Endogenous Starter Bacteria Associated to Chanterelle mycelia Enhance Aroma, color and growth of mycelia

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Abstract— **Chanterelle (*Cantharellus cibarius*) mushroom can be cultured from its fruit body on agar medium. The present study showed that the growth rate of chanterelle mycelia in agar medium is slow whereas the pigment of the cultured mycelia was medium dependent. Different mycelia colors were detected in this study: from orange to pink and brown.This study also revealed bacterial growth near mycelia fragments, which appeared only at the initial phase of mycelia growth after which the mycelia continued to grow, blocking bacterial growth in the center of the agar plates. Therefore, we presumed that these bacteria were able to transfer the color to the chanterelle mycelia and may serve as fungal growth helper bacteria.**

The first step was to isolate these accompanying bacteria in pure culture and relate its phenotypical aspect to the mycelia aspect. The second step consisted of chemically treating the mycelia to suppress bacteria around and verify the mycelia's ability to enhance or decrease color production. As a third step, the Chanterelle mycelia were treated separately with different chemical reagents [Sodium nitrate, Potassium phosphate monobasic, Ammonium nitrate, Citric acid, Acetic acid, Boric acid (0.05 g/ml), 1% NaOH, 1% KOH and 0.5%.HCl] followed by incubation in different agar plates. We demonstrated that some treatments killed all bacteria after which the mycelia lost its growth capacity. As a final step, agar plates showing no development of mycelia were inoculated with bacteria. After this inoculation, mycelia growth resumed and obtained the color of the inoculated bacteria.

The results clearly showed that endogenous bacteria present in Chanterelle mycelia serve to initiate mycelial growth and impart color to the Chanterelle mycelia. The isolated bacteria produced aromas, lecithinase, amylase and laccase as well. However, these bacteria were unable to produce oxidase, catalase or protease.

Index Terms— **Chanterelle, endogenous bacteria, color, pink, brown, orange, perfume, enzymes.**

I. INTRODUCTION

Chanterelles are ectomycorrhizal fungi growing in a mutually and beneficial association with certain trees. Ectomycorrhizal fungi in general are a subset of mycorrhizal fungi that form sheaths over the root tips of their host trees. Chanterelles are highly prized for their flavor and can be safely collected and consumed because they are easily identified [1]. For the propagation of fungal mycelia in laboratory an optimum medium is required, the composition of which provides all the essential nutrients offered via symbiosis naturally by host trees. Routinely used media such as Malt Extract Agar (MEA), Potato Dextrose Agar (PDA) and Potato Dextrose Yeast Agar (PDYA) may lack certain essential growth components required for the growth of Chanterelle mushrooms. Other commonly observed factors inhibiting its growth include unknown specific nutritional requirements, difficulty in using complex carbohydrate sources, and high natural contamination of sporocarps by moulds and bacteria [2, 3].

However, several attempts have been made for aseptic cultivation of pure mycelium and fruiting bodies of Chanterelle mushrooms. Nylund (1982) [4] supplemented the agar medium used for chanterelle mycelium production with several antibiotics. Antibiotics kept the co-inhabiting bacteria and fungi sequestered for 17-53 days following which a pure mycelium was obtained which was confirmed by genetic sequencing.

Danell & Camacho (1997) [5] produced the first cultivated *Cantharellus cibarius* in a potted 16-month-old *Pinus sylvestris* with mycelium grown for only one year in culture. Chanterelle mushrooms may serve as an important source for natural pigments since fungi can be grown in higher yields using biotechnological techniques, compared with higher plants whose growth rates are slow. Different species of *Cantharellus* produce carotenoid pigments, which produce yellow, orange and red colors. The natural pigment of orange yellow chanterelle mushroom is canthaxanthin. Major applications of this pigment are in the cosmetic and food industries including dairy products, confectionary, fish meat and poultry products, beer and wine [6].

Chanterelle mushrooms also produce some enzymes such as polyphenol oxidases [7], superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) [8].

Due to high nutritional and gourmet values, wide applications in food and cosmetic industries, as well as production of several biologically important metabolites, the demand for chanterelle mushrooms is on the rise.

Based on the background provided, this study aims to demonstrate the role of endogenous bacteria associated with

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chanterelle in color production and growth of the Chanterelle mycelia. In addition, the study tested the ability of isolated strains for enzyme production.

II. Material and Methods

A. Fragment of Chanterelle body inoculation in solid medium

Chanterelle fruits were harvested from Juva forest located close to Juva Truffle Center, Finland. They were brushed and washed several times with tap water. One piece of 0.5 x 0.5 cm was cut under sterile conditions and placed on the surface of four different solid agar media.The following agar media were used: Potato Dextrose Agar (Potato extract, 4 g; Glucose, 20 g; Agar, 20 g; Distilled water, 1 L), Yeast extract Agar (Yeast extract, 20 g; Sucrose, 150 g; Agar, 20 g; Distilled water, 1 L), Malt Extract Agar (Malt extract, 20 g; Peptone, 1 g; Glucose, 20 g; Agar, 20 g; Distilled water, 1 L), and Nutrient Agar.

B. Mycelia and associated bacteria observation

Three observation methods were performed to investigate the existing of other microorganisms than Chanterelle mycelia growing on the agar media. The first method was based on visual observation of bacterial colonies developing around the chanterelle mycelia. The second observation method was performed by placing the agar cultures containing the chanterelle mycelia under the microscope to check the existence of bacterial colonies around the mycelia. The third observation technique was based on gram staining method.

After microscopic observation, *Actinomycete* cultures were inoculated in *Actinomycete* Isolation Agar: sulfate, 0.001 g/L, magnesium sulfate, 0.1 g/L. *Serratia spp*. were identified using API 20E; and *Staphylococcus spp*. were confirmed using API Staph.

C. Chemical treatment of Chanterelle fragments to destroy associated bacteria

Different compounds were used such as sodium nitrate, potassium phosphate monobasic, ammonium nitrate, citric acid, acetic acid and boric acid (0, 05 g/ ml) as chemical treatments in order to destroy the existing bacteria. Other treatments with 1% NaOH, 1% KOH and 0.5% HCl were performed. The treatment protocol involved immersing 10 chanterelle fragments (0.5 x 0.5 cm) in a 9 ml assay tube containing 1 ml of the respective chemical for 2 hours at room temperature. The fragments were then washed several times with sterilized water followed by re-streaking on PDA, YEAST, and NA medium.

D. Detection of mycelial growth in absence of bacteria

The treatment showing inhibition of all bacteria around the mycelia was considered for artificial inoculation by arbitrarily choosing strain 4 (red in color), after which mycelia were grown by incubation for 1 month.

E. Enzymatic assays

The qualitative investigation of enzymes was carried out according to methods based on [9, 10]. A 5-microliter volume of bacterial suspension was spotted in the centre of agar plates containing substrates for specific enzymes dissolved in growth media. After 2-3 days of incubation at room temperature, the plates were flooded with the suitable indicator. Formation of clear zones or zones with different colors around the bacteria colony indicated the presence of the enzyme activities.

Amylase

Amylase activity was assessed by culturing bacterial suspensions on TSA (MPA, Sigma-Aldrich, Germany) medium supplemented with 0.2% soluble potato starch (Sigma-Aldrich, Germany) at pH 6. After incubation for 2 days, the plates were flooded with 1% iodine in 2% potassium iodide solution. Formation of a clear zone surrounding the colony was considered as positive result for amylase production.

Protease

For proteolytic activity, TSA medium amended with 0.4% gelatine was used for growing corresponding bacteria. Degradation of gelatine was seen as clear zone around the colonies. Plates when flooded with aqueous ammonium sulphate resulted in the formation of a precipitate making the agar opaque and enhancing the clear zone.

Lipase

For lipase activity measurement, the bacteria were grown on Peptone Agar Medium (Peptone 10g, NaCl 5g, Agar 16g, Distilled water added to make 1L) supplemented with 1% w/v tween 20 (separately sterilized). Positive lipase activity was indicated by the formation of a visible precipitate of calcium salts of lauric acid.

Peroxidase

For peroxidase activity, TSA Medium was used to culture fungal isolates. After 4 days of incubation the presence of peroxidase was evaluated by flooding the plates with a freshly prepared mixture of 0.4 % H2O2 and 1 % pyrogallol dissolved in water. Plates were checked 3 and 24 hours after applying the indicator agent. The formation of a dark yellow brown color around the mycelium indicated peroxidase activity.

Laccase

Laccase activity was determined by culturing fungal isolates on TSA medium amended with 0.05g/L 1-napthol. As the mycelia grows, the colorless medium changes to blue due to oxidation of 1-naphthol by laccase enzyme.

Catalase

For catalase activity, TSA medium was used for bacteria and Potato Dextrose Agar used for fungi essay. After 4 days of incubation the presence of catalase was evaluated by flooding the plates with a freshly prepared 0.4 % H2O2. The presence of bubbles indicated the presence of catalase activity.

Data Presentation

Mycelial and bacterial growth associated with the chemical treatments were scored for presence or absence of growth in culture and detection of aromas were noted and recorded. Reactions and aroma production in enzyme assays were scored on a scale from (-) to (++) with (-) indicating absence and (++) indicating excessive production.

III. RESULTS

A. Effect of medium agar composition on the color of Chanterelle mycelia incubated in Petri dishes

Chanterelle fragments incubated at room temperature in PDA, YEA and NA, showed a change in mycelia color related to the change of culture media used (Figure 1). This change in color may differ according to the biochemical pathway used by the mycelia for nutrient consumption. For example, YEA medium has high content in nitrogen and in this medium the mycelia appeared brown. It is important to mention that the brown color was only found in YEA. However, Chanterelle fragments incubated in PDA showed the development of two colors (white and taupe). When incubated in Malt extract agar, chanterelle mycelia developed two colors: orange or pink. This change in mycelia color compared across different growth media may result from differences in specific nutrients available for bacterial development and secondary metabolites produced relative to the specific culture medium. This is the first study reporting results on the description Chanterelle mycelia color relative to associated bacteria in different culture media.

Danell (2001) [11] found that the initial white color of chanterelle mycelium changed to yellow when growing in the dark on MFM (Modified Fries Medium), although carotenoid concentrations within the mycelium varied from time to time, depending on carbon dioxide levels and incubation temperature. However, different aspects of some fungus species related to their medium culture were assumed. In fact, the studies of Zain et al. (2009) [12] showed that *Aspergillus terreus*, *Penicillium janthinellum* and *Penicillium duclauxii* cultivated on different growth media including yeast extract, malt extract, yeast-malt extract, and potato dextrose, demonstrated that the growth and the secondary metabolites of these three fungal strains were greatly affected by the culture medium types.

These authors showed that the color of the culture and secondary metabolites were noticeably altered and changed according to the growth medium used. Alam et al. (2001) [13] used different types of media for the study of mycelia pigments and pycnidia of the fungus *Botryodiplodia theobromae* Pat, and revealed that pigmentation of the fungus increased with the increase in glucose, however growth rate gradually decreased.

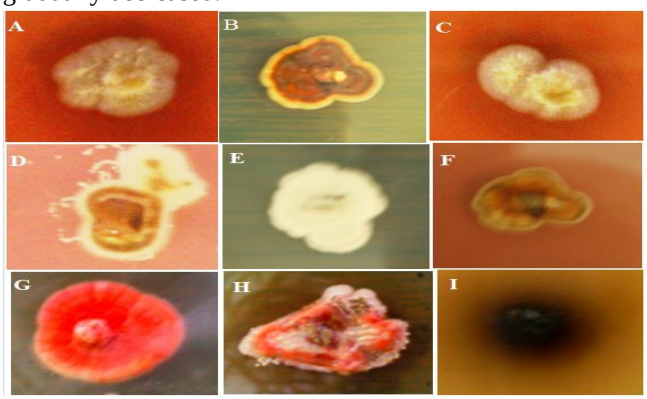

Figure 1. Phenotypic aspect of fragments of Chanterelle after growing in different culture media A, B, C: PDA medium; D, E, F: Nutrient broth; G and H: Malt extract agar; I: yeast extract agar.

In this study, almost similar cultural characteristics were observed in all the media with slight variation in PCM; the color of the mycelia was white to light grey at the beginning and became darker with the age; maximum pigmentation was observed in PDA (Potato Dextrose Agar) (Black 75% of the total mycelia surface) and moderate (15-25%) in PSA (Potato Sucrose Agar). Additionally, pink color development was low (5-10%) in Richard's agar and Czapek's agar. According to Shresta et al. (2006) [14] , various degrees of colony pigmentation were produced by incubating all isolates of *Cordyceps militaris* in all media exposed to light, except for poor or nutrient-deficient media (water agar :WA), which produced no pigmentation.

Results of this study revealed that nutrition source was the main factor responsible for the degree of pigmentation in *C. militaris* under incubation in light.

However, the exact nutrient factor responsible for the induction of pigmentation in *C. militaris* was not known, although observations showed that peptone and yeast extract were the main components in inducing pigmentation. Media without peptone or yeast extract or other organic nitrogen sources produced lighter pigmentations than the media prepared with standard concentrations of these substances. The specific role of yeast extract on pigmentation of *C. militaris* was evaluated by observing the difference in pigmentation between CDA (Czapek-Dox agar) and CZYA (Czapek Yeast Extract agar). Besides, a single amino acid, DL-asparagine, was found to be as efficient as peptone or yeast extract for inducing pigmentation, as revealed by production of similar types of pigmentations on MM (*Schizophyllum* (Mushroom) Genetics Minimal Medium) and MCM (*Schizophyllum* (Mushroom) Genetics Complete Medium plus Yeast Extract). The authors suggested that incubation period was also an important factor for the development of pigmentation in *C. militaris* under light incubation. In most of the media, pigmentation was most pronounced after three weeks of incubation when compared to early periods, but tended to fade after four weeks in some media. Deep pigmentation during late incubation periods of *C. militaris* could be due to different phases of photo-induced carotenogenesis such as light reaction, protein synthesis and accumulation of carotenoid pigments [15].

Friederichsen & Engel (1958) [16] reported carotenoid as the compound, which produces orange color of *C. militaris*.

Boonyapranai et al. (2008) [17] reported that PDA is one of the most commonly used culture media, because of its simple formulation and its ability to support mycelial growth and pigment production for a wide range of fungi.

Maheshwari et al. (1999) [18] stated that PDA and PDB could be the best culture media for mycelial growth and pigment production. While UKNCC (1998) [19] suggested that most fungi thrive on PDA, but this can be too rich in nutrients, thus encouraging the mycelia growth with ultimate loss of sporulation. Tseng et al. (2000) [20], investigating the growth and pigment production of the edible mushroom *Monascus purpureus*, suggested that pigment production and mycelia growth ran in parallel in all cultures.

B. Presence of bacteria around Chanterelle

After plating the Chanterelle fragment at the surface of the three considered Agar medium (PDA, YEA and NA) , and after 2 days of incubation at room temperature, approximately 25°C, we noticed that some bacteria grow around the Chanterelle fragment at the center of the Petri dishes (Figure 2).

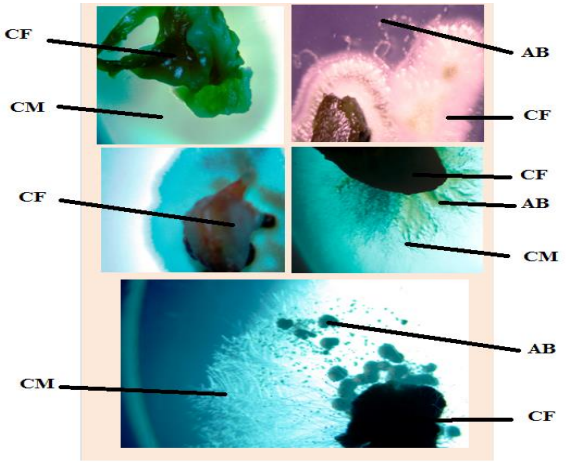

Figure 2. Photo illustrating the presence of bacteria around a fragment of Chanterelle located in the centre of Petri dishes, and the presence of mycelia only from the center to the periphery of the Petri dishes.

AB: Associated bacteria, CF: Chantarelle Fragment, CM: Chantarelle mycila

When the incubation time was extended to one month, it was noticed that the bacteria preserved its place in the center of the Petri dishes. However, the mycelia continued growing alone to reach the peripheral agar surface without the associated bacteria (Figure 2).

After two nights incubation at room temperature, approximately 25°C, every bacteria isolated from the area around the mycelia revealed the same color that occurred in the mycelia (Figure 3).

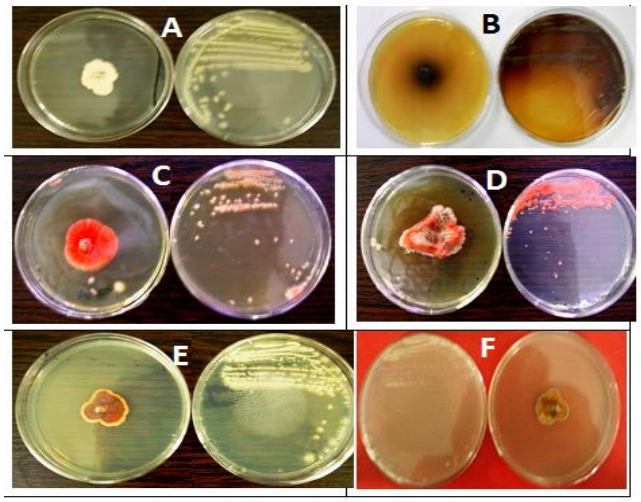

Figure 3. Photo of bacteria associated with the Chanterelle mycelia and as purified isolates.

Each photo A, B, C, D, E and F present two Petri dishes the left one or the right one shows the inoculated plates with the fragment of the mycelia, the second Petri dish presents the purified corresponding bacteria.

A: Strain 1, B: Strain 2, C: Strain3, D: Strain 4, E: Strain 5, F: Strain 6

This experiment showed clearly that when the fragment of Chanterelle was plated at the surface of the Petri dishes, some endogenous bacteria associated to Chanterelle may grow in symbiosis with the mycelia (Figure 4).

Different aspects of bacteria associated to Chanterelle mycelia were observed by microscopy (Figure 5).

bacteria were incorporated in fungal tissues during primordial formation, thus growing actively between the cells without harming the mushroom. In addition, Rangel- Castro (2001) [22] showed that amino acids, organic acids, and sugars released by chanterelles serve as nutrient source for the bacteria. Bacterial contamination presents a potential problem that has plagued the culturing of chanterelles [23, 24].

Pilz et al. (2003)[21] showed that Chanterelle tissue may serve as nutrient media more favorable for bacteria originating from the chanterelles than fungal hyphae, thus precluding subsequent isolation of uncontaminated chanterelle hyphae. Recently, Dutch scientists used an antibacterial nutrient media formulation [25] to grow and isolate pure chanterelle mycelium from chanterelle tissues [26, 27].

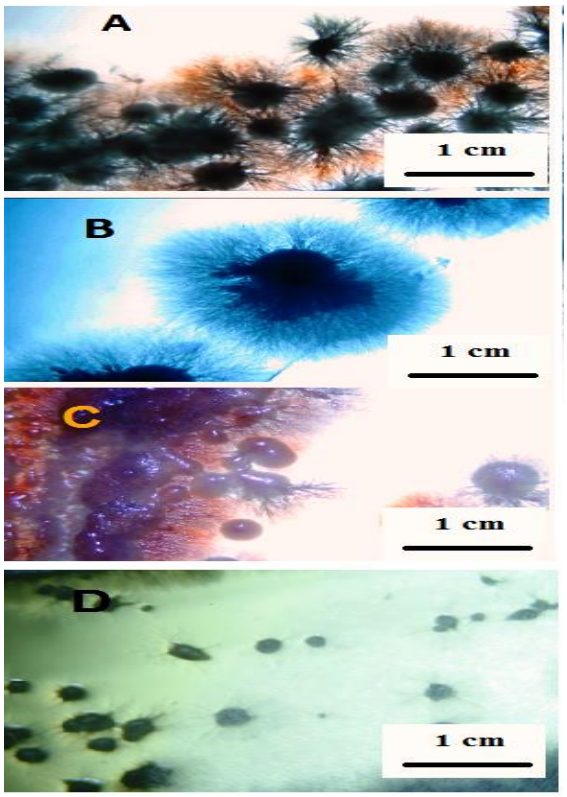

Figure 4. Depiction of bacteria around mycelia, Microscopic observations conducted in Petri dishes including agar medium.

A : Strain 3,B: Strain 2, C: Strain 4, D: Strain 1

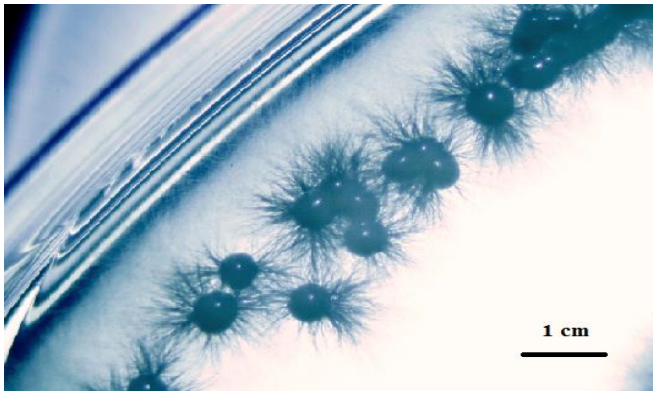

Figure 5. Depiction of bacteria developed around the mycelia, (Microscopic observation conducted in Petri dishes including agar medium).

In fact, the bacteria first appeared around the mycelia was isolated and purified. In total, six bacterial isolates were obtained, noted as S1, S2, S3, S4, S5 and S6.

Danell et al. (1993) [3] found that the presence of bacteria and other microorganisms within the sporocarp tissues is one reason chanterelles have been so difficult to cultivate. Bacteria are present in millions per gram of fresh weight).

Pilz et al. (2003) [21] suggested that these associated

C. Bacteria Gram staining and identification

Four Bacteria S1, S2, S3 and S5 were Gram positive. According to the Gram reaction, it seems that these bacteria belong to the *Actinomyces* group (Figure 6). However, two other bacteria S4 and S6 were Gram negative. These two bacteria, when streaked in Asparagine and Acetamid mediums, produced a fluorescent color therefore they belong to the fluorescent *Pseudomonads*. Strain S4 produced red pigment and was identified as *Serratia maresens* using API 20 E and the strain S6 was identified as *Staphylococcus* using API Staph.

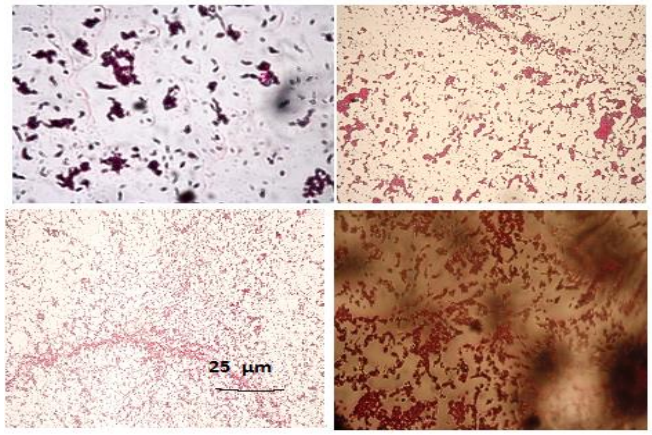

Figure 6. Gram staining of bacterial strains 1, 4, 5 and 6 objectives, Scale 1 cm line 25 µm

Danell et al. (1993) [3] isolated and identified aerobic bacteria from *C. cibarius* (fruit body) FB and found that most of them belong to the fluorescent *Pseudomonas* group. *Bacillus spp.*, *Xanthomonas spp.*, and *Streptomyces spp.* were also found, though in significantly lower amounts, they also observed that the proportion of fluorescent *Pseudomonas* in soil samples of FB growing sites was only 12%, while inside the FB these bacteria represented an average of 78% of the total culturable community. Utilization of mannitol and trehalose, which are carbohydrates exuded by *C. cibarius* mycelia, was a common trait among *Pseudomonas spp*. from all environments studied.

Trehalose degradation is not a common trait among bacteria, and exhibited by only a limited number of fluorescent *Pseudomonas spp* [28, 29].

A helper effect of the bacteria on fungal growth was examined by Danell (1994) [30] in *C. cibarius* but no helper effect was found. Danell et al. (1993) [3] suggested that the fungus probably produced trehalose, and this was involved in the selection of bacteria associated with the fruiting body. It has been observed that bacteria located in different environments have different capacities to utilize diverse substrates [31]. Rangel-Castro et al. (2001a) [32] found that mannitol and trehalose are exuded by *C. cibarius* mycelia in vitro cultures. This may support the suggestion that bacteria growing inside FB have a greater capacity to utilize these exudates than bacteria from other environments. However, the association between *C. cibarius* and bacteria is not well understood (Figure 6).

D. Chemical treatment of Chanterelle fragments

The Chanterelle fragment treated with different chemical reagents did not show the same microbial biomass behavior (Table 1).

In fact, the treatments by sodium nitrate, potassium hydroxide, ammonium nitrate suppressed the mycelia's growth, and allow bacteria to grow (Figure 7).

In addition, some bad odors were detected following these treatments. In PDA, only black fungi possessing prompt radial grew and filled all the Petri dishes. Additionally, after boric acid, acid acetic and potassium hydroxide treatments, it was noticed that only fungi development enabled growth of both the Chanterelle mycelia and the endogenous bacteria.

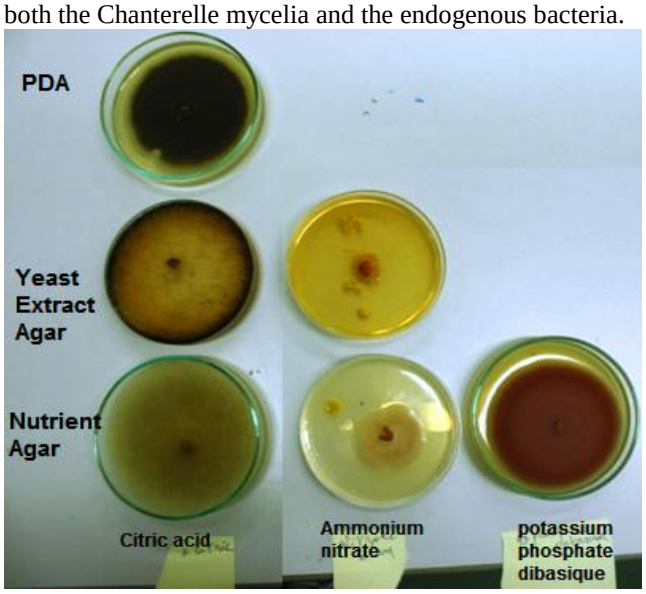

Figure 7. Example of treatments showing bacterial or fungal development with bacteria enabling the development of Chanterelle mycelia.

Only the treatment of the Chanterelle fragments with hydrochloric and acetic acids and sodium hydroxide prevented the development of both bacteria and fungi. These plates were inoculated with the red bacteria strain 4 (Figure 8).

Incubation of mycelia without bacteria at room temperature, revealed no development of the Chanterelle mycelia growth (Figure 9A). However, after one week incubation at room temperature, approximately at 25°C, the inhibited mycelia associated with strain 4 showed regrowth (Figure 9B). The growth kinetics of the mycelia when associated with strain4 was enhanced over time after two and three weeks (–Figure 9C – 9D, respectively).

Effective use of hydrogen peroxide as a chemical sterilant in mushroom production and selection of cultivable mushroom strains for tropical conditions was tested on *Pleurotus* mycelium [33]. when eight strains were cultured aseptically on agar at six hydrogen peroxide concentrations (0–0.1%, v/v) at 27 °C and another fast growing strain *P. sajor-caju* strain 1, was cultured non-aseptically at six hydrogen peroxide concentrations (0–0.1%, v/v) at 27 °C. Results showed that the mycelial growth rate in all strains increased when hydrogen peroxide was increased from 0 to 0.001% (v/v), and then decreased with further increments in hydrogen peroxide concentration. The hydrogen peroxide concentration associated with 50% reduction in maximum mycelial growth rate due to toxicity (EC50) ranged from 0.009 to 0.045% (v/v); in non-aseptic culture of *P. sajor-caju* strain 1, bacterial growth was observed at concentrations ≤0.016%, whilst the upper hydrogen peroxide concentration limit for fungal growth was 0.025% (v/v).

The highest hydrogen peroxide concentrations 0.016% (v/v) and 0.025% (v/v) in which bacteria and fungi, respectively, were observed to grow were within the concentration range 0.009–0.028% (v/v).

Wong & Preece (1985) [34] revealed the effect of N-cetylpyridinium chloride, benzalkonium chloride, Cetrimide, bronopol (2-bromo-2-nitropropane-1,3-diol), Panacide and Chloramine T tested as possible disinfectants for use in growing mushrooms (*Agaricus bisporus*) where *Pseudomonas tolaasii* blotch is prevalent. The most effective materials in vitro against *P. tolaasii* were quaternary ammonium compounds and bronopol.

In 8 min 'clean' and 'dirty' tests incorporating yeast cells showed that only bronopol failed to kill the pathogen. If mushroom casing (peat plus limestone) was added to these short duration tests the pathogen survived all six disinfectants. When tests with added casing were extended to 20 h, bronopol was very effective (biocidal value 100 µg/ml) and the pathogen was not killed by the other five disinfectants. In experiments on agar plates, bronopol and chloramine T were stimulating to the growth of *A. bisporus*. Growing mushroom caps treated with bronopol remained white, whereas caps treated with the other five disinfectants turned brown within 30 min. It is thus likely that bronopol could be used to control the source of bacterial blotch epidemics in mushroom growing.

(Tseng el al., 2000)[20] noted the effect of different levels of sodium chloride, sodium nitrite, polyphosphate on growth and pigment production of the edible mushroom *Monascus purpureus*.

The addition of sodium chloride (>50•0 g/l) and polyphosphate (>3•0 g/l) to broth medium decreased mycelial growth andpigment production, whereas low concentrations of sodium nitrite (<0•2 g/l) promoted mycelial growth and pigment production. The fungus showed more tolerance to salt and polyphosphate in ground meat than in broth medium and used sucrose as a carbon source as well as glucose for growth and pigment production.

Acetic acid and sodium hydroxide added to cultures on PDA, MYE and NA inhibited Chanterelle mycelial growth in the absence of associated bacteria may serve for further studies considering effects of artificial contamination by bacteria.

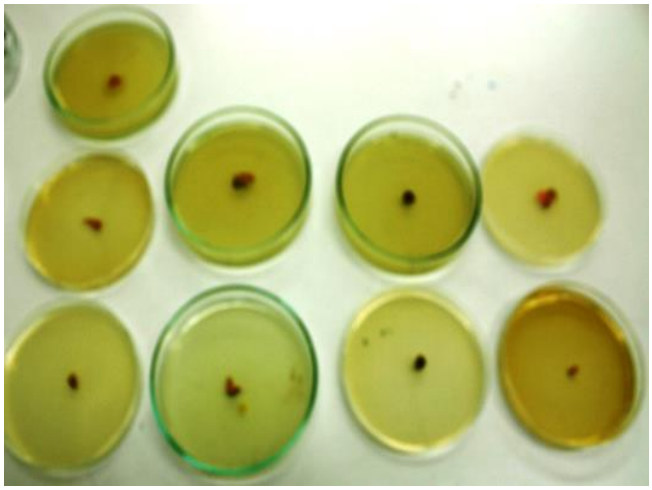

Figure 8. Photo presenting a collection of Chanterelle ycelia treated by hydrogen chloride.

E. Bacterial enzymes

Results gathered in Table 2 show that bacteria isolated from Chanterelle were also able to produce perfume, lecithinase, amylase and laccase. However, these bacteria were unable to produce oxidase, catalase and protease.

According to Toljander et al., (2007) [35] the mycelial exudates not only increased bacterial growth and vitality but also influenced the bacterial community composition. This suggested that some bacteria preferentially utilized different compounds available in exudates. To capture and utilize resources from the fungal partner, particular enzyme complexes may be necessary for the fungal-associated bacteria [36]. Thus, the efficient use of such enzyme systems to obtain essential energy and carbon sources from the fungal partner emerges as a key mechanism involved in the bacterial interaction with soil fungi.

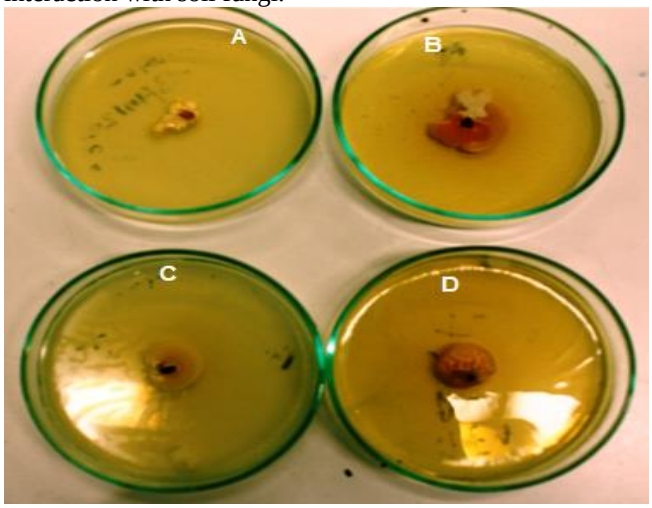

Figure 9. Example of treated Chanterelle fragment with chemical substances in order to suppress all microorganisms' development which have received artificial contaminant bacteria (strain 4).

(A): mycelia which did not receive the bacteria serving as a control (B): After one week of growing at room temperature approximately at 25°C. (C): After two weeks. (D): After three weeks.

It has been demonstrated by Rangel-Castro et al. (2001b) [29] that *C. cibarius* has a limited capacity to utilize organic N. To compensate for this, the fungus may access recalcitrant sources of N by taking advantage of the bacterial enzymatic capacity. However, the ability of bacteria to secrete lytic enzymes, such as proteases, might be very important for the vegetative soil mycelium.

IV. Conclusion

This study is the first one showing the contribution of endogenous bacteria of chanterelle in Chanterelle mycelial growth and pigmentation. Is it possible that the orange color of the Chanterelle is related to soil culture? Furthermore, under hydroponic conditions and considering different substrates, is it possible that these different colored bacteria have the ability to yield different Chanterelle fruits? To answer these questions, more in-depth studies must be considered. In addition, the present study provides six strains of bacteria, which are able to produce color, perfume and enzymes suggesting possible exploitation in further biotechnology studies.

Table 1: Effects of Chemical reagents in fragmented body fruits of Chanterelle and behavior of associated mycelia and bacteria.

	Hydrogen chloride	Acetic acid	Citric acid	Boric acid	Sodium Nitrate	Sodium Hydroxyde	Phosphate Potassium	Hydroxyde Potassium	Nitrate Ammonium
Chemical Formula	HCl	C2H4O2	C6H8O7	H3BO3	NaNO3	NaOH	K3PO4	KOH	(NH4)(NO3)
PDA	My - Bact -	My - Bact -	Fungi +	Fungi +	My - bact + Bad odor	My - Bact -	Fungi +	My - bact + Bad odor	My - bact + Bad odor
YEA	My - Bact -	My - Bact -	Fungi +	Fungi +	My - bact + Bad odor	My - Bact -	Fungi +	My - bact + Bad odor	My - bact + Bad odor
NA	My - Bact -	My - Bact -	Fungi +	Fungi +	My - bact + Bad odor	My - Bact -	Fungi +	My - bact + Bad odor	My - bact + Bad odor

Different treatments were applied (Sigma) (Sodium nitrate, Potassium phosphate monobasic, Ammonium nitrate, Citric acid, Acetic acid, Boric acid) (0.05 g/ml). Also, other treatments with 1% NaOH, 1% KOH and 0.5%.HCl were considered. The experiment consisted on putting 10 fragments chanterelle bodies having 0.5 x 0.5 cm in size in 1 ml of each treatment in 9 ml liquid essay tube. After storage during 2 hours at room temperature, several washings with sterilized water were performed and fragments were restreaked in PDA, YEAST, NA. My-: inability of Chanterelle Mycelia to grow. Bact-: inability of associated bacteria to grow, Bact +; ability of associated bacteria to grow. Fungi+: The specific treatments enhance the development of some fungi. Bad odor: The associated treatment induce bad odor.

Table 2: Ability of selected strains for enzyme and perfume production in three different media (PDA, YEA, NA)

	Perfume			Lecithinase	Amylase	Laccase	Oxydase	Catalase	Protease
	PDA	YEA	NA						
Strain 1	+++	++	-	++	++	++	-	-	-
Strain 2	+++	++	-	++	++	++	-	-	-
Strain 3	+++	+	-	++	++	++	-	-	-
Strain 4	++	+	-	+	+	+	-	-	-
Strain 5	++	+	-	+	+	+	-	-	-
Strain 6	++	+	-	+	+	+	-	-	-

For perfume: +++: Refers to high production; ++: average production; +: very low level smelling; -: Absence of smell perfume [no quantifying test was performed but the detection of the smell is only the method adopted in the present study in order to verify whether the bacteria is producer or not of perfume].
For Enzymes production: +: Refers to the presence of the activity; −: Refers to absence of the activity; ++: Refers to an excessive presence of the activity.

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