



Trametes elegans: a fungal endophytic isolate from Otoba gracilipes as biocatalyst for natural flavors production

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1. Introduction

Flavoring production by biotechnological processes has recently been a target for academic and industrial research, in which Fungi, which produces volatile flavor compounds by microbial methods and by biotransformation, have been recognized as excellent candidates. One of them belongs to the Phylum Basidiomycete, which normally grows in decaying wood but few times reported as endophyte. In this work, a fungal isolate from the cortex of the medicinal plant *Otoba gracilipes* (tropical rainforest, Colombia), was identified by ITS sequence analysis as *Trametes elegans*. This unusual plant-associated microorganism was screened as a pleasant flavor resource by cultivating it on different broths at pH=6.0, during 15 days. Then, the exhausted media was filtered and recovered to vacuum-rotary evaporation at 40 °C and tested for aroma profile. Volatile compounds produced as secondary metabolites were extracted with dicloromethane and analyzed with TLC. Furthermore, lipoxygenase activity on linoleic acid was estimated for fungal biomass.

2. Materials and Methods

2.1 Isolation and molecular identification by ITS sequence analysis

2.3 Thin layer

chromatography

(silica gel; hexane:

ethyl acetate [95:5])

2.2 Induction of aroma production through broth culture [1] (Table 1)

2.4 Estimation of LOX activity in linoleic acid [2]

Table 1. Broth's composition. * indicates aroma inductor.

| Broth | Composition | | |
|-------|--|--|--|
| Α | Potato-dextrose | | |
| В | Chontaduro's (<i>Bactris gasipaes</i>) peel, L-fenilalanine* | | |
| C | Glucose, CaCl.2H ₂ 0, MgSO ₄ .7H ₂ 0, yeast extract, NH ₄ Cl, tween 80, mineral oil*, coconut oil* | | |

3. Results

3.1 ITS sequence analysis (Figure 1) identified the isolated fungi as *Trametes elegans* (Figure 2).



Figure 1. ITS sequence.



Figure 2. Axenic culture of *T. elegans*.

3.2 Aromas perceived during different sampling times are shown in Table 2.

3.3 Dicloromethane extracts of exhausted broths exhibit similar composition (Figure 3 and 4). No reaction observed with Vanillin test.

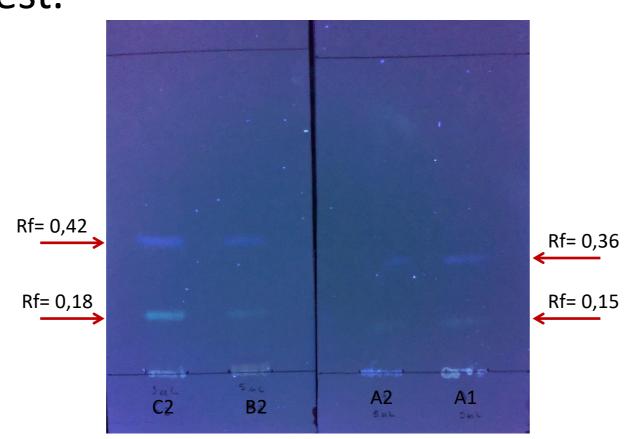


Figure 3. TLC chromatoplate 365 nm.

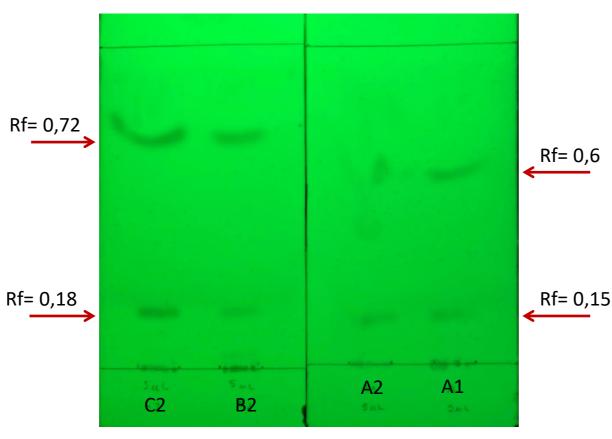


Figure 4. TLC chromatoplate 254 nm.

3.4 LOX activity on linoleic acid from *T. elegans* biomass was estimated in 1,32E-02 nkat (Figure 5).

Table 2. Aromas perceived in different broths and sampling times. Numbers 1 and 2 indicate replicates; + indicates intesity of the aroma.

| | Aromas | | |
|-----------|-------------------|-------------------------------|---------------------------------------|
| Broth | Day 5 | Day 11 | Day 15 |
| Control A | Potato (++) | Potato (++) | Potato (++) |
| A1 | Floral, rose (++) | Floral, rose (++) | Sweet (+++) |
| A2 | Floral, rose (++) | Floral, rose (+) Sweet (+) | Sweet (+++) |
| Control B | Honey (+) | B. gasipaes (++) | B. gasipaes (++) |
| B1 | Honey (+) | Floral, rose (+) | Green pea (+) <i>B. gasipaes</i> (++) |
| B2 | Honey (+) | Green pea (+) | Green pea (+) <i>B. gasipaes</i> (++) |
| Control C | No odor | No odor | No odor |
| C1 | Sweet (++) | Floral, rose (+) | Sweet (+) Citric (+) Floral, rose (+) |
| C2 | Sweet (++) | Floral, rose (+) Sweet (+) | Sweet (+) Citric (+) Floral, rose (+) |

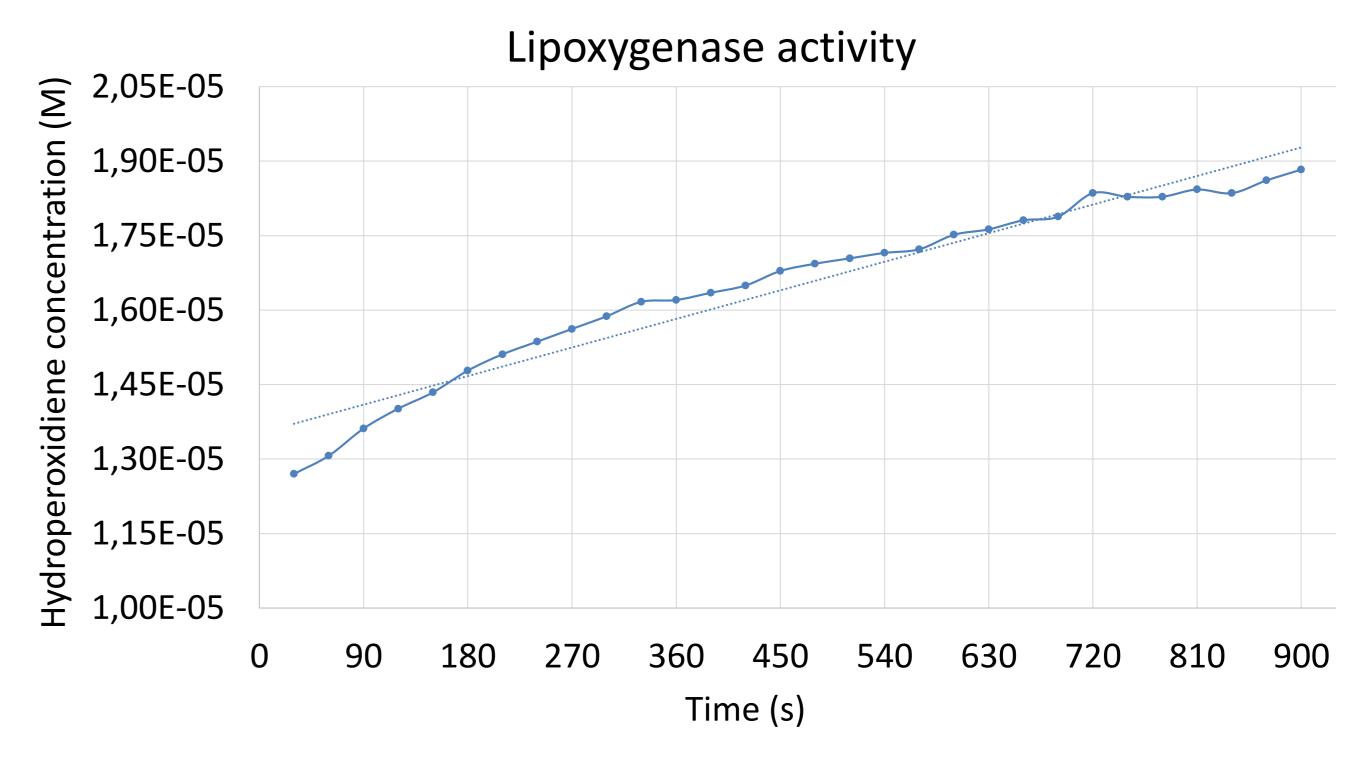


Figure 5. LOX activity on linoleic acid measured by hydroperoxidiene formation during 15 minutes.

5. Conclusions

- Currently there is no report of an endophytic Basidiomycete (T. elegans) as a natural biocatalyst for pleasant aroma compound production during fermentation [3,4]. Simultaneously, biotransformation of chontaduro's peel (B. gasipaes) for pleasant aroma production was evidenced.
- The estimated enzymatic LOX activity of the strain growing on different media suggests it has potential as biocatalyst.
- TLC analysis shows at least three groups of compounds of intermediate polarity, coconut and chontaduro's broth exhibit similar composition.
- Further studies on the separation and chemical structure dilucidation of aroma compounds will be made using GC-MS.

6. References



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[4] Abrahão, M. R., Molina, G., & Pastore, G. M. (2013). Endophytes: Recent developments in biotechnology.

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