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Enhancement of eritadenine production using three carbon sources, immobilization and surfactants in submerged culture with shiitake mushroom (*Lentinula edodes*) (Berk.) Singer)

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In this study, the effect of three carbon sources (mannitol, minced potato and sucrose), two immobilization substrates (alginate and wood cylinders), and three surfactants (Tween 20, Tween 40 and Tween 80), were evaluated on eritadenine production using shiitake (*Lentinula edodes*) mycelium under submerged cultivation, in shake flasks within 20 days. Eritadenine and biomass were measured by HPLC and gravimetrically, respectively. Alginate immobilization of mycelium promoted significant enhancement of eritadenine yields of 88 mg/L, compared to the control (8.7 mg/L) and wood immobilization (14.8 mg/L). Likewise, eritadenine yields (72.4 mg/L) were enhanced by adding surfactant tween 20 to the broths in 0.5%, than control (8.7 mg/L) without surfactant. Tween 40 and 80 did not improve eritadenine yields, but both produced better biomass values (superior to 5 g/L) than the control (3.9 g/L). All carbon sources (sucrose, mannitol, mince potato, and glucose as control) produced similar low eritadenine yields, with best results (10.2 mg/L) by sucrose, although glucose produced the best biomass yields of 3.9 g/L. Also, carbon sources and the best biomass values did not show significant effect on eritadenine production. pH values in the best eritadenine yielding fermentations went down from 6 to 3-4, but pH had a low correlation with eritadenine yields. Finally, all data obtained in the present study are useful for optimizing culture conditions, towards industrialization of this important health improver metabolite (eritadenine).

Key words: Eritadenine, shiitake mushroom, submerged culture, immobilization, surfactants.

INTRODUCTION

Edible shiitake mushroom has been massively consumed for thousands years in East Asian countries especially in China, Korea and Japan. This mushroom is the second most consumed in the world after the bottom mushroom

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(*Agaricus* spp.), with annual production of two millions of tons (Hwang et al., 2012), mainly because the mushroom contains various bioactive substances very beneficial to health. Among other benefits, shiitake is well known as lipid-lowering agent, antibacterial, antiviral, anticancer, and by its excellent nutritional properties (Bisen et al., 2010; Rasmy et al., 2010; Yang et al., 2013; Gil-Ramirez et al., 2018).

Eritadenine from shiitake is a secondary metabolite consisting of a purine alkaloid with an oxidized sugar fragment, which has been shown to be successful in decreasing lipids like cholesterol and triglycerides levels in blood (Yang et al., 2013). Thus, in mice, eritadenine prevents hyperhomocysteinemia, because of its action on triglycerides metabolism (Yang et al., 2013). Similarly, a reduction in serum cholesterol up to 20% in a week is obtained in rats, daily supplemented with 0.005% eritadenine in their food (Shimada et al., 2003; Shu-Lei et al., 2012). Also eritadenine has other interesting biological effects, like inhibition of parasite *Cryptosporidium* sp. (Čtrnáctá et al., 2010), and inhibition of the enzyme, S-adenosyl-l-homocysteine hydrolase (SAHH) (Yamada et al., 2007) and angiotensine (Afrin et al., 2016). In that sense, eritadenine has been proposed as pharmaceutical ingredient (Enman et al., 2011).

Eritadenine have been obtained successfully by using submerged culture of shiitake mycelium; however yields are still unsatisfactory for an industrial scale production. For instance, Enman et al. (2008) reported yields of 10.2 mg/L after 20 days of incubation in a simple broth composed of malt extract and yeast extract in shake flasks. Later, better yields of 25 mg/L were obtained in similar broth, but supplemented with a dried distillers grain and soluble water extract (DDGS), incubated at similar conditions in shake flasks and bioreactors (Enman et al., 2012).

Nutritional conditions, fungal immobilization and surfactants could increase eritadenine production in shiitake mycelium under submerged cultivation, as well as some reports have demonstrated improved yields of metabolites in many filamentous fungi (Kirby et al., 2014; Noreen et al., 2016; Hameed et al., 2017). For example, nitrogen and carbon sources influence positively the production of the antioxidant ergothioneine in shiitake (Tepwong et al., 2012a; b), as well as the production of the anticancer alkaloid chaetominine with *Aspergillus fumigatus* (Zhang et al., 2016). Additionally, fungal immobilization have improved activity and production of laccase enzyme by *Trametes versicolor* and *Coriolopsis polyzona* cultures (Alaoui et al., 2008; Ünal and Kolankaya, 2013; Noreen et al., 2016); have enhanced penicillin production with *Penicillium* (Weber et al., 2012), and gluco-amylases used in food industry by *Aspergillus* cultures (Papagianni et al., 2002). Furthermore, the addition of surfactants has promoted higher yields of terpenes in *Saccharomyces* sp. (Kirby et al., 2014), has enhanced significant laccases activity in *Armillaria* sp. (Hadibarata and Kristanti, 2013), and have improved yields of monakolin K in *Monascus* (Zhang et al., 2014). Nevertheless, little is known about the effect of surfactants, cell immobilization and various carbon sources in shiitake submerged culture for eritadenine production. This study aimed to evaluate eritadenine yields obtained with shiitake mycelium in submerged cultures, employing three carbon sources, three surfactants, and two fungal immobilization supports.

MATERIALS AND METHODS

Fungal strain and propagation

Shiitake mushroom strain LEUCO4, was obtained by culturing the mycelium cloned from carpophores bought in a local supermarket in Colombia. The mycelium was identified taxonomically by 18SrRNA method, sequencing the ITS1 and ITS4 regions. The sequences obtained corresponded to a *Lentinula edodes* (Berk.) Singer strain (data not published yet), after alignment of sequence in BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A portion of mycelium was transferred to petri dishes with malt extract agar (MEA) (Oxoid Limited, Hampshire, UK), and incubated at 24°C within 12 days to grow (Stamets, 2000), and were then cut out to make 0.6 cm diameter agar-mycelium disks, used in all the inoculations.

Liquid basal media

As liquid basal media, a broth composed of 20 g/L of malt extract, 2 g/L of yeast extract (Oxoid Limited, Hampshire, UK) and 20 g/L of D-glucose were used, at a pH of 6±0.1. This was prepared according to the method described by Enman et al. (2008), and used as control.

Carbon sources assay

Flasks of 250 mL were filled with liquid basal media and used to evaluate different carbon sources. Instead of D-glucose, flasks were supplemented with three different carbon sources; D-mannitol, sucrose (both from Sigma-Aldrich Corp., USA) and mince potato. Minced potato was obtained by blending potatoes (from local markets, Colombia) for 5 min, according to method described by Sambamurthy and Nageswarra (1971). All the flasks were inoculated with six agar-mycelium disks and incubated within 20 days in an orbital shaker at 120 rpm and 24°C.

Surfactants assays

The effect of surfactants on eritadenine production was evaluated using tween 20, 40 and 80 (Merck Corp., Germany). Surfactants were added in a concentration of 5% to flasks with 100 mL of liquid basal media. Flasks were inoculated with six 0.6 mm agar-mycelium disks, and incubated within 20 days in an orbital shaker at 120 rpm and 24°C.

Immobilization assays

Two different fungal immobilization supports were used to produce eritadenine: (1) wood and (2) calcium alginate. Wood immobilization method was carried out using wood [*Jacaranda copaia* (Aubl.) D.

Don] disks, with 0.6 cm diameter and 0.5 cm of thickness. Then disks were soaked in distilled water until they reached 50% of moisture, using gravimetric method. Next, the autoclaved cold disks were placed over solid sterile MEA in a petri dish, and inoculated with a fragment of mycelium from a MEA culture; then the disks were incubated for 16 days at 24°C, to obtain wood-mycelium disks. Six wood disks were used to inoculate 250 mL Erlenmeyer's containing 100 mL basal broth and placed in orbital shaker for 20 days, at 24°C and 120 rpm. Alginate immobilization method was prepared by using a solution of calcium alginate (Phytotechnology Laboratories, Shawnee Mission, KS, USA) at 3% and circular agar-mycelium disks cut from a MEA culture (0.6 cm diameter and 0.5 cm of thickness). The disks were submerged in the alginate solution and mixed with 0.1 M $CaCl_2$ for 40 min, until the polymerization reaction occurred, according to the method described by Shide et al. (2004). The 1 cm in diameter beads obtained were put inside 250 ml flasks containing 100 ml of basal broth, and incubated together with the wood immobilization flasks in identical conditions.

Biomass measurement

Biomass was separated from broths using vacuum filtration using Wattman #4 filter. The biomass obtained was dried at 60°C in an oven for 24 h, and weighted in a Sartorius Practtum 313-1s scale (Germany). Biomass was expressed as dry weight (DW).

HPLC analysis

Filtrated broths from the last step were employed for eritadenine quantification. HPLC analysis were achieved adapting the method described by Enman et al. (2008), using a HPLC Agillent Technologies 1200 Series apparatus (USA). Samples were injected through a C18 column (5 µm, 150 mm×4.6 mm) using a mobile phase consisting of acetronitrile with a gradient from 2 to 60%, during the first 10 min; and 0.1% trifluoroacetic acid (TFA) from 60 to 2%, from 10 to 11 min (both solvents from Merck Corp., Kenilworth, NJ, Germany). Temperature was kept at 23°C and detection wavelength was 260 nm. An eritadenine standard (Santa Cruz Biotechnology Inc., Dallas, TX) was used for the calibration curve.

Statistical methods

All data were tested using variance analysis ANOVA and Tukey multiple range test ($p<0.05$), using R project 3.1.3 software (https://www.r-project.org/).

RESULTS AND DISCUSSION

Eritadenine yields by carbon sources

Eritadenine from three carbon sources (mannitol, sucrose and minced potato) were compared with the eritadenine standard, as shown in Figure 1. All carbon sources produced eritadenine after 20 days of incubation, as shown in Table 1. Maximal yield was obtained with sucrose, although, there were non-significant differences between the carbon sources and the control composed by D-glucose ($P<0.05$). These yields were similar than those reported by Enman et al. (2008) who obtained 10.23 mg/L of eritadenine after 20 days of incubation, with a shiitake strain cultured in the same basal broth of the present experiments. These results contrast with the improved ergopeptides production, promoted by using mannitol and potato mince in broths, as carbon sources in submerged cultivation of *Claviceps* fungi (Sambamurthy and Nageswara, 1971). The results indicated that carbon source is not a very determinant factor for eritadenine production with shiitake, but because eritadenine contains five nitrogen atoms, then probably nitrogen and other complex nutritional sources play a more important role in biosynthesis of such molecule than carbon sources (Enman et al., 2012). For instance, Enman et al. (2012) obtained slightly better yields of eritadenine (25 mg/L) than the present study, adding 10% of a cereals water extract (DDGS) to the broths. Since the extract contains proteins, fats and ashes, the authors hypothesized that some of those substances have a stimulatory effect on eritadenine biosynthesis. Similarly, higher ergothioneine (a potent antioxidant) yields, are produced by shiitake mycelium, by the addition of some amino acids (Tepwong et al., 2012b), or ammonium sulphate (Jang et al., 2016) as nitrogen sources in broths. Also, Mantle (2009) enhanced significantly the production of indolediterpenoid metabolites with *Claviceps* fungi under submerged cultivation, by adding tryptophan as precursor in the biosynthesis pathway and Zhang et al. (2016) demonstrated the importance of amino acids addition as precursors for the anticancer alkaloid chaetominine production with *Aspergillus* sp., in a dosage dependent manner, wherein high dosages probably produce inhibition of enzymes of the pathway, and low yields of the metabolite.

Biomass yields by carbon sources

All carbon sources tested produced different biomass yields compared to the control, as shown in Table 1. In this study, glucose (control) generated the highest yields, followed by potato mince; while sucrose and mannitol produced similar lower values. Biomass production under submerged culture was similar and slightly lower than that reported in previous studies (Tepwong et al., 2012b; Enman et al., 2008). The production of biomass with mince potato was higher than sucrose and mannitol. This is probably due to the protein contents of the potato tuber, which is a suitable nutritional source. Further, mince potato, is commonly used to produce shiitake biomass under submerged culture (Aminuddin et al., 2007) and in petri dishes (Mahamud and Ohmasa, 2008), in the form of potato dextrose agar (PDA), the most used media for edible mushrooms (Zagrean et al., 2017). For shiitake, potato acts mainly as a carbon source, due to its high starch content, but also it contains 2.4% of protein (Loyola et al., 2010). In submerged cultures, it is well established that nitrogen is a limiting factor to shiitake

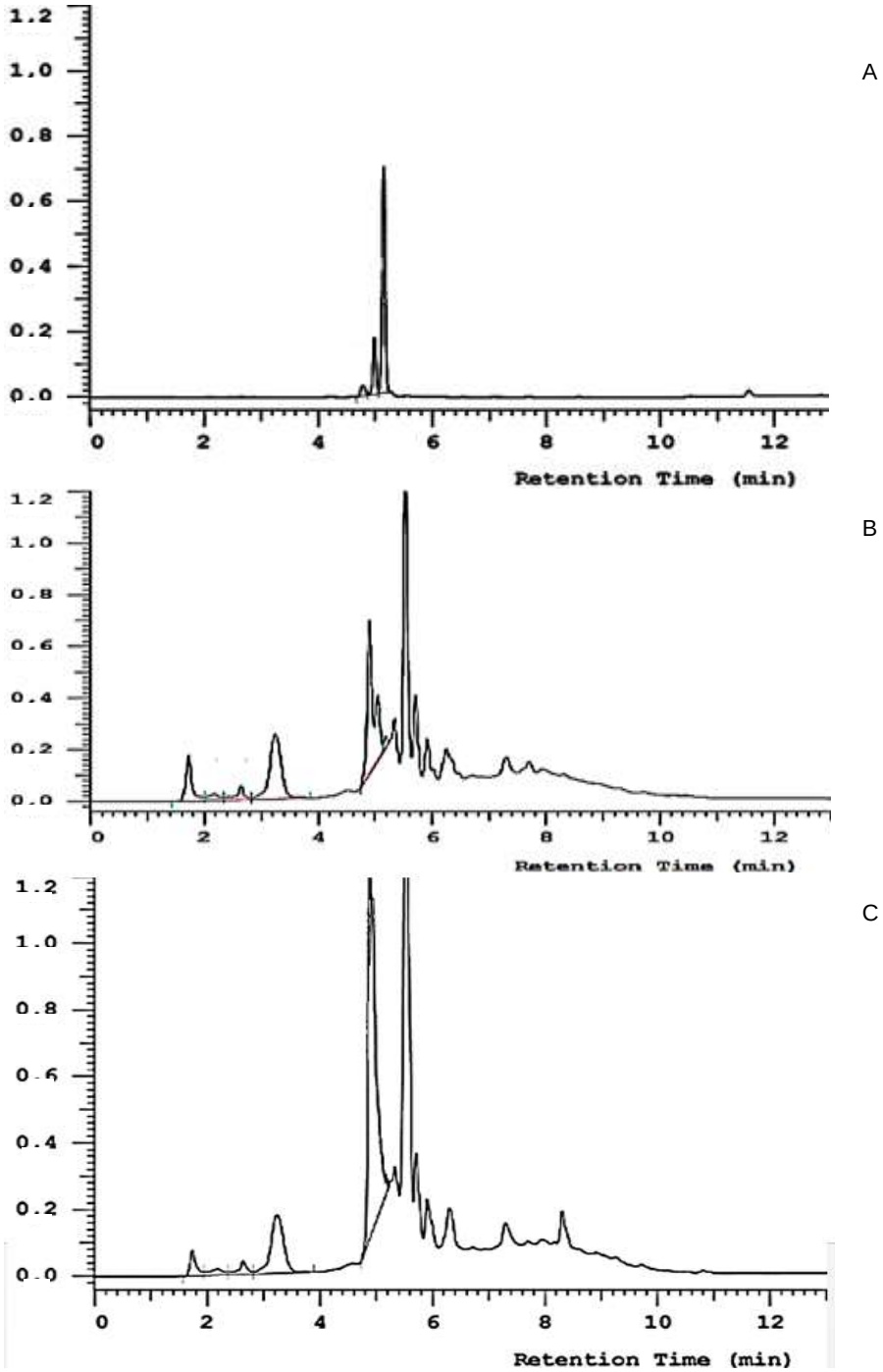

Figure 1. HPLC Chromatograms. (A) Standard reactive (Santa Cruz Biotecnology Inc), eritadenine corresponds to the peak at 4.5-4.6 min. (B) Chromatogram for mycelium cultured with surfactant tween 40 in basal broth. (C) Chromatogram for immobilized mycelium assay in alginate.

growth, even more than carbon sources (Tepwong et al., 2012b), moreover various hydrolytic and oxidative enzymes are more active, when nitrogen sources are provided to shiitake, which enhance metabolism and consequent growth (Pedri et al., 2015). Furthermore, in the case of D-mannitol these results are contradictory to others studies. D-Mannitol is reported as one of the best carbon sources to produce biomass in submerged cultures with important medicinal fungi like shiitake (Tepwong et al., 2012b), *Hydnum repandum L.* (Peksen

Table 1. Eritadenine and biomass yield by shiitake mycelium with three carbon sources, three surfactants and two immobilization supports in submerged cultivation.

Treatment	Eritadenine yield (mg/L)	Biomass (g/L)	Final pH
Alginate immobilization	88.06 ± 3.61[a]	-*	3.21 ± 0.07
Wood immobilization	14.83 ± 2.01[cd]	-*	3.36 ± 0.36
Tween20	72.40 ± 3.11[b]	2.90 ± 1.08[bc]	4.00 ± 0.14
Tween 40	13.06 ± 1,58[cd]	5.68 ± 0.23[a]	3.59 ± 0.34
Tween 80	18.66 ± 0.90[c]	5.00 ± 0.16[a]	3.25 ± 0.21
Sucrose	10.20 ± 1.91[d]	1.39 ± 0.04[d]	3.44 ± 0.01
Mannitol	8.43 ± 0.72[d]	1.09 ± 0.07[d]	3.37 ± 0.02
Potato, minced	8.00 ± 1,40[d]	2.35 ± 0.11[c]	3.57 ± 0.00
Control	8.73 ± 6.16[d]	3.93 ± 0.25[b]	3.55 ± 0.02

Values show averages of three data with their standard deviation. Similar groups have the same letter according to Tukey test (P≤0.05). *Not evaluated

et al., 2013) and *Amanita caesarea* (Scop: Fr.) (Daza et al., 2006). In this study D-mannitol generated lower biomass values, probably due to the complexity of the structure (an alditol), which is more difficult to metabolize than simple saccharides. Mannitol must be broken down to monosaccharides before entering the respiratory pathway. Accordingly, Tepwong et al. (2012b) obtained slightly better shiitake biomass yields with fructose or glucose than mannitol in submerged culture.

Finally, shiitake biomass in submerged culture has been obtained in higher yields using alternative nutritional sources. Lopez-Peña et al. (2013) obtained 9.5 g/L of biomass using a vid wood polar extract rich in polyphenols and some protein content. Harris-Valle et al. (2007) obtained 7 g/L using a polar vid wood extract. Nevertheless, those authors do no mention clearly the substance in the extracts responsible for the enhancement of growth, but possibly in polar vegetal extracts, polar molecules like polyphenols promote growth in shiitake (Beltrán-García et al., 2001). Supplementary, Hassegawa et al. (2005) reaffirmed the enhancement of shiitake biomass production by adding molasses to the broths, although the same result is not obtained when sucrose (main component of molasses) is used.

Eritadenine yields with surfactants

Similarly to others, surfactants have specific effects on fungal metabolism (Jakovljevi et al., 2014; Lazim et al., 2016). In this study, surfactant Tween 20 enhanced significantly eritadenine yield, as shown in Table 1. Eritadenine yield obtained with surfactant Tween 20 was two times that reported previously (Enman et al., 2008, 2012). Tween 20 did not enhance biomass production. Similarly, Kirby et al. (2014) found higher production of terpene in surviving yeast cells (*Saccharomyces* sp.), after being submitted to an inhibitory concentration of Tween 20 (up to 10%). As in *Saccharomyces* sp., maybe in shiitake, Tween 20 acts as selective pressure factor, stimulating growth of resistant cells that are producer of higher quantity of metabolites like eritadenine.

On the other hand, the positive enhancing effect on eritadenine yields promoted by Tween 20, lies also in the consequent permeation of fungal cell membranes. Surfactants in liquid media change the permeability of cellular membranes, which promote oxygen and nutrients to enter at a faster rate to cells, releasing metabolites continuously in the media (O´sulivan et al., 2004). Also, Tween 20 can acts as stress factor, stimulating shiitake to produce eritadenine. Accordingly, in *P. chrysogenum* metabolic changes are observed as stress result of adding detergents to liquid broths, with consequent production of different organic acids (Jakovljevi et al., 2014)

Finally, Tween 40 and 80 did not enhance eritadenine yields, but biomass production did significantly. Similar growth stimulation by Tween 80 in submerged cultures have been observed in *Beauveria* (Mwamburi et al., 2015), and *Armillaria* sp. (Hadibarata and Kristanti, 2013). Probably, surfactants enable mycelial cells to absorb nutrients at a faster rate promoting better cell multiplication (Lazim et al., 2016), which can be happening here for shiitake. The present results with tweens found an interesting tendency: Tween 20 promotes more metabolites, but not the growth, whilst tween 80 promotes good growth, but few metabolite production. Accordingly, in *Saccharomyces* sp. occurs the same: Tween 80 has no growth inhibitory effect, but tween 20 inhibits it, although the tolerant cells are at the same time good terpene producers (Kirby et al., 2014).

Effect of immobilized conditions on eritadenine yields

Calcium alginate promoted significantly eritadenine

Table 2. Correlation matrix for eritadenine content, biomass and pH, after 20 days of submerged shiitake mycelium cultivation.

	Eritadenine (mg/L)	Biomas	pH
Eritadenine (mg/L)	1	-0.019	**0.516**
Biomass		1	-0.068
pH			1

production to 88 mg/L as shown in Table 2. This concentration is more than eight times the eritadenine concentration reported by Enman et al. (2008), and almost three times that reported by the same authors in 2012. Nonetheless, fungal immobilization on wood disks was not successful and produced similar yields than carbon sources (Table 1). This is probably the first time calcium alginate is used for immobilization of shiitake mycelium under submerged conditions for eritadenine production. Maybe in shiitake cells, the alginate gel matrix produce some type of enzyme protection when conditions become unfavorable, resulting in a promoted eritadenine biosynthesis pathway. A superior stability of Aspergillus glucosydases exposed to pH 3,5 have been obtained by enzyme immobilization (Gonzales-pombo et al., 2014); similarly immobilized L-asparaginases from *Penicillium* show higher activity in pH 9 and 60°C in comparison to the same enzyme not immobilized (El-Refai et al., 2016). Besides, alginate is known as better support than others for immobilization of microorganisms and enzymes, for example Ünal and Kolankaya (2013) found that the superior activity and stability in a longer time period in *Trametes* sp. laccase, immobilized in alginate compared to kappa-carrageenan. On the contrary, wood seems to be a non-favorable support for eritadenine production, as various authors describe usage of different common immobilization supports to produce metabolites in submerged cultures with filamentous fungi, but with very different results, as higher enzymes production by immobilizing in polyurethane (PUF) rather than pine wood (PW) with *Dichomitus squalens* (P. Karst.) D.A. Reid, (Susla et al., 2007); also fivefold manganese peroxidase production was observed when *Irpex lacteus* (Fr.) Fr cultures were immobilized in PUF than in PW (Kasinath et al., 2003). Polyurethane like other polymers (e.g. Alginate) acts only as attachment place to the fungi, while wood acts not only as attachment place, but also as nutrient source to the fungi (Susla et al., 2007), nonetheless wood acts only as a carbon source mainly made of cellulose and other difficult to break polymers; then possibly its very low nitrogen content limits the shiitake mycelium to grow and its production of some metabolites, besides shiitake breaks wood slower in cultures with no nitrogen, than in nitrogen supplemented cultures, because some nitrogen sources stimulate more expression of enzymes like oxidases and hydrolases (Pedri et al., 2015). Besides, significant growth and enhanced production of the antioxidant erothioneine by shiitake in submerged culture have been evidenced, when monosaccharides based broths were supplemented with various amino acids as nitrogen sources (Tepwong et al., 2012b). The present results suggested that nutritional and culture conditions for high biomass yields, are not necessarily the same conditions for high eritadenine yields.

Finally, fungal immobilization has been a very well established technique for culturing different fungi in submerged culture, which enhances fungal metabolites yields; like the enzymes laccases with *Coriolopsis polyzona* (Pers.) with Ryvarden (Alaoui et al., 2008); the antibiotic peniciline with *Penicillium* (Weber et al 2012), and gluco-amylases with *Aspergillus* sp. (Papagianni et al., 2002). Additionally, immobilization offers various advantages like greater resistance of entrapped biomass to sudden physical-chemical changes, with consequent recyclability of biomass for various batches before losing metabolite productivity (Rodriguez, 2009; Gonzales-pombo et al., 2014, El-Refai et al., 2016). in this sense, Noreen et al. (2016) reported optimal activity at pH 3 and 60°C for alginate immobilized *Trametes* sp. laccase, compared to non-immobilized laccase with optimal activity in pH 4.5 and 45°C; and similarly better stability of alginate beads have been observed during *Aspergillus* fermentations to produce glucoamylases (Papagianni et al., 2002). In the present study, this resistance and stability of biomass and beads, seems to be present in shiitake submerged cultures, according to the improved yields of eritadenine obtained in alginate immobilization experiment.

Effect of final pH on eritadenine and bimass yields

pH was reduced from the initial value after 20 days of fermentation, as reported previously in fermentations with shiitake (Enman et al., 2012; Pedri et al., 2015), as a direct consequence of active aliphatic acids production, typical of wood decomposer fungi like shiitake (Hakala et al., 2005). The samples with tween 20 that produced the best eritadenine yield (72.40 mg/L) had final pH of 4.0, and samples with alginate immobilization (88 mg/L) of 3.2, indicating a reduction in pH, as shown in Table 1. Similarly, the best eritadenine yields of 25 mg/L obtained by Enman et al. (2012), reduced pH to 3.5 to 3.6. These

results indicate that the optimal pH for eritadenine production is in the range of 3 to 4.

On the other hand, in the case of biomass production there was a pH reduction as well. The best treatments for biomass production were tween 40 and tween 80 (5.68 and 5 g/L, respectively)and reduced pH to the range of 3.25 to 3.59. Similar results were reported previously, establishing superior growth rate at a pH of 3.5 to 4, as optimum values for shiitake biomass production in submerged culture (Hassegawa et al., 2005; Quaicoe et al., 2014).

In that order of ideas, pH values to obtain eritadenine and biomass are different. As shown in Table 2, the correlation analysis for pH and biomass did not give a significant value, while pH and eritadenine yield presented a medium correlation of 0.5. These results corroborate the conclusion of Enman et al. (2008 and 2012) that is not possible to obtain high eritadenine and biomass yields at the same time. Thus, the implementation of different incubation and nutritional factors, are necessary to obtain high eritadenine or biomass yields. This situation seems to happen with the surfactants evaluated. For instance, tween 20 produced the highest eritadenine yields, but not the higher biomass productions, while tween 80 was vice versa.

Additionally, in this study, possibly the more favorable pH value to produce eritadenine may stimulate certain growth morphology, that influences the pathway in a positive manner. pH is known as a very important factor affecting morphology and growth (Gibbs et al., 2000), at the same time morphology affects metabolites production by shiitake in submerged cultivation, such as higher quantities of ergothioneine are produced, when bigger pellets are formed, in dependent manner of stirring speed (Tepwong et al., 2012a). Similarly, when shiitake is cultured in bioreactors and shake flasks, pellets and free dispersed mycelium are formed respectively, with certain differences in eritadenine yields not statistically analyzed (Enman et al., 2008), with higher yields in free dispersed mycelium cultures. On the other side, as the purine molecules (like in eritadenine) are biosynthesized from aminoacides, the different yields of eritadenine in different final pH may be a result of the different profile of amino acids that are present in different pH values (Aminuddin et al., 2013).

Conclusion

Addition of surfactants to broths enhanced significantly eritadenine and biomass yield after 20 days of fermentation. Tween 20 increased eritadenine yields with about eight times, compared to broths without the surfactant. Tween 40 and Tween 80 incremented biomass yields (two-folds), but these surfactants did not increase eritadenine yields. Similarly, immobilization of the mycelium in alginate enhanced significantly eritadenine production in submerged culture by six times, compared to non-immobilized fermentations, whilst wood immobilization did not promote these same results. Finally, to enhance significantly eritadenine yields, it is necessary to bear in mind a correct pH value (in the range 3-4), combined with optimized broths, and incubation parameters like immobilization and surfactants towards obtaining eritadenine in producing good yields. Future studies regarding eritadenine production by submerged cultures should explore the relationship between yields and some nitrogen sources, lipids, salts, and growth morphology of shiitake mycelium. In that sense, the knowledge of the role of these parameters could offer new approaches towards improvement of this important biotechnological process. This information will be very useful in future commercial eritadenine production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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