culture was observed with CMCase, reaching 1938 U/l in the Tx5 medium. This was followed by b-D-glucosidase in the Tx5 medium (1294 U/l) and avicelases in the Tx1 medium (890 U/l). These levels represented an increase in the co-culture compared to the monoculture: 36-fold for CMCase (maximum levels in Tx6), 2.5-fold for b-D-glucosidase (Tx4), and 2.3-fold for avicelases (Tx2) (Figure 2B-D). Unlike amylase and cellulase activities, xylanase activity showed higher levels in the monoculture compared to the co-culture. Tx1, Tx3, Tx4, and Tx9 media from the monoculture exhibited an average production of 830 U/l (Figure 2E), like the average levels in both co-culture media (802 U/l). The highest pectinolytic activity was observed in the Tx4 medium of the co-culture, with 1082 U/l, compared to 705 U/l in the Tx3 medium of the monoculture (Figure 2F). This difference also represents a rise in the enzyme production of the co-culture.

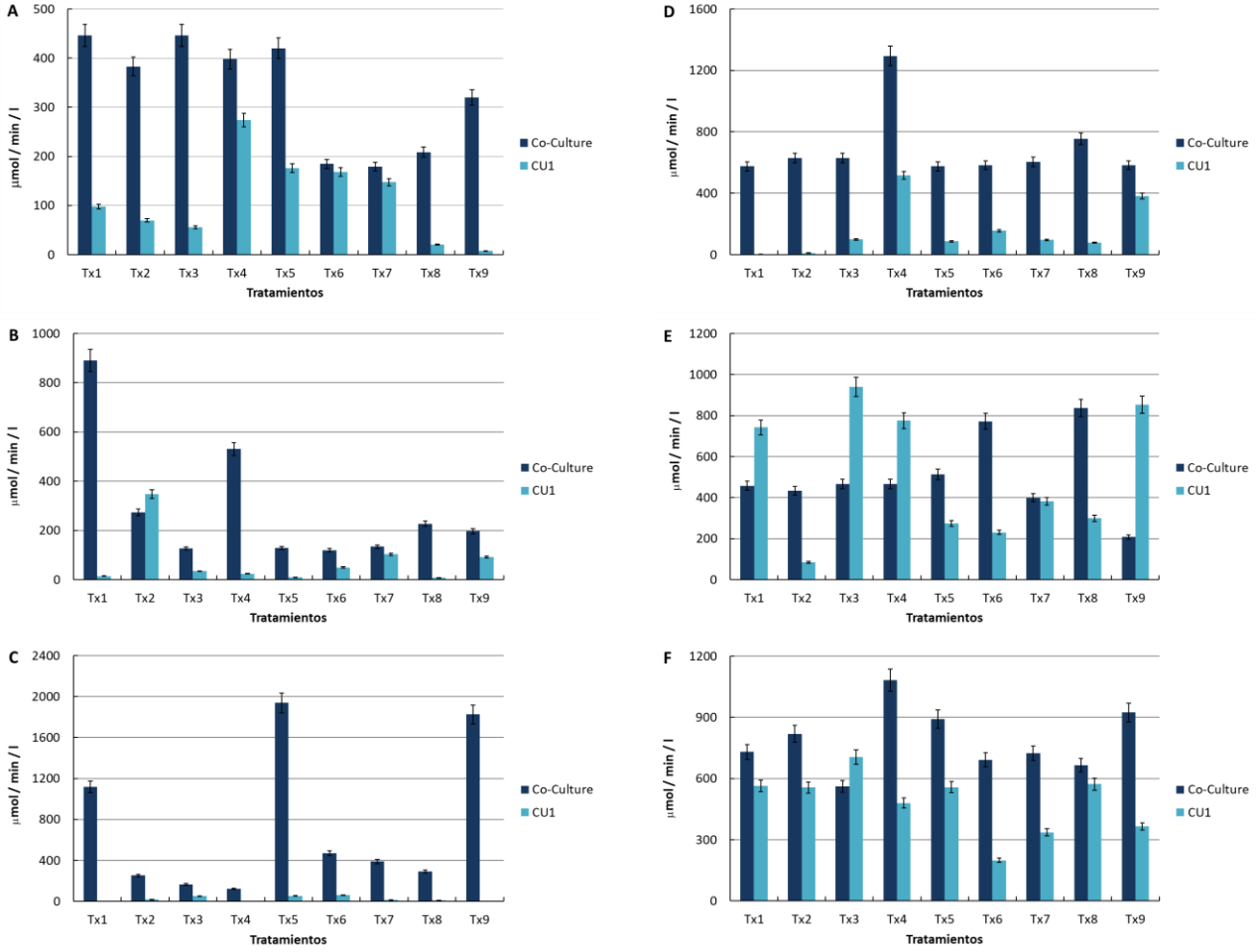

Figure 2. Enzyme production. Where A) corresponds to amylases, B) Avicelases, C) CM cases, D) β-D-glucosidases, E) xylanases and F) pectinases. All treatments showed statistically significant differences ($p \leqslant 0.05$).

Figure 3 presents the kinetics of laccase production in the co-culture of *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2, and the monoculture of *Trametes maxima* CU1. The co-culture consistently maintained higher laccase production levels, a finding that has significant implications for biotechnology and microbiology research. The first 16 days saw similar behavior in both treatments, but with significantly higher production levels in the co-culture. From day 18th, the monoculture treatments experienced a gradual decline in laccase activity, while the co-culture continued to produce the enzyme at a constant rate. In both cases, laccase production was maintained after day 20th, and no significant changes were observed at the end of the experiment.

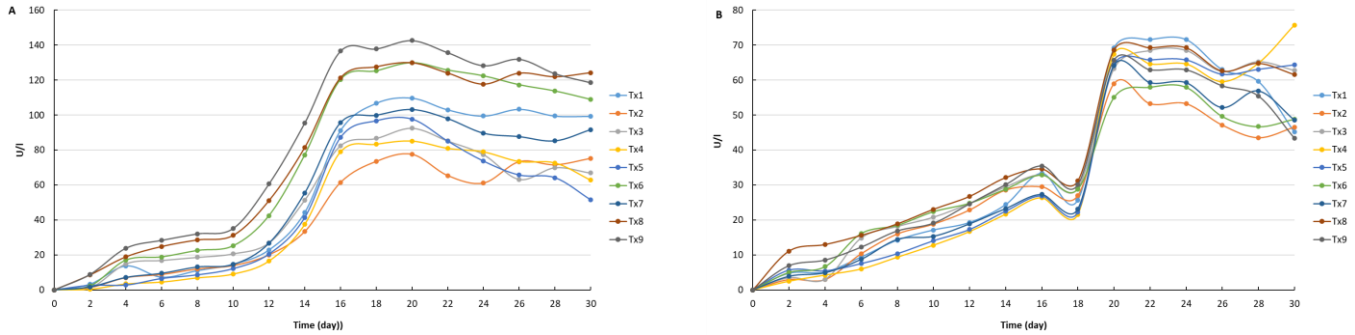

Figure 3. Kinetics of laccase production in co-culture (A) and in monoculture (B).

3.2. Efecto de la temperatura sobre la actividad enzimática de carbohidrolasas.

Trametes maxima CU1 has shown significant potential in several biotechnological applications due to its robust enzymatic profile. As mentioned above, under the conditions studied, it was able to produce amylases, cellulases, xylanases, pectinases, and laccases. The latter has been reported as a thermotolerant enzyme (Gutiérrez Soto. 2009). Therefore, the effect of temperature on the detected carbohydrase activities was evaluated since the laccase produced by *Pycnoporus sanguineus* CS2 is considered thermostable (Martínez-Salcedo et al., 2015; Gutiérrez Soto et al., 2015).

To determine the effect of temperature on the enzymes present, the supernatants of the Tx1, Tx2, and Tx3 media were selected since they presented most of the enzymes at elevated levels. Figure 4 shows that the co-culture supernatants showed a significant change in absorbance, which the higher concentration of enzymes could explain. However, xylanases, although showing the highest titers in monoculture compared to co-culture, did not present a significant increase in absorbance. Therefore, the increase in activity with increasing temperature could be attributed to the production of thermostable enzymes by *Pycnoporus sanguineus* CS2.

It is noteworthy that the supernatant of co-culture Tx2 showed the highest enzymatic activity at 70°C, while the supernatant of monoculture Tx3 presented the highest change in absorbance (although lower than co-culture) also at 70°C, demonstrating the thermotolerance of *Trametes maxima* CU1 enzymes. Consequently, combining these strains to recover cocktails of thermostable amylases, cellulases, xylanases, pectinases, and laccases has excellent potential for industrial applications.

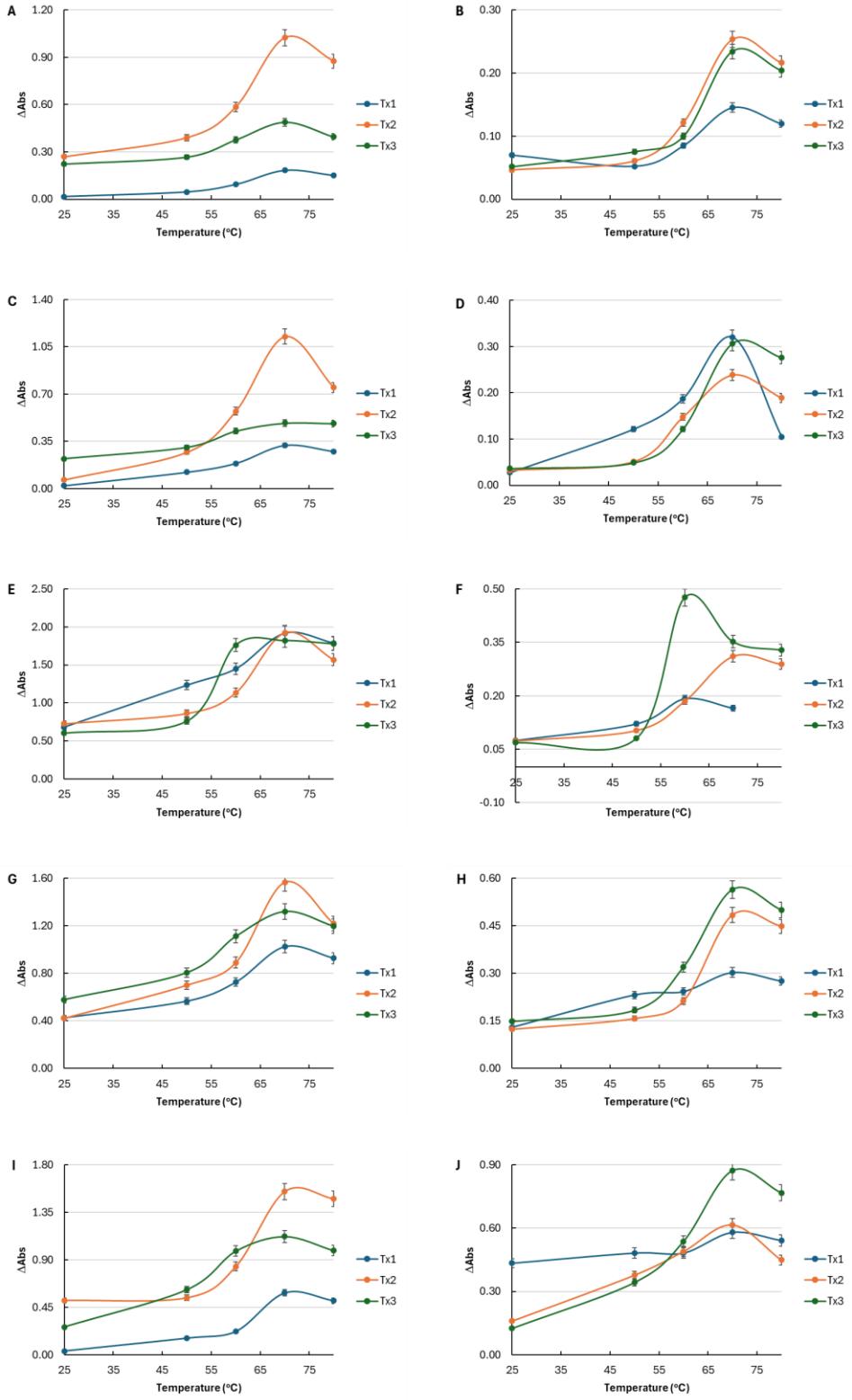

Figure 4. Effect of temperature on carbohydrolases. Where A, C, E, G and I correspond to amylases, Avicellases, CMCases, ⍟-D-glucosidases, xylanases and pectinases of the co-culture respectively, while B, D, F, H and J correspond to the monoculture activities. All treatments showed statistically significant differences ($p \leqslant 0.05$).

4. Discussion

Evaluating the co-culture of *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2 versus the monoculture of *T. maxima* CU1 for enzyme production on low-cost agro-industrial substrates revealed promising results. Co-culture induced higher cellulolytic activity on various substrates, including orange peel, wheat straw, and peanut shell. This synergistic approach increased cellulase, xylanase, and laccase enzyme production. The enzymatic activity observed in co-culture was significantly higher than in monoculture on most substrates evaluated. The synergy observed in co-culture could be attributed to several mechanisms (Zhang et al., 2020a). The interaction between fungal strains could stimulate enzyme production through the secretion of inducing metabolites or competition for nutrients (Dullah et al., 2021). Furthermore, co-culture could favor the degradation of lignin, a recalcitrant biomass component, allowing greater access to cellulose and hemicellulose for cellulolytic enzymes (Asiegbu et al., 1996). It is worth mentioning that enzyme production in co-culture was affected by several factors such as substrate type, humidity, temperature, pH, the concentration of additional nutrients, and fermentation time (Borchers et al., 2013; Fang & Xia, 2013; Abdu et al., 2022). Co-culture compatibility may also depend on the time and amount of inoculum, considered critical factors (Hibbing et al., 2010). The choice of substrate had a significant impact on enzyme production. In this sense, with its low lignin content, orange peel was a favorable substrate for pectinase production. In contrast, with its high cellulose content, wheat straw induced a higher production of cellulases.

In general, the kinetics of enzyme production revealed distinctive patterns for each enzyme and treatment. In the case of amylase and cellulase production, it reached its maximum during the first 0-20 days, followed by a gradual decrease. This behavior has been attributed to the initial depletion of soluble sugars and the repression of enzyme production promoter genes (Gutiérrez-Rojas et al., 2015; Nazir et al., 2010). On the other hand, laccase production showed a continuous increase during the first 16 days, followed by a plateau in co-culture and a slight decrease in monoculture. This behavior could be related to the gradual degradation of lignin and the release of aromatic compounds that induce laccase production (Valmaseda et al., 1991; Sharma & Arora, 2010).

Concerning thermotolerance, the results observed in the *Trametes maxima* CU1 monoculture were consistent with the findings of other studies, where *Trametes* strains have been shown to produce enzymes with high activity and stability over a range of temperatures and pH levels, making them suitable for industrial applications (Ado et al., 20218). It is worth mentioning that analysis of the secretome of *Trametes hirsuta* has revealed a diverse array of extracellular enzymes, including ligninolytic, cellulolytic, and hemicellulolytic enzymes, which varied significantly with growth conditions, indicating the adaptability of *Trametes* species to different substrates and environments (Vasina et al., 2016). In addition, it will explain the differences in the levels and activities present in the different media used. On the other hand, results with other co-cultures have shown greater thermostability than a monoculture. For example, the co-culture of halophilic bacteria *Marinirhabdus* sp. and *Marinobacter hydrocarbonoclasticus* demonstrated significantly enhanced protease activity and stability over a wide range of environmental conditions, including temperatures from 25 °C to 60 °C, compared to monocultures (Anh et al., 2021), which explains the higher activity in the co-culture *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2.

Finally, the results of this study demonstrate the potential of co-cultivation of *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2 for efficient and economical enzyme production from low-cost agro-industrial substrates. The synergy observed in the co-cultivation could lead to faster and more robust development of biotechnological processes for biomass degradation and obtaining high value bioproducts.

5. Conclusions

The results of this study demonstrate the synergistic potential of the co-culture of *Trametes maxima* CU1 and *Pycnoporus sanguineus* CS2 to produce a broad spectrum of cellulolytic and hemicellulolytic enzymes. The combination of both strains significantly increased enzyme activity, especially in the degradation of complex lignocellulosic substrates. The thermotolerance of the co-culture enzymes and their ability to act on various substrates suggests a wide range of industrial applications. These findings highlight the importance of exploring co-cultivation strategies to optimize enzyme production and promote the development of more efficient and sustainable biotechnological processes for converting biomass into high-value-added products.

6. Acknowledgments

The authors acknowledge the support received from the Universidad Autónoma de Nuevo León.

7. Conflicts of Interest:

The authors declare that they have no conflicts of interest.

References

1. Abadulla, E., Tzanov, T., Costa, S., Robra, K.H., Cavaco-Paulo, A. and Gübitz, G.M., 2000. Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta*. *Appl Environ Microbiol*, *66*, 3357-3362.
2. Abduh, M.Y., Ramadhan, C., Fadhlilah, A.P., Abdul, S.D., & Burhan, K.H. (2022). Solid-state fermentation of groundnut (Arachis hypogaea) shell using *Trichoderma* sp., tape yeast, and tempeh yeast to produce cellulase. Journal of Applied Biology & Biotechnology.Vol. 10(4), pp. 153-160. DOI: 10.7324/JABB.2022.100421.
3. Ado, B. V., Amande, T. J., Ebah, E. E., & Mabitine, D. M. (2018). Screening, production and partial characterization of a thermostable laccase from *Trametes* sp. isolate B7 with biotechnological potentials. *Biotechnology Journal International*, *22*(4), 1-16.
4. Anh, H. T. H., Shahsavari, E., Bott, N. J., & Ball, A. S. (2021). Application of co-culture technology to enhance protease production by two halophilic bacteria, Marinirhabdus sp. and Marinobacter hydrocarbonoclasticus. *Molecules*, *26*(11), 3141.
5. Asiegbu, F. O., Paterson, A., & Smith, J. E. (1996). The effects of co-fungal cultures and supplementation with carbohydrate adjuncts on lignin biodegradation and substrate digestibility. *World Journal of Microbiology and Biotechnology*, *12*, 273-279.
6. Bader, J., Mast-Gerlach, E., Popović, M. K., Bajpai, R., & Stahl, U. (2010). Relevance of microbial coculture fermentations in biotechnology. *Journal of applied microbiology*, *109*(2), 371-387.
7. Baldrian, P. (2004). Increase of laccase activity during interspecific interactions of white-rot fungi. *FEMS microbiology ecology*, *50*(3), 245-253.
8. Borchers, S., Freund, S., Rath, A., Streif, S., Reichl, U., & Findeisen, R. (2013). Identification of growth phases and influencing factors in cultivations with AGE1. HN cells using set-based methods. PLoS One, 8(8), e68124.
9. Brijwani, K., Oberoi, H. S., & Vadlani, P. V. (2010). Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. *Process Biochemistry*, *45*(1), 120-128.
10. da Silva, Y. H., de Oliveira, T. B., Lima, M. S., Pasin, T. M., de Almeida Scarcella, A. S., de Moraes, M. D. L. T., ... & de Lucas, R. C. (2022). Co-Culture of *Trichoderma reesei*, *Talaromyces* sp. and Aspergillus spp. Produces A Multi-Enzyme Cocktail for the Hydrolysis of Sugarcane Bagasse Pretreated with Piperonilic Acid (PIP) and Methylenedioxycinnamic Acid (MDCA).
11. Dullah, S., Hazarika, D. J., Parveen, A., Kakoti, M., Borgohain, T., Gautom, T., ... & Boro, R. C. (2021). Fungal interactions induce changes in hyphal morphology and enzyme production. *Mycology*, *12*(4), 279-295.
12. Dwivedi Brijwani, K., Oberoi, H. S., & Vadlani, P. V. (2010). Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. *Process Biochemistry*, *45*(1), 120-128.
13. Fang, H., & Xia, L. (2013). High activity cellulase production by recombinant Trichoderma reesei ZU-02 with the enhanced cellobiohydrolase production. *Bioresource technology*, *144*, 693-697.
14. Gutiérrez Soto, J. G. (2009). *Purificación y caracterización parcial de las lacasas de Trametes maxima CU1 y su aplicación en la degradación de colorantes textiles* (Doctoral dissertation, Universidad Autónoma de Nuevo León).
15. Gutiérrez Soto, J. G., Medina González, G. E., García Zambrano, E. A., Treviño Ramírez, J. E., & Hernández Luna, C. E. (2015). Selection and characterization of a native *Pycnoporus sanguineus* strain as a lignocellulolytic extract producer from submerged cultures of various agroindustrial wastes. *BioResources*, *10*(2).
16. Gutiérrez-Rojas, I., Moreno-Sarmiento, N., & Montoya, D. (2015). Mecanismos y regulación de la hidrólisis enzimática de celulosa en hongos filamentosos: casos clásicos y nuevos modelos. Revista Iberoamericana de Micología, 32(1), 1-12
17. Hibbing, M. E., Fuqua, C., Parsek, M. R., & Peterson, S. B. (2010). Bacterial competition: surviving and thriving in the microbial jungle. Nature reviews microbiology, 8(1), 15-25.
18. Javed, M. M., & Khan, T. S. (2006). An innovative approach for hyperproduction of cellulolytic and hemicellulolytic enzymes by consortium of *Aspergillus niger* MSK-7 and *Trichoderma viride* MSK-10. *African journal of Biotechnology*, *5*(8), 609-614.
19. Martínez, S. M. S., Gutiérrez-Soto, G., Garza, C. F. R., Galván, T. J. V., Cordero, J. F. C., & Luna, C. E. H. (2013). Purification and partial characterization of a thermostable laccase from *Pycnoporus sanguineus* CS-2 with ability to oxidize high redox potential substrates and recalcitrant dyes. *Applied Bioremediation-Active and Passive Approaches*, *351*.
20. Nazir, A., Soni, R., Saini, H. S., Kaur, A., & Chadha, B. S. (2010). Profiling differential expression of cellulases and metabolite footprints in *Aspergillus terreus*. Applied biochemistry and biotechnology, 162, 538-547
21. Reyes-Calderón, A., Garcia-Luquillas, K. R., Ludeña, Y., Hernández-Macedo, M. L., Villena, G. K., & Samolski, I. (2020). A simple and accurate method for specific quantification of biomass in mixed cultures of filamentous fungi by quantitative PCR. *Revista peruana de biología*, *27*(1), 085-090.
22. Sarris, D., Philippoussis, A., Mallouchos, A., & Diamantopoulou, P. (2020). Valorization of low-cost, carbon-rich substrates by edible ascomycetes and basidiomycetes grown on liquid cultures. *FEMS microbiology letters*, *367*(20), fnaa168.

23. Sharma, R. K., & Arora, D. S. (2010). Production of lignocellulolytic enzymes and enhancement of in vitro digestibility during solid state fermentation of wheat straw by *Phlebia floridensis*. Bioresource Technology, 101(23), 9248-9253.
24. Valmaseda, M., Martínez, M. J., & Martinez, A. T. (1991). Kinetics of wheat straw solid-state fermentation with *Trametes versicolor* and *Pleurotus ostreatus*—lignin and polysaccharide alteration and production of related enzymatic activities. Applied microbiology and biotechnology, 35, 817-823.
25. Vasina, D. V., Pavlov, A. R., & Koroleva, O. V. (2016). Extracellular proteins of *Trametes hirsuta* s t. 072 induced by copper ions and a lignocellulose substrate. *BMC microbiology*, *16*, 1-14.
26. Zhang, J., Ke, W., & Chen, H. (2020a). Enhancing laccase production by white-rot fungus *Trametes hirsuta* SSM-3 in co-culture with yeast sporidiobolus pararoseus SSM-8. *Preparative Biochemistry & Biotechnology*, *50*(1), 10-17.
27. Zhang, Q., Zhao, L., Li, Y., Wang, F., Li, S., Shi, G., & Ding, Z. (2020b). Comparative transcriptomics and transcriptional regulation analysis of enhanced laccase production induced by co-culture of *Pleurotus eryngii* var. ferulae with *Rhodotorula mucilaginosa*. *Applied microbiology and biotechnology*, *104*, 241-255.

Citation: López-Sandin, I., Gutiérrez-Soto, G., Elizondo Luevano, J. H., Parra Saldívar , R., Franco Flores, M., Castillo Martínez1, D., & Garza Hernandez, D. M. (2024). Aprovechamiento de residuos agroindustriales para la obten-ción de enzimas termoestables degradadoras de pared celular vegetal utilizando un co-cultivo de basidiomicetos . *Scientia Agricolis Vita*, *1*(2), 12–21. https://doi.org/10.29105/agricolis.v1i2.18

Received: date
Revised: date
Accepted: date
Published: 31-05-2024