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Fermentation of Residual Wheat Straw by *Penicillium chrysogenum and Streptomyces griseus* Generates Phytohormones

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Abstract

Residual lignin from wheat straw (RELIWS) is burned, that causes environmental pollution; as well as global warming by releasing greenhouse gases, an ecological alternative is its microbial by double fermentation by monosporic common fungi and native actinomycete that are able to transform it to phytohormones. The objectives of this work were: i) depolymerization of RELIWS by Penicillium chrysogenum, b) conversion of aromatics of RELIWS into phytohormones by Streptomyces griseus and c) effect of transformed REWSLI into phytohormones of S. griseus on the primordium growth of Phaseolus vuglaris (bean). In that se, the RELIWS was extracted and depolymerized by P. chrysogenum, the depolymerized RELIWS broth was inoculated with S. griseus, the conversion into phytohormone on the growth of P. vuglaris was analyzed using the response variables: days to emergence, in the stem and root primordium phenology: height and length of root in biomass: aerial fresh/dry weight (AFW/RFW), fresh/dry weight (ADW/RDW). The experimental data were analyzed by ANOVA/Tukey HDS in Statgraphics Centurion. According to the results, S. griseus transformed aromatics from the depolymerization of RELIWS by P. chrysogenum into a phytohormone that caused a positive effect on the phenology and biomass of P. vulgaris primordia, compared to the response of fed P. vulgaris. with a 100% mineral solution and pure gibberellin. It was demonstrated that through double fermentation it is possible to convert RELIWS from P. chrysogenum into a base to transform it from S. griseus into a phytohormone similar to gibberellin. Ongoing research will define what type of phytohormone it is. Although this double fermentation proves the potential of both microorganisms to give added value to RELIWS, a natural resource considered agricultural waste that causes environmental problems, when burned it increases greenhouse gases. It is concluded that microbial potential is an environmental aid tool to prevent global warming.

Keywords: Actinomycete, gibberellin, Lignin, Phytohormone, Soil Monosporic Fungus,

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INTRODUCTION

A byproduct of wheat crops is wheat straw, of that an approximate production of 1,105 million tons is estimated. In addition, 55% of the wheat crop is grain and the rest is waste, contrary to what is believed, farmers believe that it is garbage [12], subjecting it to incineration "in situ" that causes a harmful environmental impact [3]. Wheat straw is distributed in 38% cellulose, 25% hemicellulose, 8% lignin and 0.2% cutin [4]. Residual lignin from wheat straw (RELIWS), is recalcitrant due to the complexity of its chemical structure, which mainly consists of three types of repeating units: coumaryl, guaiacil and syringyl [5]. RELIWS is usable if it is degraded, but not by chemical synthesis due to its high value and its byproducts that contaminate the

environment [6]. An alternative is the mitosporic fungi, *Penicillium chrysogenum* and *Aspergillus fumigatus*, which have the ability to depolymerize RELIWS in a relatively short time [7]. The depolymerization of RELIWS involves a group of extracellular enzymes: lignin-peroxidase, laccase and manganese-peroxidase. Lignin does not contain hydrolysable bonds, so the enzymes involved in depolymerization are oxidative [8,9]. Furthermore, lignin is stereo-irregular which implies that enzymatic attacks are not specific [10]. Laccase is a phenol oxidase enzyme with copper; therefore, it catalyzes the oxidation of RELIWS, into orthophenol, paraphenol, aminophenol, polyphenol, polyamine, aryl-diamine with reduction of O_2 to H_2O [5,8,10], this enzyme exists in basidiomycetes, mitosporic fungi, higher plants, insects and some bacterial genera [7,10, 11]. While one mechanism for the conversion of RELIWS into phytohormones is to use the genus *Azotobacter armeniacus* phythormones [12,13,14). Also, in the supernatant of an *A. chroococcum* culture some unidentified derivatives of auxins and gibberellin were detected; aspect still unknown in species of the genus actinomycetes such as *S. griseus* [13,15-17]. Therefore, the objectives of this work were: i) depolymerization of RELIWS by *P. chrysogenum*; ii) conversion of RELIWS aromatics into phytohormones by *S. griseus*; iii) effect of RELIWS transformed into *S. griseus* phytohormone on the phenology and biomass of the primordium of *P. vuglaris*.

Materials and Methods

Activation of *P. chrysogenum*

It was used from the collection of the Environmental Microbiology Laboratory of the Chemical-Biological Research Institute of the UMSNH. RELIWS was extracted from dry wheat straw, ground and sieved on a 0.0841 mm mesh, 10% acetic acid was added in a 1:2 (w/v) ratio, and it was neutralized with 10% NaOH (w/v), it was sterilized at 120°C/60 min. and dried at 70°C/24 h. *P. chrysogenum* was activated on RELIWS-agar [1].

Kinetics of RELIWS depolymerization by P. chrysogenum

To separate the mycelia of *P. chrysogenum* from the RELIWS-agar in a 15.0 mL Petri dish. of sterile saline-detergent solution, removed with a loop and recovered with a pipette. 12.5 mL were inoculated of *P. chrysogenum* in 500.0 mL Erlenmeyer flasks. with 250.0 mL of RELIWS broth, were incubated on a rotary shaker for 1 week, at 30°C and 150 rpm. A sample was taken at week 1 to measure Laccase activity; of the depolymerization of RELIWS [11,16].

Determination of Laccase activity

The RELIWS broth depolymerized by *P. chrysogenum* was centrifuged at 8000 rpm at 4°C/15 min. to eliminate the mycelium. Laccase activity was measured in a spectrophotometer by the oxidation of 2,2'-acyno-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at 420 nm, 25°C at 0 minute, at 3 minutes and at 5 minutes, with a molar extinction coefficient of ε420=3600 M-1 cm-1; A reaction mixture was prepared with 2.4 mL. of 25 mM sodium acetate buffer, pH 3.0, 300.0 µL of 10 mM ABTS and 300.0 µL of the sample [1,11]. To determine the laccase units, the following equation was used [1,15,16].

$$UL^{-1} = \frac{A(1x106)(Vt)(C)}{t(\varepsilon)(Vm)}$$

- C = Cell size (1.0 cm)
- Ɛ = Molar extinction coefficient of ABTS
- Vm = Sample volume (mL.)
- Vt = Total volume of the reaction (mL.)
- t = Reaction time (min.)
- A= Δ Abs 420 nm = Final Abs - Initial Abs

Conversion of RELIWS depolymerization into phytohormones by *S. griseus*

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The genus *S. griseus* belongs to the collection of the Environmental Microbiology Laboratory. To activate *S. griseus*, it was cultured in Oat Agar [11,15] and subsequently each isolate was seeded in depolymerized RELIWS broth, previously transformed by: *P. chrysogenum*, the transformation of each depolymerization was centrifuged at 8000 rpm/15 min, then adjusted with casein peptone, yeast extract, glucose and $CuSO_4$ $5H_2O$, the pH was adjusted to 6.8 - 7.0 and sterilized at 121°C/20 min. *S. griseus* was grown in the supernatant of the RELIWS broth for 12 days, at 30°C/150 rpm, samples were taken on days 6 and 12; After the depolymerized transformation of RELIWS by *S. griseus* into phytohormone, it was centrifuged at 10,000 at 8°C/20 rpm/20 min., the supernatant was frozen to perform the corresponding bioassay on *P. vulgaris* seeds [1].

Effect of RELIWS transformed into phytohormones by *S. griseus* on the phenology and biomass of the primordium of *P. vuglaris.*

The *P. vulgaris* seeds were disinfected with sodium hypochlorite for 10 minutes, rinsed with sterile water 6 times, then 5 minutes with 70% ethanol and rinsed 6 times with sterile water. 6 treatments were used with 2 controls: AC or absolute control of *P. vulgaris* seeds irrigated only with water; RC or relative control seeds fed with a 100% mineral solution recommended for *P. vulgaris*; T1 *P. vulgaris* seeds treated with 0.01 ml/4 seeds of S. griseus cell-free filtrate at 6 days of conversion, T2 *P. vulgaris* seeds with 0.01 of *S. griseus* cell-free filtrate 6 days of conversion, T3 seeds of *P. vulgaris* with 0.1 ml of 6-day-old *S. griseus* cell-free filtrate, T4 *P. vulgaris* seeds with 0.01 ml of 12-day-old S. griseus cell-free filtrate, T5 *P. vulgaris* seeds with 0.1ml 12-day *S. griseus* cell-free filtrate. T6 *P. vulgaris* seeds with 0.01 ml of pure gibberellin, T7 *P. vulgaris* seeds with 0.1 pure gibberellin, with 5 repetitions per treatment, subsequently, the supernatants of the transformed *S. griseus* isolates into phythormones were applied with two doses: 0.1mL. and 0.01mL/*P. vulgaris* seed. It was placed in sterile Petri dishes on a cotton bed moistened with sterile distilled water as a support, covered by sterile filter paper. The seeds as an absolute control were irrigated with distilled water and as a relative control the seeds were fed with mineral solution at 100%.The seeds were left in a solarium in the dark for 5 days, when seeds germinated seeds were left in a solarium for another 10 - 15 days, after this time the following was measured: phenology of primordium of *P. vulgaris*: plant height, root length; biomass: aerial fresh weight, radical fresh weight, then the aerial and radical part was dried (80°C/24 h.) in an oven, to obtain the aerial and radical dry weight [1,14].The experimental data were analyzed by ANOVA/Tukey with a significance level α of 0.05 using the Statgraphics Centurion 16.103 ® program [18].

Result and Discussion

Laccase enzymatic activity in wheat straw lignin by *P. chrysogenum* for conversion to aromatics

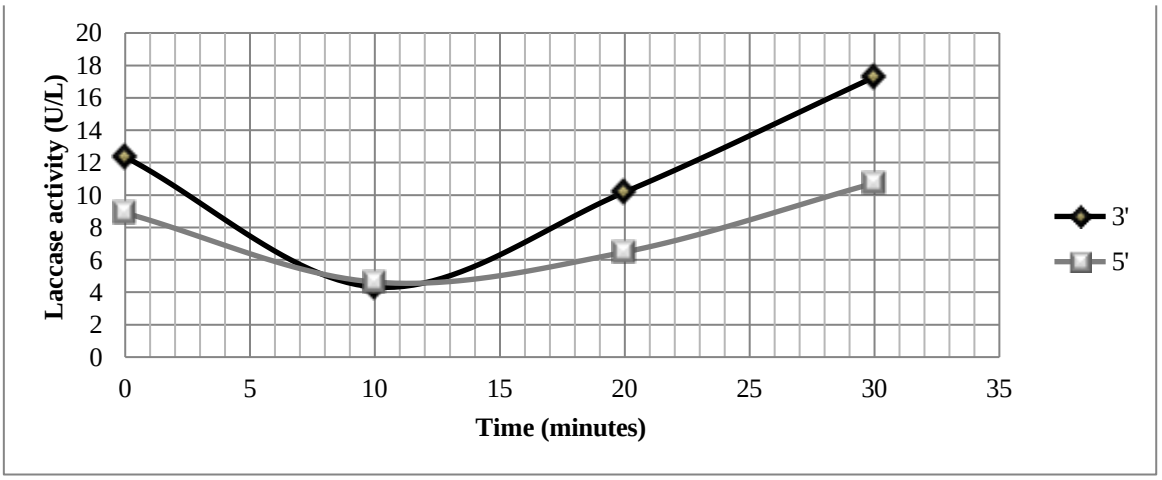

Figure 1. Laccase activity of *Penicillium chrysogenum* in depolymerization of residual lignin in wheat straw.

In Figure 1, it was observed that *P. chrysogenum* showed a decrease in its laccase activity from minute 0 to minute 10, in minutes 3 and 5 of reading in the spectrophotometer, while it indicted an increase in laccase activity from minute 10 onwards. The highest laccase activity was detected at minute 3 of reading in the spectrophotometer with 17.28 U/L at minute 30. The laccase activity of *P. chrysogenum* is due to its origin, this is agricultural soil, where it normally uses as a source of carbon and energy lignin, so it is suggested that at minute 5 of reading in the spectrophotometer it no longer had enough lignin and therefore showed lower enzymatic activity [10, 11,16] Figure 1

Treatments* *P. vulgaris* 'seeds	*P. vulgaris* percentage (%) germination and days to emergency+
P. vulgaris only water Absolute control (AC)	61.8^{c**} $^{+}6^{b**}$
P. vulgaris fed mineral solution at 100% relative control (RC)	69.1^{b} 6^{b}
T1 Sterile filtrate free of *S. griseus* cells 0.01 mL (6 days)	68.7^{b} 4^{a}
T2 Sterile filtrate free of *S. griseus* cells 0.1 mL (6 days)	69.3^{b} 4^{a}
T3 Sterile filtrate free of *S. griseus* cells 0.01 mL (12 days)	100^{a} 4^{a}
T4 Sterile filtrate free of *S. griseus* cells 0.1 mL (12 days)	100^{a} 4^{a}
T5 gibberellin 0.01 mL (10 ppm)	100^{a} 4^{a}
T6 gibberellin 0.1 mL (10 ppm)	100^{a} 4^{a}

Table 1. Effect of *Streptomyces griseus* transformed from RELIWS

*n = 20, **different letters had a statistical difference according to ANOVA, p > 0,05, Tukey HSD. ** residual liginin from wheat straw

Table 1 shows the evidence that *S. griseus* transformed the depolymerization products of RELIWS into compounds analogous to gibberellin equivalent to a dose of 10 ppm of pure gibberellin. Given that the positive effect of sterile filtrates free of cells of *S. griseus* on the germination percentage and days to emergence of *P. vulgaris* seeds, which was similar to the positive effect registered of 10 ppm of gibberellin [2, 8,13]. The above supports that these *S. griseus* cell-free filtrates, given its chemical nature, had a phytohormone-like effect compared to *P. vulgaris* seeds irrigated only with water or absolute control (AC) and fed with a 100% mineral solution. %. While the seeds of *P. vulgaris* irrigated only with water used as an absolute control and those of P. vulgaris fed with the 100% mineral solution germinated in 6 days a numerical value statistically different from the 4 days of the seeds treated with the filtrates. free of *S. griseus* cells of 6 and 12 incubations, thus there is a difference with the doses used of 0.01 and 0.1 ml, especially without statistical difference with the germination time of *P. vulgaris* seeds treated with pure gibberellin. The 4-day time of the seeds treated with the cell-free filtrates supports that the depolymerization of RELIWS, were converted to gibberellin-like compounds, indicating that double fermentation is an option to give residual lignin to wheat straw by depolymerization due to *P. chrysogenum* a biotechnological value, when *S. griseus* converted it into a gibberellin-type compound [14,15], research in progress by chromatography and other analytical tests may demonstrate that this is the case.

Effect of the transformed *S. griseus* derived from depolymerization of RELIWS by *P. chrysogenum* on the phenology and biomass of the *P. vulgaris* primordium.

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	Phenology		Biomass			
Treatment*/parameters *P. vulgaris*	Plant height (cm)	Radical length (cm)	Aerial fresh weight (g)	Radical fresh weight (g)	Aerial dry weight (g)	Radical dry weight (g)
P. vulgaris only water absolute control (AC)	10.3^{d**}	6.27^{d}	0.73^{d}	0.08^{d}	0.17^{b}	0.010^{b}
P. vulgaris fed mineral solution at 100% relative control (RC)	13.0^{c}	15.1^{b}	0.96^{c}	0.10^{c}	0.27^{a}	0.022^{a}
Sterile filtrate free of *S. griseus* cells 0.01 mL (6 days)	16.22^{b}	17.71^{a}	1.48^{b}	0.31^{a}	0.15^{b}	0.030^{a}
Sterile filtrate free of *S. griseus* cells 0.01 mL (12 days)	16.45^{b}	15.86^{b}	1.17^{b}	0.14^{b}	0.15^{b}	0.022^{a}
Gibberellin 0.01mL/10 seeds	18.22^{a}	15.18^{b}	1.74^{a}	0.28^{a}	0.22^{a}	0.022^{a}

Table 2. Effect of *Streptomyces griseus* transformed from RELIWS.

*n = 20, **different letters had a statistical difference according to ANOVA, p > 0,05, Tukey HSD. **residual liginin from wheat straw

Table 2 shows the effect of *S. griseus* cell-free filtration from the depolymerization of RELIWS by *P. chrysogenum* on the phenology and biomass of *P. vulgaris* at seedling stage. In plant height (PH), the positive effect of the transformed *S. griseus* was evident in comparison with the PH of *P. vulgaris* irrigated only with water and with the PH of *P. vulgaris* fed with the 100% mineral solution with statistically different numerical values, except when pure gibberellin was used on the PH of *P. vulgaris*. Something similar was observed with the cell-free filtrates of *S. griseus* regarding root length compared to that recorded in RL in *P. vulgaris* treated with pure gibberellin, with numerical values statistically higher than RL in *P. vulgaris* irrigated only with water. and the RL fed a 100% mineral solution. A positive effect of the *S. griseus* cell-free filtrates that were taken at 6 and 12 day of incubation both, registered in the fresh (FAW/FRW) and dry (ADW/RDW) weight of the aerial and radical part of *P. vulgaris* in general non-statistical difference compared to the biomass of *P. vulgaris* treated with pure gibberellin. The above supports that the depolymerization of RELIWS by *P. chrysogenum* generated compounds that allowed *S. griseus* to transform them into gibberellin-type phythormones. Based in the ability genetic capacity of this plant growth promoting bacteria or actinomycete to transform agriculture waste into a phytohormone [6,16,17]. Which ongoing research attempts to verify, so far, this organic bacterial product looks like gibberellin obtained from a large agriculture waste [7-9, 14].

CONCLUSION

This work proposes an option for agriculture by giving added value to the residual lignin of wheat straw (RELIWS) as a raw material so that by double microbial fermentation with *P chrysogenum and S. griseus* it becomes a useful phytohormones for agriculture. At the same time, it is an ecological option to avoid the burning of RELIWS and thus greenhouse gases preventing global warming from agriculture. The phytohormone-rich product derived from this process has the potential to enhance crop growth, yield, and resilience, thereby promoting sustainable and efficient agriculture. Furthermore, by reducing the reliance on synthetic phytohormones, this approach can contribute to a more environmentally benign agricultural landscape. Comprehensive studies on the phytohormone composition, efficacy, and application methods are necessary to fully realize the potential of this innovative process.

Overall, this research presents a promising strategy for sustainable waste management and agricultural enhancement. By harnessing the capabilities of microorganisms to convert a problematic

waste product into a valuable resource, this work contributes to a more sustainable and resilient agricultural system.

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Conflicts of Interest

The authors declare no conflicts of interest.

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