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REVIEW ARTICLE

Beauveria bassiana secondary metabolites: a review inside their production systems, biosynthesis, and bioactivities

Metabolitos secundarios de *Beauveria bassiana*: una revisión dentro de sus sistemas de producción, biosíntesis y bioactividades

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ABSTRACT

The application of entomopathogenic fungi as biological control agent is an activity that has been carried out for a long time because they can regulate insect pests through specific formulations of spores and blastospores as infective units, as well as their endophytic ability in some plants offers protection against plant pathogens. Due to its wide distribution and antagonistic ability, *Beauveria bassiana* is widely used all around the world. Among its main mechanisms of infection is the

production of secondary metabolites that have antimicrobial properties and interfere with the immune response of insects. Currently, the fungal secondary metabolites have attracted the attention of the scientific community. The pigments produced by *B*. *bassiana*, mainly red and yellow, are relevant in the pathogenic process against insects and have antiviral, cytotoxic as well as antimicrobial properties against bacteria of clinical interest and phytopathogenic fungi. Therefore, this review details and compiles important aspects of the properties of the main *B. bassiana* metabolites, focusing on the fermentation systems used to obtain them, biosynthesis, aspects of toxicity and pest biocontrol.

Keywords: Bassianin, Biocontrol, Fermentation process, Oosporein, Pigment synthesis, Tenellin.

RESUMEN

La aplicación de hongos entomopatógenos para el control biológico es una actividad que se ha llevado a cabo desde hace mucho tiempo debido a que pueden regular plagas de insectos a través de formulaciones específicas de esporas y blastosporas como unidades infectivas, así mismo, su capacidad endofítica en algunas plantas ofrece protección contra microorganismos patógenos de las mismas. Debido a su amplia distribución y a su capacidad antagónica, *Beauveria bassiana* es ampliamente utilizado en todo el mundo. Dentro de sus principales mecanismos de infección está la producción de metabolitos secundarios que tienen propiedades antimicrobianas e interfieren con la respuesta inmune de insectos. Actualmente, los metabolitos secundarios de hongos han llamado la atención de la comunidad científica.Los pigmentos que produce *B*. *bassiana*, principalmente de color rojo y amarillo son importantes en el proceso patogénico contra insectos y tienen propiedades antivirales, citotóxicas, así como antimicrobianas contra bacterias de interés clínico y hongos fitopatógenos. Es por ello por lo que en esta revisión se detallan y recopilan aspectos importantes de las propiedades de dichos pigmentos, enfocándose en sistemas de fermentación utilizados para su obtención, biosíntesis, aspectos de toxicidad y biocontrol de plagas.

Palabras clave: Bassianina, Biocontrol, Proceso de fermentación, Oosporeína, Síntesis de pigmentos, Tenelina.

1. INTRODUCTION

Beauveria bassiana (Bals-Criv.) Vuill. 1912, is an entomopathogenic fungus with a wide spectrum of action against arthropods and insects (Mascarin & Jaronski, 2016). It is found in the soil and colonizing plants in an endophytic way (Uma Devi *et al*., 2008). Its entomopathogenic mechanism involve the infection, invasion and kill against target organisms. It depends on the ability of the fungus to evade the immune response of the insect. Cells such as spores or blastospores are used as infective propagules (Gibson *et al*., 2014). However, *Beauveria* also has the ability to produce bioactive metabolites to inhibit the growth of several pathogenic fungi (Narayanasamy, 2013). Bassianolides, beauvericin, oxalic acid, oosporein, bassianin, and tenellin are the prominent metabolites. The last three pigments have been reported with biological activities (Mascarin & Jaronski, 2016). Hence,

the use of entomopathogenic fungi as an alternative in pest control has advantages over other pathogens such as bacteria or viruses since its large-scale production is cheap and simple (Ramanujam *et al*., 2014). *Beauveria bassiana* has high virulence and a wide range of action against different arthropods (Kos & Celar, 2013).

The production systems of any fungal biopesticide are traditionally carried out by two systems: solid-state fermentation (SSF), to obtain aerial conidia, and the submerged fermentation (SmF), for the production of blastospores (both products are infective propagules used in commercial products) (Mascarin & Jaronski, 2016). Both systems are also suitable for the production and recovery of secondary metabolites, such as fungal bioactive pigments (Subramaniyam & Vimala, 2012). More than 40 years ago, the pigments produced by *Beauveria bassiana* were characterized and published (Basyouni *et al*., 1968; Jeffs & Khachatourians, 1997; Wat *et al*., 1977). However, recent publications have proved the role of each metabolites in the *B*. *bassiana* infection process as well as the importance of considering them as practical alternatives in biological control (Eley *et al*., 2007; Valencia *et al*., 2011).

The present document reviews information about the *Beauveria bassiana* secondary metabolites, its production, biosynthesis, toxicity and security aspects, as well as some essential characteristics to better understand the role of these secondary metabolites as a biological control agent.

2. *BEAUVERIA BASSIANA*, A FUNGAL ENTOMOPATHOGEN

Entomopathogenic fungi (EF) have the ability to infect their host directly through the integument causing several internal damages and consequently the death of target insect (Carrillo-Rayas & Blanco-Labra, 2009; Mascarin *et al*., 2013). Currently, the most important and studied genera of EF includes *Metarhizium*, *Beauveria*, *Paecelomyces*, and *Lecanicillium* (Borges *et al*., 2010). They are widely used around the world, because of its high insect infective and its minimal risk for non-target organisms and human health (Luo *et al*., 2014; Sahab, 2012). The EFs are mesophilic (25 °C - 30 °C) and aerobic. Their optimal growth depends on the appropriate conditions of humidity, nutrients (carbon and nitrogen sources), temperature, pH, water activity as well as quality and quantity of the inoculum (Muñiz-Paredes *et al*., 2017). Greater global awareness of the uncontrolled use of traditional chemical pesticides, coupled with new policies and regulations have generated the rise of biopesticides including fungal entomopathogens (Mahdavi *et al*., 2016). Particularly, *Beauveria bassiana* has a wide variety of target insects of different orders (Zimmermann, 2007). Its genetic variability is an important advantage to adapt to different environmental conditions. It attacks successfully several populations of insects. Thus, under natural conditions it produces aerial conidia as propagules responsible for reproduction and infection. This feature is exploited for its use as a biocontrol agent, commonly applied as a spore suspension on crops (El Kichaoui *et al*., 2017; Lohse *et al*., 2015; Lopez-Perez *et al*., 2015).

2. 1. GENERAL OVERVIEW ON THE PATHOGENESIS OF *BEAUVERIA BASSIANA*

To understand the production of pigments, first it must be understood the fungal metabolism and how this favors the infection process of *Beauveria bassiana*. The fungal primary metabolism handles cell proliferation and growth which encompasses all biochemical pathways, cell reproduction, viability and obtaining energy such as primary metabolites (organic acids, vitamins, amino acids, among others). However, once nutrients are depleted in the medium and differentiation and sporulation occur, the excretion of secondary metabolites begins (Cortés-Sánchez & Mosqueda-Olivares, 2013). These metabolites are peptides, enzymes, organic acids, antibiotics, pigments, among others (Lopes *et al*., 2013). *Beauveria* species have the ability to secrete beauvericin, bassianolide, beauveriolide, bassiacridin also yellow pigments like tenellin and bassianin. In addition, it produces the non-peptide red pigment oosporein which are biologically active metabolite that have not been reported to enter the food chain or accumulate in the environment. Also, it presents low toxicity in humans (Amin *et al*., 2010; Chávez-Ibañez *et al*., 2014; Patocka, 2016; Vega *et al*., 2008). These metabolites are not essential for *B. bassiana* growth and development. In fact, they are exploited for their antifungal, antitumor or antibacterial activity (Chintapenta *et al*., 2014). The secondary metabolites have an important role in fungal diversification and adaptation to several ecological niches (da Costa Souza *et al*., 2016).

The infection process of *Beauveria bassiana* in insects (Fig. 1) begins with the adhesion of the spores to the cuticle through hydrophobins and adhesins such as Mad 1, Mad2 Adhesin-like proteins y Hyd 1, Hyd 2, Hyd 3—Hydrophobins. The formation of a germinal tube or appressorium, develops the penetration peg which exerts mechanical pressure perforating the cuticle supported by hydrolytic enzymes including lipases, proteases (serine endoprotease, serine elastase) and also chitinases (endochitinases and exochitinases). Then, there is a morphogenetic differentiation of the spores to blastospores. It colonizes the hemocoel consuming the nutrients of the hemolymph as well as secreting toxins that contribute to the modification of its structural integrity by inhibiting the selective process or its enzymes besides interfering with its regulatory system accelerating the death of the insect. Once the insect is dead, the cuticle is breached again from the inside out in order to emerge conidiophores that sporulate on the body of the insect and can begin the infection in other arthropods by spreading the spores (Borges *et al*., 2010; Mascarin & Jaronski, 2016; Valero-Jiménez *et al*., 2016; Daza *et al*., 2019; Litwin *et al*., 2020) The production of specific pigments confer to the spores resistance to ultraviolet radiation, and to environmental conditions that could be detrimental to the fungus (Chintapenta *et al*., 2014). The infection process lasts up to 14 days and depending on the fungal species the first symptoms of infection occur 7 days after the first contact of the fungus with the insect (Litwin *et al*., 2020).

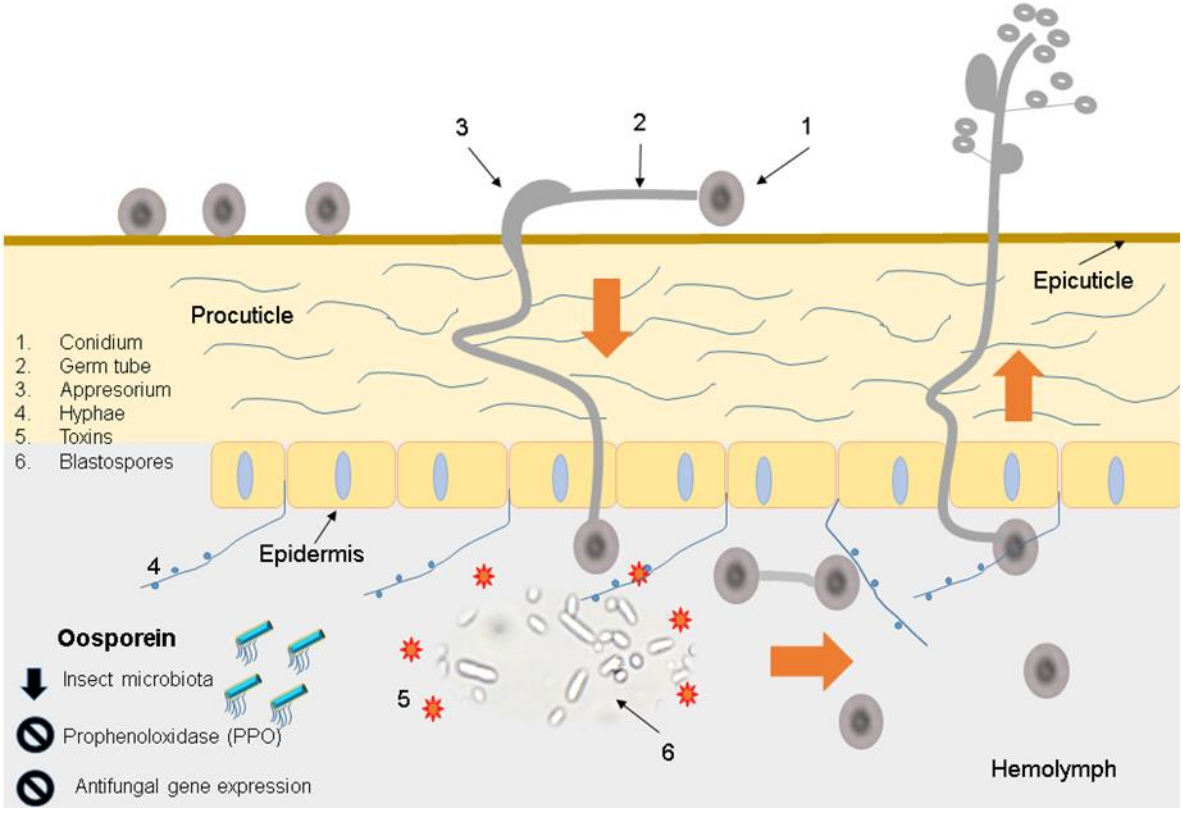

Fig. 1. General overview of the infection process in insects by *Beauveria bassiana*. The cycle of infection (showed by orange arrows) begins with the adhesion of asexual spores to the insect (dispersed by air, rain, etc.) which attack the cuticle germinating and forming the germinal tube and either an appressorium or penetration peg. From these structures, begins the penetration of the cuticle layers by enzymatic attacks and mechanical pressure until reaching the hemolymph. Once in the hemolymph, the spores suffer a morpho-genetic differentiation to blastospores to consume nutrients, colonize internal tissues and evade the immune response supported by the secretion of toxins. Once the above is achieved, the insect die emerging conidiophores that will produce a new cycle of infection on other insects.

The insects have the capability to defend themselves from the attack of entomopathogenic fungi. However, the environmental conditions (temperature, sunlight, humidity, rain, wind) determine the success of the contact of the spores to the insect cuticle and its possible penetration and germination. In the cuticle, insects promote the release of benzoquinones, glandular secretions of volatile antifungal compounds such as iridoid monoterpenes (epi-chrysomelidial) and salicylaldehyde, antimicrobial peptides such as β-1,3-glucanases, chitinase and protease inhibitors. In addition, the melanization contributes to the inhibition of hyphae growth and spore germination. Furthermore, the synergism between

grooming, burrowing and even temperature rise induced by sun can limit the infection of entomopathogenic fungi (Ortiz-Urquiza & Keyhani, 2013). Internally, the insect's immune system plays an important role in regulating the response to infection by entomopathogenic fungi by involving hemocytes (granulocytes and plasmocytes) in hemolymph, which promotes the encapsulation of *B*. *bassiana* hifal bodies (Vertyporokh *et al*., 2019). In terms of humoral immunity, the induction of heat shock (43 °C/15 min) in *Galleria mellonella* larvae promotes the expression of antifungal compounds such as gallerimicin and galiomicin, and also increases the activity of lysozyme (Wojda *et al*., 2009). Also, the enzyme prophenoloxidase plays an important role in the defense against *B*. *bassiana* by promoting melanization, phagocytosis, encapsulation, cytotoxic reactions, among others (Mc Namara *et al*., 2019). Meanwhile, the expression of antifungal peptides such as Tenecin 3 in *Tenebrio molitor* improves its survival from *B*. *bassiana* infection by decreasing the presence of blastospores in hemolymph (Maistrou *et al*., 2018).

3. *BEAUVERIA BASSIANA*, A FUNGAL ENDOPHYTE

Endophytic fungi are those that have the ability to live in plant tissues without causing damage in addition to obtaining benefits, both nutrients for growth and resistance against pathogens and can live in internal and external tissues of the plant (roots, bark, stem and leaves) (Hu & Bidochka, 2019). *Beauveria bassiana* is endophyte on more than 20 plants, incluiding *Zea mays*, *Solanum lycopersicum* and *Phaseolus vulgaris* (McKinnon *et al*., 2017). The sites of location of *B*. *bassiana* are the leaves, stems and roots and internally leaf apoplast, xylem, stomatal openings, parenchyma and vascular tissue (Vega, 2018).

Beauveria bassiana has shown antifungal activity against some phytopathogenic fungi such as *Gaeumannomyces graminis* var tritici J. Walker (Ascomycota: Sordariomycetidae), *Fusarium oxysporum* E.F. Smith & Swingle (Ascomycota: Hypocreales), *Fusarium oxysporum* f. sp. cepae (Hanzawa) W.C. Snyder & H.N. Hansen (Ascomycota: Hypocreales), *Fusarium oxysporum* f. sp. lycopersici (Sacc.) W.C. Snyder & H.N. Hansen (Ascomycota Hypocreales), *Armillaria mellea* (Vahl) P. Kumm (Basidiomycota: Agaricales), *Rosellinia necatrix* Berl. ex Prill. (Ascomycota: Xylariales), *Botrytis cinerea* Pers. (Ascomycota: Helotiales), *Pythium ultimum* Trow (Oomycota: Pythiales), *Pythium debaryanum* R. Hesse (Oomycota: Pythiales), *Pythium myriotylum* Drechsler (Oomycota: Pythiales), *Pythium myriotylum*, *Septoria nodorum* (=*Phaeosphaeria nodorum* (E. Müll.) Hedjar. (Ascomycota: Pleosporales) and *Rhizoctonia solani* (Ownley *et al*., 2010). The main defense mechanisms against those fungi are antibiosis (secondary metabolites), competition (for both plant establishment sites and nutrients), plant growth promotion, and induction of systemic resistance (Jaber & Ownley, 2017). Barra-Bucarei *et al*. (2019) evaluated the antifungal capacity of native strains of *Beauveria bassiana* (RGM393, 461,547, 557, 565, 570, 632, 644, 657, 731 and 2519) in tomato plants (*Solanum lycopersicum* L.) and chili pepper (*Capsicum annuum* L.) against the phytopathogenic fungus *Botrytis cinerea*. They found in an antagonism test that radial growth inhibition of the pathogen (PRGIP) by *B*. *bassiana* was up to 39% with strain RGM 644 based on mechanisms of antibiosis or competition rather than mycoparasitism. In the case of *in plant* assessment, 1 ×

10^6 conidia/mL^{-1} of *B*. *bassiana* was inoculated into the plant growth substrate (300 mL; 2: perlite, 2: peat, 2: compost and 1: vermiculite) of both tomato and chili pepper, using a concentration of the pathogen of 1×10^5 conidia/mL^{-1} which was sprayed on the leaves of each plant, including a control (without the endophyte). The percentage of surface area affected by the pathogen (PSAP) in chili pepper leaves was lower with the RGM 547 strain (2%) compared to the control inoculated with the pathogen (63%). As for the leaves of tomato plant, the lowest percentage of PSAP was 2.5% (strain RGM 644) compared to 40% of the pathogen. In each case, signs of necrosis and chlorosis were found on the leaves inoculated with *B*. *cinerea* (control). The endophytic effect of *B*. *bassiana* in the plants showed a pattern of colonization of roots, stems and leaves. On the other hand, Rivas-Franco *et al*. (2019) evaluated the effect of maize seeds covered by *Metarhizium* spp. and *Beauveria bassiana* Bb21 with the presence of *Fusarium graminearum* and second to third instar larvae of *Costelytra giveni*. Their study showed that *B*. *bassiana* Bb21 exerts a negative effect on the growth of coated spore maize seeds at a concentration of 1×10^8 conidia/mL. This was due to a significant reduction in dry weight of shoots and roots suggesting that *B*. *bassiana* is benefited from nutrients without synergistic benefit to the plant, possibly due to the lack of specificity of the entomopathogenic fungus to the maize plant. Meanwhile, it is not capable of infecting *C*. *giveni* but it decreases the symptoms of necrosis by *F*. *graminearum* in the roots by less than 30 %. A study by Tall & Meyling (2018) found that nutrient availability is an important factor in the growth of corn plants. In their work they used seeds soaked with *B*. *bassiana* GHA spores (5.9×10^4 conidia/mL) and with sterile Triton-X (0.05%) as control. At the beginning of the experiment all samples (N=54 treated and N=54 untreated) were fertilized (16.9 mg N per plant) and then fertilized daily (high concentration of nutrients) and unfertilized daily (low concentration of nutrients), watering both daily. At the end of the experiment it was concluded that the greater quantity of nutrients resulted in greater biomass concentration. Therefore, *B*. *bassiana* only exerts a positive effect on the growth of corn plants when the availability of nutrients is high.

Then, what is the relationship with other plant endophytes? In the study conducted by Senthilraja *et al*. (2010), it was found that *Beauveria bassiana* (strain B2) together with *Pseudomonas fluorescens* (TDK1 and Pf1 strains) have the ability to control the insect pest *Aproaerema modicella* as well as the plant pathogenic fungus *Sclerotium rolfsii*, which causes the collar rot disease. Likewise, Shrivastava *et al*. (2015) determined that *Rhizophagus intraradices* together with *B*. *bassiana* have the ability to induce the production of new terpenoid compounds in tomato plants (*Solanum lycopersicum* Mill cv. Castlemart). *Rizophagus intraradices* produced the terpenes myrcene and α-phellandrene while *B*. *bassiana* the terpene myrcene, and even increase 3-fold times the concentration of those produced by the plant itself (monoterpenes: δ-2-carene, sabinene; sesquiterpenes: δ-elemeney, β-elemene, (E)-β-caryophyllene and α-humulene). Also, the combination of both fungi decreases the weight (<0.07 g) of second instar larvae of *Spodoptera exigua* Hübner compared to the control (0.084 g). Interestingly, the symbiotic fungus-plant relationship can improve the production of secondary plant metabolites. In a study by Espinoza *et al*. (2019) showed that *B*. *bassiana* (1×10^4 conidia/mL^1) increased

the concentration of secondary metabolites of *Allium schoenoprasum* (polyphenols, flavonols and alkaloids) more in leaves than in roots. This represents an opportunity as a defense mechanism of the plant against pathogens and an alternative for its use in traditional medicine.

Other endophytic applications of secondary metabolites are as plant detox. Haruma *et al*. (2019) demonstrated that oosporein produced by *Chaetomium cupreum*, a root endophyte fungus of *Miscanthus sinensis*, has the ability to detoxify Aluminium with a stability constant in the Al-oosporein complex of 12.1, which was higher than phytosiderophores produced by the plant as citric acid (8.0) and malic acid (5.4). Relevantly, in conjunction with chlorogenic acid (stability constant 15.1) the detoxification of Al is higher (0.11 ± 0.03 µmol/100 mg Dry Weight) compared to the control, i.e. without the presence of *C*. *cupreum* (0.03 ± 0.01 µmol/100 mg Dry Weight). Also, such Al detoxification contributes to the growth of the plant, since otherwise high amounts of Al in the cell walls of *M*. *sinensis* would inhibit its growth as observed in the root length (control= 3.5 ± 0.4 cm, inoculated= 36.5 ± 3.4 cm). These results suggest that *M*. *sinensis* inoculated with *C*. *cupreum* can be used as a strategy for phytoremediation of mine site soils.

4. CULTIVATION SYSTEMS OF *BEAUVERIA* SPP. AND ITS APPROACH FOR THE PRODUCTION OF SECONDARY METABOLITES

Different culture media and fermentation systems have been used to culture *B*. *bassiana*. Depending on their nutrient composition, pH, temperature, light, availability of water and the mixture of surrounding atmospheric gases, changes in vegetative growth, colony morphology, pigmentation and sporulation have been observed (Pradeep *et al*., 2013). In laboratory scale, different pigments can be produced depending on the nutrients of culture media, temperature of incubation, and fermentation system (Kulandaisamy-Venil & Lakshmanaperumalsamy, 2009). Most fungal strains are capable to use various carbon and nitrogen sources, as these nutrients influence their growth and the type and yields of pigments produced (Celestino *et al*., 2014). It should be taken into account that for the production of pigments, glucose is the ideal carbon source, and nitrogen sources such as ammonium and peptone confer good growth and increase the concentration of the pigments (Kumar *et al*., 2015). In addition, different fermentation systems are able to provide high yields of pigments (Velmurugan *et al*., 2010). It should be mentioned that in order to obtain high production of pigments, the physicochemical conditions, as well as the culture conditions, must be kept under control (Neera *et al*., 2017).

4.1. Submerged fermentation (SmF)

SmF is the method used by the biotech industry, to produce most of the biological control agents (BCAs) because the process is short and easy. The bioprocess could be carried out under controlled physicochemical conditions such as nutrients, oxygen diffusion, heat transfer, temperature, aeration, agitation and pH (Mascarin *et al*., 2015; Pham *et al*., 2009). Under SmF *B. bassiana* produces yeast-like cells called blastospores, which germinate in less than 10 hours. The blastospores are suitable to be used as an infective structure in control pests (Gibson *et al*., 2014;

Hussain *et al*., 2012). In addition, under SmF conditions certain strains can secrete important metabolites such as pigments (Fig. 2) (El-Ghany, 2015).

The production of *B*. *bassiana* pigments has been reported by Amin and coworkers (2010) who obtained a red extract from a culture medium consisting of 40 g/L of glucose, 5.0 g/L of yeast extract and enriched with several salts at conditions of 27 °C, pH 6.0 and 300 rpm. In addition, there are reports on the production and characterization of the pigment oosporein obtained from *Lecanicillium aphanocladii* (oosporein is also produced by *Beauveria* spp). It was used potato dextrose broth as culture media, agitation at 150 rpm and 30 °C for 7 days in dark conditions (da Costa Souza *et al*., 2016). Also, it has been reported the production of oosporein using potato dextrose broth from the fungus *Cochliobolus kusanoi* (Alurappa *et al*., 2014) as well as from *Chaetomium cupreum* in liquid culture using the same media at 150 rpm at 25 ° C for 4 days (Mao *et al*., 2010) (Fig. 2 A).

Regarding to the yellow pigments tenellin and bassianin, their production has been achieved by liquid medium composed of glucose, ammonium tartrate and some salts at 25 °C, 220 rpm for 14 days (Basyouni *et al*., 1968). On the other hand, tenellin production also has been possible through a liquid culture medium from mannitol, boric acid and some salts (Eley *et al*., 2007).

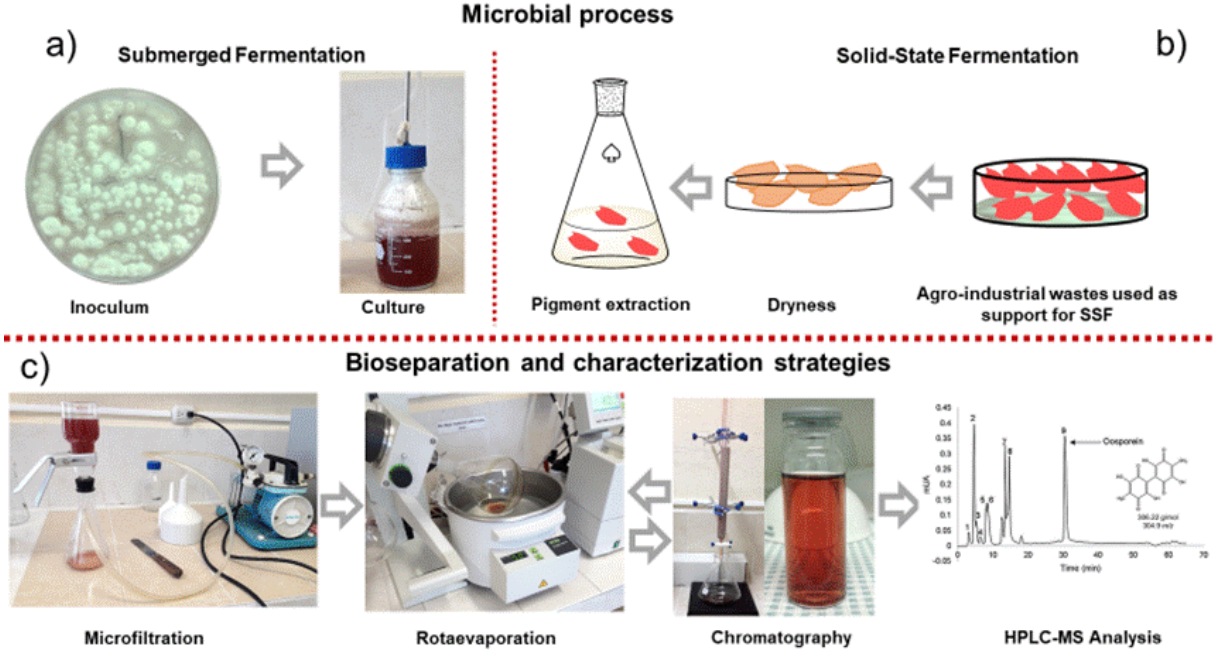

Fig. 2. Schematic diagram of the bioprocess for the production, bioseparation and characterization strategies of secondary metabolites from *Beauveria bassiana* with emphasis on oosporein. A) SmF. B) SSF using agro-industrial wastes as solid support-substrate. C) The bio separation and characterization processes proposed for both SmF and SSF.

Table 1. Fungal secondary metabolites produced by submerged (SmF) and solid-state fermentation (SSF).

Culture system	Strain	Metabolite	Culture media composition	Yield	Reference
SmF	***Cochliobolus kusanoi***	Oosporein	Potato dextrose broth	Not reported	(Alurappa *et al*., 2014)
	Beauveria bassiana	Red pigment	Glucose (40g/L), yeast extract (5.0g/L), $NaNO_3$ (1.0g/L), KH_2PO_4 (2.0g/L), KCl (0.5g/L), $MgSO_4 \cdot 7H_2O$ (0.5g/L), $FeSO_4 \cdot 7H_2O$ (0.02g/L).	480 mg/L	(Amin *et al*., 2010)
	Chaetomium cupreum	Oosporein	Potato dextrose broth (Potato, 200g/L and glucose, 20g/L)	Not reported	(Mao *et al*., 2010)
	Lecanicillium aphanocladii	Oosporein	Potato dextrose broth	8.5 mg/100mL	(da Costa Souza *et al*., 2016)
	Beauveria caledonica	Oosporein	Sabouraud dextrose liquid medium	5 mg	(Mc Namara *et al*., 2019)
	Beauveria bassianal B. tenella	Oosporein	D-Glucose (20 g/L), Difco neopeptone (20 g/L), glycine (5 g/L), KH_2P0_4 (2 g/L), $MgS0_4 \cdot 7H_20$ (1 g/L).	100mg/L	(Basyouni *et al*., 1968)
	Beauveria bassiana	Tenellin	d-manitol (50 g/L), KNO_3 (5 g/L), KH_2PO_4 (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), NaCl (0.1 g/L), $CaCl_2$ (0.2 g/L), $FeSO_4 \cdot 7H_2O$ (20 mg/L), mineral ion solution-2 (10 mL, $ZnSO_4 \cdot 7H_2O$ (880 mg/L), $CuSO_4 \cdot 5H_2O$ (40	Not reported	(Eley *et al*., 2007).

			mg/L), $MnSO_4 \cdot 4H_2O$ (7.5 mg/L), boric acid (6 mg/L), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (4 mg/L).		
	Beauveria bassiana*l *B. tenella	Tenellin/ Bassianin	D-Glucose (20 g)/L, ammonium tartrate (4.6 g/L), KH_2PO_4 (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), NaCl (0.1 g/L), $CaCl_2$ (0.1 g/L), $CuSO_4+5H_2O$ (3.93 x 10^4 g/L), H_3BO_3 (5.7 x 10^{-5} g/L), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (3.68 x 10^{-5} g/L), $MnSO_4 \cdot H_2O$ (6.1 x 10^{-5}g/L), $ZnSO_4 \cdot 7H_2O$ (8.79 x 10^{-3}g/L), $FeSO_4 \cdot 7H_2O$ (9.96 x 10^{-4}g/L).	Both up to 60 mg/L (depending salts and glucose concentration used)	(Basyouni *et al*., 1968)
SSF	***Beauveria bassiana* Nov. EO-1**	Oosporein	Vermiculite 20%, wheat bran 30%, aqueous sodium alginate 1%/ YM agar	Not reported	Eyal *et al*., 1994
	Acremonium cavaraeanum	Oosporein	Rice 200 g, soybean meal 6 g and pH 6.8	Not reported	Xu *et al*., 2017
	***Fusarium proliferatum* CECT 20569**	Beauvericin	Solid wheat 100 g	Not reported	Meca *et al.,* 2010
	***Fusarium subglutinans* ITEM-1434**	Beauvericin	Different grains (oat, maize, rye, barley, wheat and rice kernels)	From 93-704 µg/g of substrate	Kostecki *et al*., 1999

The characteristics of the liquid culture media used to produce pigments from *B. bassiana* commonly oosporein are already reported (Table 1). It is important to highlight that most of the culture condition mentioned above reports similar range of temperature, pH, agitation, as well as nutrients. However, to produce pigments as tenellin and bassianin, there is little information available in the literature. So, it somehow defines the optimal production conditions according to their cultivation requirements.

4.1.1. Downstream process for *B. bassiana* pigments

The extraction process of beauvericin and the yellow pigments (tenellin and bassianin) have been carried out by Soxhlet. The pigments are extracted from the mycelium using polar solvents such as acetone. Then, the obtained extract usually evaporated in a vacuum in order to dry it by removing lipid with petroleum ether, removing mannitol with water and drying with benzene. The latter solution is submitted into a column of silicic acid, then chloroform is used to obtain yellow pigment (Basyouni *et al*., 1968). Another study suggests centrifugation of the culture medium in order to precipitate the mycelium and recover the pigment with acetone. The chromatographical analysis coupled to mass spectrometry (HPLC-MS) of the acetone extract allowed to identify tenellin at λ_{max} 342 nm and *m*/*z* $[M+H]^+$ 370 (Eley *et al*., 2007).

The oosporein extraction have been carried out from the culture media by filtering and using ethyl acetate followed by acetic acid at pH 3 to obtain the crude red extract. Subsequently, it is fractionated with methanol and concentrated in a rotary evaporator. The use of equal amounts of ethyl acetate in relation to the liquid culture allows to obtain the red crystals. These crystals are soluble in non-polar solvents such as dimethyl sulfoxide (DMSO) and ethyl acetate but no in distilled water (Fig. 2 C) (Mao *et al*., 2010). This pigment is also extracted from the culture broth with ethyl acetate 1:2 v/v followed by evaporation with the aid of a vacuum evaporator until obtaining dry pigment. It is important to mention the pigment is soluble in water due to it is produced extracellularly, which would facilitate its production at a bigger scale (Amin *et al*., 2010). However, it is necessary to optimize the production conditions by designing experiments that allow the validation, through small-scale experiments, of the ideal conditions for the production of pigments above mentioned such as temperature, pH, agitation and the composition of the culture medium so they are carried on an industrial scale, allowing savings of both time and resources (i.e. economic, material, etc.) (Aguilar-Zarate *et al*., 2014).

The purification of oosporein is developed by using a preparative reverse phase high-performance liquid chromatography (RP-HPLC) and then the compound is crystallized with a methanol-water system (95:5). On the other hand, it is possible to use a Sephadex LH-20 column to purify the compound (previously concentrated on a rotary evaporator with ethyl acetate) and then use thin layer chromatography (TLC) for characterization. As a recommendation, the identification of the compound could be carried out by an MS/MS and NMR analysis (da Costa Souza *et al*., 2016). Also, it is possible to characterize the compound by Fourier transform

infrared (FT-IR), proton nuclear magnetic resonance (HNMR) and heteronuclear single quantum coherence (HSQC) spectral analysis (Alurappa *et al*., 2014).

4.2. Solid-state fermentation (SSF)

SSF involves the growth of microorganisms on solid substrate particles under no visible water as well as nutrients and pH conditions. The solid material on SSF can act at the same time as a carrier and a source of carbon but sometimes is only used as a carrier (inert material or not) which is impregnated with liquid medium. Furthermore, natural or synthetic materials can be used as a substrate (Lopez-Perez *et al*., 2015). The feasibility of those substrates for SSF is variable and depending on the physicochemical features as porosity, water absorption and packing density (De la Cruz Quiroz *et al*., 2014). The main substrate used for spore mass-production of *Beauveria bassiana* on SSF is rice, but also, agro-industrial wastes have been used. In terms of yield, is been proved the use of agro-industrial waste is higher more than obtained using rice (Fig. 2 B, C) (Lopez-Perez *et al*., 2015).

There is little information related to the production of secondary metabolites by *B. bassiana* under SSF. However, it is well known its capability for growing up in cereals and producing mycotoxins (EFSA, 2014). Hence, it is capable to produce secondary metabolites by SSF (Table1). It has been reported the production of oosporein in SSF by *B. bassiana* Nov. EO-1 using wheat bran as substrate and vermiculite as solid support (Eyal *et al*., 1994). Also, species such as *Fusarium* and *Acremonium* are capable to produce oosporein and beauvericin in SSF (Xu *et al*., 2017; Meca *et al*., 2010; Kostecki *et al*., 1999). The extraction of metabolites from solid fermented mass is labor intense. It involves the solubilization of metabolites, the filtration and/or centrifugation and sometimes a chromatographical separation (Fig. 2).

5. RESPONSE MECHANISMS OF *B. BASSIANA* FOR THE SYNTHESIS OF METABOLITES

Beauveria bassiana can secrete small molecules derived from secondary metabolism once an infection begins in different organisms to generate a toxic response (Valencia *et al*., 2011). Once germinal tube penetrated the cuticle of the insect the blastospores releases some toxins that contribute to the death of the insect by modulating both the cellular and humoral immune response and decreasing the insect's nutrient and microbial load (Patocka, 2016; Ortiz-Urquiza & Keyhani, 2016; Mc Namara *et al*., 2019) such as the case of oosporein who is not directly related to the pathogenicity mechanism of *B. bassiana* but has antimicrobial activity against insect microbiota (Fan *et al*., 2017).

5.1. Oosporein: a pigment of benzoquinone nature

Once the infection in the host is started, *B. bassiana* gradually begins to release into the interior of the hemocoel the red pigment, that subsequently causes a pathological state due to its biological activity as a mediator of pathogenesis (Mosqueira *et al*., 2015; Liu *et al*., 2017).

Oosporein is a molecule derived from the C2 symmetrical 2,5-dihydroxybenzoquinone (Fig. 3 A), found in several soil fungi, usually in some *Beauveria* species (Luo *et al*., 2015) such as *B*. *brongniartii*, *B*. *bassiana* and *B*. *caledonica* (Zimmermann, 2007; Mc Namara *et al*., 2019), in addition to some fungi considered as biological control agents (Love *et al*., 2009). It was first identified in 1960 as a symmetric 1,4-dibenzoquinone derivative from *B*. *bassiana*, which has relevant bioactive activities (Feng *et al*., 2015). However, by 1944 it had already been described as a colorant produced by *Oospora colorans* (Molnár *et al*., 2010). The purified compound was identified with the empirical formula $C_{14}H_{10}O_8$ and by means X-ray diffraction analysis of a single crystal, this molecule was identified as 3,3',6,6'-tetrahydroxy-4,4'-dimethyl-1,1'-bi (cyclohexa-3,6-diene)-2,2´,5,5´-tetraone (Mao *et al*., 2010; Alurappa *et al*., 2014).

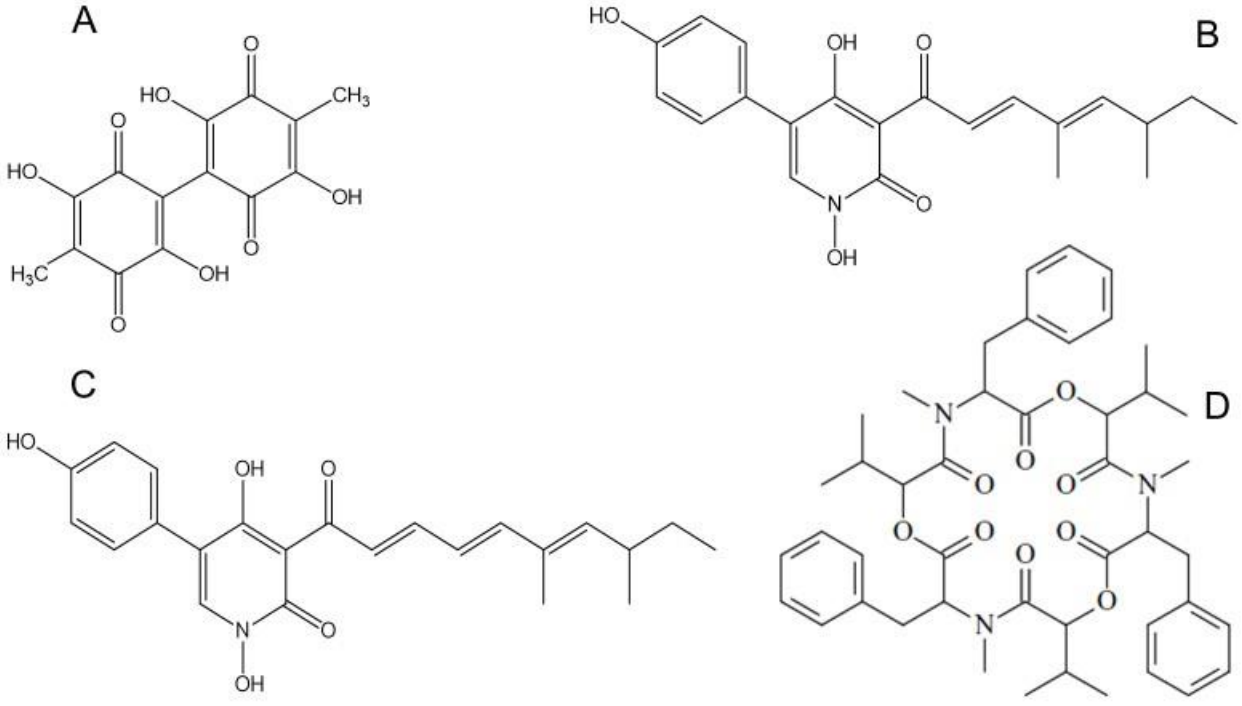

Fig. 3. Chemical structures of A) oosporein; B) tenellin, C) bassianin and D) beauvericin, produced by *Beauveria* spp.

The physicochemical properties of oosporein indicate that at pH values above 3, the solubility (basal solubility Cs0 =24.8 ± 0.3 µM) and the degree of dissociation (ionization constant pKa =2.42 ± 0.02) increases exponentially. In addition, depending on the temperature the solubility increases in acidic aqueous solutions. Oosporein has three levels of deprotonation [oosporein]$^{-}$, followed by [oosporein]$^{2-}$ (pK_a =6.79 ± 0.08) and [oosporein]$^{3-}$ (pK_a =9.19 ± 0.03). As for stability, the influence of pH and temperature decrease of the half-life of the compound. For

example, under conditions of 23 °C to pH 8 only 12 days and at 53 °C with the same pH value, 8 hours. While in moderate acidic conditions it is more stable (for example pH <5). This shows that the nature of the compound is a strong organic acid with very low lipophilicity (Seger *et al*., 2005).

5.2. Tenellin and bassianin: pigments derived from 2-pyridone

Tenellin and bassianin are yellow 1,4-dihydroxy-2-pyridone pigments isolated from several species of *Beauveria*. They are formed from a chain of polyketide reduced (pentaketide in the case of tenellin and hexaketide for bassianin) (Fig. 3 B and C), with the portion amide of the tyrosine (Molnár *et al*., 2010). These pigments are produced by *B*. *bassiana* and *B*. *tenella* (Patocka, 2016).

The yellow pigment tenellin can be isolated from the mycelium of *Beauveria* spp. and has an empirical formula $C_{21}H_{23}NO_5$ (Jirakkakul *et al*., 2015). From chemical and spectroscopic evidence, the nomenclature for tenellin and bassianin are 3-[(E,E)-4,6-dimethylocta-2,4-dienoyl] and 3-[(E,E,E)-6,8-dimethyldeca-2,4,6-trienoyl], respectively. They are derivatives of 1,4-dihydroxy-5-(p-hydroxyphenyl)-2(1H) –pyridine (Patocka, 2016). However, structurally they only differ in one extension of the side chain (Gibson *et al*., 2014).

To date, there is no documented information on the physicochemical characteristics of both tenellin and bassianin. Hence, it is important their study to know their interactions with the environment (Seger *et al*., 2005). After analyzing the production conditions and the types of pigments produced by *B*. *bassiana*, the chemical characteristics of the metabolites are shown in Table 2.

Table 2. Chemical information on the main pigments produced by *Beauveria bassiana*.

Pigment name	IUPAC name	Formula	Molecular weight	Reference
Oosporein	2,2',5,5'-Tetrahydroxy-4,4'-dimethyl-1,1'-bi(1,4-cyclohexadien-1-yl)-3,3',6,6'-tetrone	$C_{14}H_{10}O_8$	306.226 g/mol	(Mao *et al*., 2010)
Tenellin	3-[(2E,4E)-4,6-Dimethyl-2,4-octadienoyl]-1,4-dihydroxy-5-(4-hydroxyphenyl)-2(1H)-pyridinone	$C_{21}H_{23}NO_5$	369.417 g/mol	(Jirakkakul *et al*., 2015)
Bassianin	3-[(2E,4E,6E)-6,8-Dimethyl-2,4,6-decatrienoyl]-1,2-dihydroxy-5-(4-hydroxyphenyl)-4(1H)-pyridinone	$C_{23}H_{25}NO_5$	395.455 g/mol	(Wat *et al*., 1977)

Beauvericin	(3S,6R,9S,12R,15S,18R)-3,9,15-tribenzyl-4,10,16-trimethyl-6,12,18-tri(propan-2-yl)-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone	$C_{45}H_{57}N_3O_9$	783.9 g/mol	(Sood *et al.,* 2017)

5.3. Beauvericin: a cyclodepsipeptide

Beauvericin is a trimeric cyclodepsipeptide composed of alternated residues of methylphenylalanyl and hydroxyvaleryl. It has a molecular weight of 783.9 g/mol (table 2). It is considered as a mycotoxin with activities such as antibiotic, insecticide, apoptosis inhibitor, antifungal agent, antineoplasic agent, among others. This molecule is considered as an ionophore with the capability of transport small ions across lipid membranes (Mallebrera *et al*., 2018).

The main toxicity produced by beauvericin is due to its ionophore activity. Beauvericin has the ability to form cation-selective channels in mammalian cells and synthetic membranes (Kouri *et al*., 2003). It has direct effect on the intracellular ion concentration. This process causes disruption of the normal concentration of monovalent and divalent cations. Also, the ATP hydrolysis is an effect that contributes to the cell death (Kouri *et al*., 2005).

Beuvericin was first isolated from *B. bassiana*. However, there are reports that mentioned the production of beauvericin by *Fusarium* sp.*, Paecylomyces* sp., and *Polipovirus* sp. (Tedjiotsop-Feudjio *et al.,* 2010). The presence of beauvericin has been found as contaminant in foods such as grains, pasta, eggs, nuts, dried fruits, and medicinal herbs. Thus, the use of beauvericin has been reduced worldwide. However, studies carried out by The Food Safety Authority in the European Union (EFSA), have concluded that acute exposure to beauvericin do not indicate concern for human health (EFSA, 2014). The authority continues working on the mechanisms of action of beauvericin toxicity and occurrence in order to reduce its appearance in food and feed (Mallebrera *et al*., 2018).

5.4. Molecular bases and metabolic pathways for the biosynthesis of pigments

The production of secondary metabolites by entomopathogenic fungi is an inherent property to them because it is a genetic property (Borges *et al*., 2010). The biosynthesis of oosporein is given from a cluster of polyketide synthase genes (PKS) in *Beauveria bassiana* identified as OpS1-7. From the determination of the function of each gene, its participation in the synthesis of this compound has been verified by comparing mutants of each one (Table 3). The depletion of the OpS2 gene increases the production of oosporein in the mutant strain. On the other hand, the regulatory function of OpS3 is interesting since its depletion implies the non-expression of the other genes (except OpS5) and consequently the null

production of oosporein in mutant strains. This led to the elucidation of the oosporein biosynthetic pathway. Through the domains of OpS1 (KS, β-ketoacyl synthase, acyltransferase, dehydrogenase, acyl carrier protein, thioesterase) a series of reactions is carried out to obtain orsellinic acid, which is hydroxylated to 6-methyl-1, 2, 4-benzenetriol by OpS4. Subsequently, it is oxidized to 6-methyl-1, 2, 4, 5-benzenetetrol by OpS7, producing a dimerization by the catalase OpS5 to obtain oosporein (Feng *et al*., 2015) (Fig. 4).

The biosynthesis of tenellin has been carried out by a cluster of genes in *B. bassiana* which consists of four open reading frames (ORFs). ORF1 (tenA) and ORF2 (tenB) encode proteins homologous to CYP450 monooxygenases, ORF3 (tenC) a Zn-dependent oxidoreductase, and ORF4 (TenS =tenellin synthetase) an HR-PKS (highly reducing polyketide synthase), fused to NRPS (nonribosomal peptide synthetase). TenS contains the type I PKS domains: β-ketoacyl synthase (KS), acyltransferase (AT), dehydratase (DH), CMet, β-ketoacyl-reductase (KR) and acyl carrier protein (ACP). Also contains the NRPS domains: condensation (C), adenylation (A), thiolation (T) and thiol ester reductase (R) (Eley *et al*., 2007; Halo *et al*., 2008; Heneghan *et al*., 2011).

Table 3. Cluster function analysis of genes responsible for the production of oosporein in *Beauveria bassiana*.

Gene code	Gene	Putative function	Mutant phenotype
OpS1	BBA_08179	Polyketide synthase (PKS) type I, responsible for the biosynthesis of oosporein and orsellinic acid	ΔOpS1: No oosporein production. The addition of orsellinic acid in the culture gives the ability to produce oosporein.
OpS2	BBA_08180	MSF multidrug resistance transporter	ΔOp2: Increase in the production of oosporein.
OpS3	BBA_08181	GAL4-like Zn2Cys6 transcription factor (fungal specific transcription factor)	ΔOpS3: No oosporein production. The Ops3 deletion disabled the expression of Ops1, Ops2, OpS4, Ops6 and OpS7.
OpS4	BBA_08182	FAD binding domain-containing hydroxylase	ΔOpS4: No oosporein production.
OpS5	BBA_08183	Laccase 2/Multicopper oxidase	ΔOpS5: No oosporein production.
OpS6	BBA_08184	Glutathione-S-transferase	ΔOpS6: No oosporein production.
OpS7	BBA_08185	Cupin, Cupin_2_superfamily dioxygenase (hypothetical protein)	ΔOpS7: No oosporein production.

*Adapted from Feng *et al*. (2015).

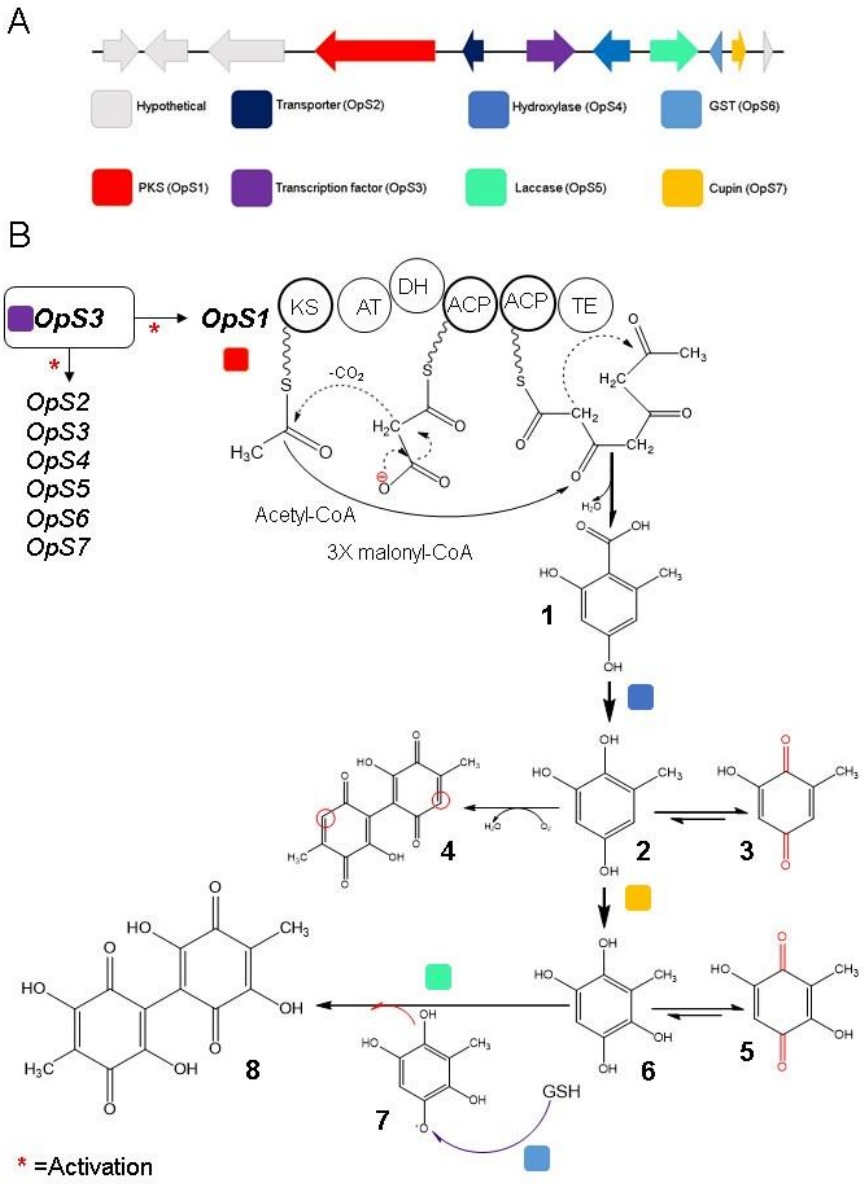

Fig. 4. Schematic representation of the biosynthetic pathway of oosporein in *Beauveria bassiana*. A) The cluster of genes involved in the production of oosporein. B) Through the domains of OpS1 (KS, β-ketoacyl synthase, AT, acyltransferase, DH, dehydrogenase, ACP, acyl carrier protein, TE, thioesterase) a series of reactions is carried out to obtain orsellinic acid (1), which is hydroxylated to 6-methyl-1, 2, 4-benzenetriol (2) by OpS4. Subsequently, it is oxidized to 6-methyl-1, 2, 4, 5-benzenetetrol (6) by OpS7, producing a dimerization by the catalase OpS5 to obtain oosporein (8). The intermediates of the reaction are 2-hydroxy-6-methyl-2, 5-cyclohexadiene-1,4-dione (3), 5, 5'-dideoxy-oosporein (4) and 2,5-dihydroxy-3-methyl -2, 5 -cyclohexadiene- 1, 4-dione (5). In the case of (7) corresponds to the free radical form of 6. The colored boxes correspond to the genes. The figure was adapted from data previously presented (Feng *et al*., 2015).

The biosynthetic processes of tenellin production begins with the fusion of a dimethylated pentacetide chain with tyrosine by a tenS synthetase hybrid to obtain pretenellin A. Subsequently, two oxidation reactions occur due to the expansion of the tetrahedral acid ring of pretenellin A to 2- pyridone pretenellin B by a ring expansion promoted by tenA, followed by an N-hydroxylation (tenB) which results in the compound tenellin. As for bassianin, the coexpression of tenS (ΔKR: dmbS-KR) together with tenC or dmbC, tenA, and tenB (P450) have led to the production of said metabolite (Boettger & Hertweck, 2013).

For the synthesis of beauvericin via fermentation process it is necessary the presence of glucose as main carbon source for fungal growth. Nevertheless, peptone and $NaNO_3$ are required as nitrogen sources (Sood *et al*., 2017). The biosynthesis of beauvericin is carried out by the action of beauvericin synthetase, an enzyme with molecular mass of 250 kDa (Peters *et al*., 1983). Also, important constituents such as AdoMet, ATP and Mg2+ are necessary for the formation of cyclic ester of a trimer of (2R)-2-hydroxy-3-methylbutanoyl-*N*-methyl-L-phenylalanine. The cyclic ester of a trimer of (2r)-2-hydroxy-3-methylbutanoyl-*n*-methyl-l-phenylalanine is synthetized by the non-ribosomal enzyme peptide synthetase. As first step, the aminoacids L-phenylalanine and valine needs a transamination reaction accepting nitrogen from any aminoacids (Sood *et al*.,2017). Prior to peptide bond formation, the aminoacids are methylated by the action of S-adenosyl-L-metionine (AdoMet). AdoMet acts as methyl donor. The interaction of the two modules leads the formation of a dipeptidol unit. Then, three successive condensation of dipeptidols leads the formation of hexadepsipeptide molecule. The biosynthetic process ends with the cyclisation reaction (Tedjiotsop-Feudjio *et al*., 2010).

5.5. Antimicrobial activity of the pigments produced by *Beauveria bassiana*

Beauveria bassiana is considered a biological control agent (BCA) for the control of pests that affect crops of economic importance around the world (i.e. coffee, cotton, etc.) (Strasser *et al*., 2000). It can produce some secondary metabolites with remarkable antibiotic, antifungal and even insecticidal properties against some insect pests as well as diseases (Sahab, 2012). Bassiacridin, bassianolide, bassianin, tenellin, oosporein, among others, have shown antimicrobial and antifungal activity *in vitro* and some have been associated with specificity and virulence because some suppress the immune response of the host, i.e. they are immunomodulators (Butt *et al*., 2016). Fan *et al*. showed that oosporein plays an important role as an antibacterial compound at the end of the infection process of *B*. *bassiana* by decreasing the microbiota of the host and helping the fungus to finish its infection cycle (Fan *et al*., 2017).

Some studies have reported the *in vitro* antibacterial activity of oosporein from fungus different than *B*. *bassiana*. The methanol purified extract by *C*. *kusanoi* showed activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Candida albicans* (Alurappa *et al*., 2014). From *Chaetomium cupreum*, oosporein have antifungal activity against *Rhizoctonia solani* and *Pytium ultimum* (Mao *et al*., 2010). In terms of the use of crude extracts from *B*. *bassiana* to evaluate antimicrobial activity, the

crude extract of ethyl acetate have antibacterial activity against *B*. *cereus*, *B*. *subtilis*, *Micrococcus leteus* and *Streptococcus aureus*, *E*. *coli* and *Aeromonas* sp. and moderate antifungal activity against the phytopathogenic fungi *Alternaria tenuis*, *Fusarium avenaceum*, *F*. *graminearum*, *F*. *moniliforme*, *F*. *oxysporum* as well as *Aspergillus paraziticus* (Sahab, 2012). The antibacterial activity of the *B*. *bassiana* ethyl acetate crude extract also had effect against *B*. *sphaericus*, *Streptococcus pyogenes* and *Chromobacterium violaceum* (Narasimha *et al*., 2010).

5.6. Other biological activities and toxicity

Tenellin, bassianin, and oosporein exert toxic activity on the membrane of equine erythrocytes by inhibiting the activity of the enzyme ATPase and causing cell lysis (Jeffs & Khachatourians, 1997). However, different properties have been evaluated to date. For example, the cytotoxic effect of oosporein, tenellin and bassianin on the cell lines of the insect *Spodoptera frugiperda*, SF- 9 (pupal ovaries) and SF-21 (primary explants of pupal tissues) in concentrations from 1 to 100 µM using controls by means of liquid media and solvents (1%) instead of toxins. The concentration at which each mycotoxin caused the 50% decrease in cell viability (CC_{50}) after 48 hours was; oosporein (SF-9 =4.23 µM ± 0.46 and SF-21 =10.43 µM ± 1.14), tenellin (SF-9 =4.84 µM ± 0.31 and SF-21 =11.95 µM ± 0.76) and bassianin (SF-9 =4.91 µM ± 0.50 and SF-21 =12.12 µM ± 1.24) (Valencia *et al*., 2011). The study showed for the first time the toxicity of the purified metabolites of *Beauveria* sp.

Scientific research on tenellin pigment has been carried out with the purpose of defining its specific role within the infection process of *B*. *bassiana* in insects. Since it has been demonstrated that this metabolite does not have the capacity to kill insect larvae (Eley *et al*., 2007). Jirakkakul *et al*. (2015) showed that in *B*. *bassiana* there are intracellular siderophores that have the capacity to bind to iron through ferricrocin, to prevent its deteriorative effects in free form serving also as storage. By means of *B*. *bassiana* mutants deficient in ferricrocin it was observed that tenellin has the capacity to act as iron (III) chelating agent and to prevent the toxic effects of iron over-accumulation. Therefore, this metabolite plays an important role intracellularly in protecting against ROS rather than causing a pathological state to insects (Jirakkakul *et al*., 2015).

Although oosporein is not the main metabolite responsible for the fungal virulence of *B*. *bassiana*. In biological systems, this is highly reactive because it possesses insecticidal activity in addition to antibiotic against Gram-positive bacteria, in addition to antiviral activity, antagonistic effect against plant pathogenic oomycetes (Feng *et al*., 2015) and it has antioxidant and cytotoxic effects according to *in vitro* tests (Alurappa *et al*., 2014). However, in relation to the insect control, it has been proven that the use of *B*. *bassiana* spores together with oosporein increases insecticidal activity. This would be a good alternative for producing biotechnological formulations on a large-scale, taking into account the cost-benefit of producing both fungal cells and pigments (Amin *et al*., 2010). One of the important findings about oosporein lies in determine how this compound contributes to the

pathogenesis process in *Galleria mellonella* larvae (wax moth). Oosporein acts inhibiting the immune response, specifically prophenoloxidase activity (PPO), as well as the regulation of antifungal gene expression, which facilitates the multiplication of *B*. *bassiana* cells in the hemocoel, allowing the infection process to be successful (Fig. 1) (Feng *et al*., 2015).

On the other hand, oosporein preferentially inhibits herpes simplex I DNA polymerase over HeLa cell lines and *E*. *coli*. In addition, at high concentrations it is toxic in some poultry including chickens for fattening and turkeys, which have been exposed in a prolonged and systemic way, causing kidney damage, gout and even death (Luo *et al*., 2015). Even more, oosporein has shown anticancer activity in HL-60 and A549 cells (IC_{50} =28 μM) (Mao *et al*., 2010). From the physicochemical characteristics of oosporein, the possibility of being strongly adsorbed by organisms has been concluded. However, there are no evidence that affect human health (Hu *et al*., 2016). Ramesha *et al*. (2015) published relevant results on the cytotoxic effects of oosporein (isolated from *C*. *kusanoi*) through different toxicity tests. In their study with Madin-Darby canine kidney cell lines (MDCK) and mouse macrophage (RAW 264.7) they found that oosporein affects cell viability (MDCK, IC_{50} =86 μM /RAW264.7, IC_{50} =78 μM). Oosporein increased the production of glutathione hydroxylase (GSH) (causing damage to the plasma membrane), and the decreases of mitochondrial membrane potential (causing DNA damage and lipid peroxidation through the formation of reactive oxygen species (ROS)). The effect increased proportionally to the doses from 25 to 200 μM. In addition, it decreases the expression of genes of the antioxidant enzymes superoxide dismutase 1 (SOD1) and catalase (CAT), and increased the expression of elements involved in apoptosis and oxidative stress such as heat shock protein 70 (HSP70), Caspase 3 (CAS3), Caspase 6 (CAS6) and Caspase 9 (CAS 9) in a manner dependent on the doses evaluated of oosporein (25-100 μM). Histological sections of the kidney and spleen of albino mouse models (Balb/C) treated orally with 50 μM showed tubular cortical dilation with epithelial vacuolation and necrosis as well as damage in germinal lymphoid centers such as splenic granulomas and infiltration macrophages, respectively.

It is important to mention that studies of biocontrol agents based on *Beauveria brongniartii* have shown residues of oosporein about 0.02 mg/m^{-2} of soil (Strasser *et al*., 2000). No studies have been reported on the production of mycotoxins by *B*. *bassiana* in plants due to their endophytic character (Hu *et al*., 2016).

6. PATENTS AVAILABILITY

The application of *Beauveria bassiana* metabolites has been documented by patents from distinct parts of the world (Table 4). As was mentioned above, the pigments are considered toxics for insects and microorganisms. In recent years, the research works have focused on the development of new biopesticides based on secondary metabolites of *B*. *bassiana* and *B*. *brongniartii*. However, scarce patents information is available due to the nature of patents for protecting the information.

Table 4. Application processes of secondary metabolites from *Beauveria* spp. patented in the last decade.

Patent No.	Country	Assignee	Title	Reference
US2011/0032088 A1	USA	PhytoMyco Research Corporation	Rust disease control by *Aphanocladium album* and/or *Beauveria brongniartii*	(Subbiah, 2011)
US2007/0044179 A1	USA	University of Tennessee Research Foundation	Insecticidal composition and methods of using the same	(Stewart & Leckie, 2007)
CN102265908A	China	University of Zhengzhou	A tobacco matrix composite and manufacturing method biopesticides	(Zhu *et al*., 2007)
CN102329837A	China	Anhui Agricultural University	Preparation method of *Beauveria* sp. extract with functions of tyrosinase inhibitor and antioxidant	(Li *et al*., 2011)
KR20160139521A	Korea	Chungbuk National University Industry-Academic Cooperation	Entomopathogenic fungi *Metarhizium anisopliae* SD4-2 and *Beauveria bassiana* SD15 having antimicrobial activities and insecticide	(Dong Woo *et al*., 2016)

The PhytoMyco research corporation in the patent US2011/0032088 A1 (Subbiah, 2011), developed a method for the recovery of low molecular weight metabolites (oosporein) produced by *Aphanocladium album* and *B*. *brongniartii* applied to the control of rust disease. The Chungbuk National University Industry-Academic Cooperation developed procedures for the control of microbes and insects by using both spores (culture concentrate) and water wastes. They used liquid cultures of *B*. *bassiana* and *M*. *anisopliae*. The procedures were protected by patent KR20160139521A (Dong Woo *et al*., 2016). The invention CN102329837A is related to the tyrosinase and antioxidant activities of *Beauveria* sp. extracts. Chromatographic procedures were applied to refine the extracts. The tyrosinase activity was 50% inhibited (IC_{50}) by using 134.5 mu g/mL and the cell lipid oxidation

of rat liver had IC_{50} of 87.2 mu g/mL. The activities were attributed to the presence of beauveriaoside (glycoside) concentrated into a brown to yellow amorphous powder (Li *et al*., 2011).

Beauvericin has been also used as a therapeutic agent. The patent US20150025219A1 applied beauvericin to human non-small-cell lung cancer cell line (A549), ovarian cancer cell line (SK-OV-3), skin cancer cell line (SK-MEL-2), and uterine sarcoma cell line MES-SA and its multi-drug resistant subline (MES-SA/DX5), colorectal carcinoma cancer cell line (HCT-15). The hexadepsipeptide showed lower IC_{50} against uterine sarcoma cell-line (MES-SA). The compound was produced by *Fusarium* sp. and are structurally the same that the produced by *Beauveria* species (Lee *et al*., 2015).

7. CONCLUDING REMARKS

The entomopathogenic fungus *Beauveria bassiana* possess special characteristics that make it one of the most used microorganisms for biological control. Its broad pathogenicity and virulence factors have a considerable effect against insects and phytopathogenic fungi. The secondary metabolites that it produces *in vivo* represents a new proposal to the use of synthetic antimicrobials. Oosporein is the most studied metabolite and has many applications, however it is crucial to investigate the role of oosporein in plants where *Beauveria* spp. is endophyte, to know if its role is only limited to counteract the immune response of insects, to have antimicrobial properties or, if there are plants where this metabolite participates in the detoxification of heavy metals. Toxicological studies have demonstrated the *in vitro* consequences of *B. bassina* secondary metabolites. However, no clinical cases have been reported where this metabolite induces a pathological state or systemic damage in humans. So, it is crucial to know detoxification pathways (both toxicokinetics and toxicodynamics) as well as the molecular and cellular interactions in the body's immune response to oosporein either by computer approximations or with the use of *in vitro* testing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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