DR. MARÍA SILVINA JUÁREZ TOMÁS (Orcid ID : 0000-0001-6982-4487)

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# Microbial production of beneficial indoleamines (serotonin and melatonin) with potential application to biotechnological products for human health

Mariana Elizabeth Danilovich[1], María Rosa Alberto[1], and María Silvina Juárez Tomás[2*]

[1]Instituto de Biotecnología Farmacéutica y Alimentaria (INBIOFAL)-CONICET. Av. Kirchner 1900.T4000ACS. San Miguel de Tucumán, Tucumán, Argentina.

[2]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI)-CONICET. Av. Belgrano y Pje. Caseros, T4001MVB. San Miguel de Tucumán, Tucumán, Argentina.

Running headline: Indoleamines production by microbes

***Corresponding author:**

Dr. María Silvina Juárez Tomás

Planta Piloto de Procesos Industriales Microbiológicos (PROIMI)-CONICET, Avenida Belgrano y Pasaje Caseros, San Miguel de Tucumán (T4001MVB), Tucumán, Argentina

Phone: +54-381-4344888

E-mail: mjuareztomas@conicet.gov.ar

**Summary**

Microorganisms have showed the ability to produce biologically active compounds associated with neurotransmission in higher organisms. In particular, serotonin- and melatonin-producing microbes are valuable sources for the development of eco-friendly bioproducts. Serotonin and melatonin are indoleamines that have received special attention due to their positive effects on human health. These biomolecules exert a critical role in several physiological or pathological processes, including some mental and neurological disorders. This article includes a review of the microbial production of serotonin and melatonin, their functions in microorganisms, and their potential uses as therapeutic and/or preventive agents to improve human health. A description of the quantification methods employed to detect indoleamines and the evidence found concerning their microbial production at lab and industrial scale -for application in biotechnological products- is also provided. The microbial ability to synthesize beneficial indoleamines should be further studied and harnessed, in order to allow the development of sustainable bioprocesses to produce foods and pharmaceuticals for human health.

**Keywords:** Microorganisms, indoleamines, serotonin and melatonin biosynthesis, biotechnological products, human health.

## Introduction

Microorganisms play a relevant role in the environment and in the different living organisms with which they interact. In several ecosystems, the interaction between microorganisms and their host depends mainly on the production of different chemicals, some of which are neurochemicals (Oleskin *et al*. 2017). Neurochemicals have been defined as chemical substances that affect the function of the nervous system (Heinbockel and Csoka 2019). These compounds, which are produced not only by animals but also by plants and microorganisms, play several roles such as neurotransmitters, neurohormones, and neuromodulators (Roshchina 2016; Heinbockel and Csoka 2019).

With respect to microorganisms, some have shown the ability to produce various biologically active compounds, which are generally associated with neurotransmission in higher organisms (Shishov *et al*., 2009; Cataldo *et al*. 2020). These compounds include small molecule neurotransmitters such as dopamine, norepinephrine, and epinephrine (catecholamines), serotonin (indoleamine), histamine (imidazolamine), and gamma aminobutyric acid, among others. Roshchina (2016) proposed the term "biomediators" for these compounds since they are considered multifunctional substances that can participate in non-nervous processes in animals, plants (Roshchina and Yashin 2014) and microorganisms (Villageliú *et al*. 2018). In addition, microbial ability to produce bioactive compounds mainly related to hormonal functions in humans (such as catecholamines and melatonin) has been also reported (Manchester *et al.* 1995; Lyte 2013).

Among the neuroactive compounds, some indoleamines (i.e., indole compounds that contain an amino group) such us serotonin (5-hydroxytryptamine) and melatonin (*N*-acetyl-5-methoxytryptamine) have aroused special interest due to their beneficial functions for living beings. These indoleamines have various relevant effects on human health in general, and on nervous system in particular (O'Mahony *et al*. 2015; Tan *et al*. 2015; O'Leary *et al*. 2020). The development of foods or pharmaceuticals including purified biomolecules from microbial origin is an eco-friendly alternative to products containing active ingredients obtained from chemical synthesis. In this sense, in the last years, few studies have described the melatonin production, mainly by genetically modified microbes (Germann *et al*. 2016; Luo and Foster, 2019; Luo *et al*. 2020). On the other hand, wild-type isolates from fermented foods, plants and environmental samples have been described as melatonin and serotonin producers (Al-Hassan *et al*. 2011; Jiao *et al*. 2016; Fernandez-Cruz *et al*. 2019; Fracassetti *et al*. 2020). Therefore, the possibility of

developing a sustainable microbial process to obtain these compounds, their precursors and derivatives constitutes a promising biotechnological perspective.

Even though several aspects of indoleamines have been already explored, in the last few years a strong interest in them has appeared in different research areas. Studies on this subject that allow the identification and harnessing of novel biotechnological potentialities associated with possible applications of microbial melatonin and serotonin in beneficial products to human health are urgently needed. This review focuses on the microbial sources of serotonin and melatonin and their effects on human health. It also provides a summary about the available analytical methods to quantify them as well as evidence related with their production at both lab and industrial scale.

## Microbial production of serotonin and melatonin

### Serotonin- and melatonin-producing microorganisms

More than two decades ago, the ability to synthesize melatonin was demonstrated in several photosynthetic microorganisms, such as the bacteria *Rhodospirillum rubrum* and *Erythrobacter longus* (Manchester *et al*. 1995; Tilden *et al.* 1997), and the protist *Lingulodinium polyedra* (formerly *Gonyaulax polyedra*) (Hardeland *et al*. 1995). Since then, the *in vitro* production of indoleamines such as serotonin and/or melatonin was evidenced in various prokaryotic and eukaryotic microorganisms (Table 1). Some microorganisms were isolated from aquatic environments, soils and plants, and other are related to fermented product making processes.

Yeasts belonging to the genus *Saccharomyces* and bacteria belonging to the genera *Geobacillus*, *Bacillus*, and *Pseudomonas* produced both serotonin and melatonin at different concentrations (Al-Hassan *et al*. 2011; Fernández-Cruz *et al*. 2016; Jiao *et al.* 2016; Ma *et al.* 2017; Muñiz-Calvo *et al*. 2019). Moreover, the production of melatonin, but not serotonin, was evidenced in cultures of some yeasts of the genera *Pichia* and *Saccharomyces*, and bacteria of the genera *Agrobacterium*, *Pseudomonas*, *Variovorax*, *Bacillus*, and *Oenococcus* (Rodriguez-Naranjo *et al*. 2012; Fernández-Pachón *et al.* 2014; Jiao *et al.* 2016).

Microbial production of melatonin has been well described in fermented drinks like wines, mainly produced by yeasts belonging to the genus *Saccharomyces* (Rodriguez-Naranjo *et al*. 2012; Fernandez-Cruz *et al*. 2019; Muñiz-Calvo *et al*. 2019). The relevance of these studies lies in the fact that melatonin affects organoleptic properties and other characteristics in fermented products (Que *et al*. 2020). In this sense, Rodriguez-Naranjo *et al*. (2012) described the synthesis of melatonin in different culture media by several *Saccharomyces* sp. strains used in the wine making

process. They concluded that melatonin production depended on the concentration of L-tryptophan and sugars in the medium and on the microbial growth phase, suggesting that melatonin acts as a growth molecule signal in yeasts. Likewise, Fernandez-Cruz *et al*. (2019) studied the intracellular biosynthesis of melatonin in *Saccharomyces* and non-*Saccharomyces* wine-yeasts using high resolution mass spectrometry to finally identify the presence of tryptophan, melatonin, serotonin, *N*-acetyl-5-hydroxytryptamine and indole-3-acetic acid in their intracellular compartment.

On the other hand, Jiao *et al*. (2016) and Ma *et al*. (2017) described the ability of *B. amyloliquefaciens* SB-9 and *P. fluorescens* RG11, two endophytic bacterial isolates, to biotransform L-tryptophan to produce melatonin. This process caused an increase in endogenous levels of melatonin in grapevines and a promotion of their growth.

**Serotonin and melatonin: biosynthesis and catabolism**

In microorganisms, plants, animals, and humans, both serotonin and melatonin are synthesized from tryptophan (Macchi and Bruce 2004; Kang *et al*. 2007; Jonnakuty and Gragnoli 2008; Back *et al*. 2016). Since the main pathway for the microbial synthesis of these indoleamines remains unresolved, biosynthetic pathways described in humans and plants are reviewed in this item.

In humans, after hydroxylation from L-tryptophan to 5-hydroxy-L-tryptophan by a tryptophan 5-hydroxylase, 5-hydroxy-L-tryptophan is transformed into serotonin via aromatic L-amino acid decarboxylase (Jonnakuty and Gragnoli 2008). For melatonin synthesis, serotonin is acetylated to *N*-acetylserotonin in the presence of serotonin *N*-acetyltransferase and then *N*-acetylserotonin is methylated to melatonin by a hydroxy indole-*O*-methyltransferase (Figure 1) (Macchi and Bruce 2004). In the human body, ninety-five percent of the serotonin is found in the gastrointestinal tract, where is produced by gut microorganisms and enterochromaffin cells (Van der Leek *et al.* 2017). Some reports highlighted the key role of human gut-associated microorganisms in regulating the biosynthesis of serotonin (Reigstad *et al*. 2015; Yano *et al.* 2015). Reigstad *et al*. (2015) showed that gut microbiota, through the production of short-chain fatty acids, promoted the transcription of *tph1* (a gene encoding the tryptophan hydroxylase) in enterochromaffin cells, regulating the serotonin production at this level. Likewise, Yano *et al*. (2015) described that indigenous spore-forming bacteria from the gut are relevant regulators of host serotonin production.

In plants, tryptophan is converted into tryptamine and, after hydroxylation by a tryptamine 5-hydroxylase, into serotonin (Kang *et al*. 2007). For melatonin biosynthesis, two main pathways

have been proposed. In the first one, serotonin is converted to *N*-acetylserotonin and then into melatonin by the action of serotonin *N*-acetyltransferase and *N*-acetylserotonin *O*-methyltransferase, respectively. In the second, serotonin is transformed into 5-methoxytryptamine by *N*-acetylserotonin *O*-methyltransferase and then this compound is converted into melatonin by a serotonin *N*-acetyltransferase (Figure 2) (Back *et al*. 2016).

In microorganisms, the production of amines is due to the decarboxylation of amino acids, which allows them to produce energy under stress conditions (Gardini *et al*. 2016). Even though similar enzymes and routes for synthetic pathways described in vertebrates participate in the process, it has not yet been completely elucidated whether 5-hydroxy-L-tryptophan (as in animals) or tryptamine (as in plants) is converted into serotonin in most of serotonin- and/or melatonin-producing microorganisms. In the last few years, 5-hydroxytryptophan, serotonin, and *N*-acetylserotonin, but not tryptamine, were extracellularly detected in the bacteria *B. amyloliquefaciens* SB-9 and *P. fluorescens* RG11 (Jiao *et al*. 2016; Ma *et al*. 2017). However, in the yeast *S. cerevisiae* QA23, tryptamine was detected as the main serotonin precursor (Muñiz-Calvo *et al*. 2019). Moreover, serotonin has been described as turning into *N*-acetylserotonin or 5-methoxytryptamine before melatonin microbial biosynthesis (Muñiz-Calvo *et al*. 2019).

In comparison with the biosynthetic processes, the catabolism of serotonin and melatonin in microorganisms is lees understood. Some amine oxidases (enzymes catalyzing the oxidation of several amines such as serotonin) or the genes encoding them were identified in various microorganisms (Yagodina *et al.* 2002; Yano *et al.* 2015). On the other hand, it has been suggested that the melatonin catabolism in bacteria is similar to that described in mitochondria (Tan *et al.* 2015). This process involves a pseudoenzymatic mechanism with the action of cytochrome C or another iron-containing hemoprotein, which degrades melatonin to $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (Tan *et al*. 2015). Also, a non-enzymatic process was described to metabolize melatonin through the interaction with reactive oxygen and nitrogen species, generating several metabolites (Tan *et al*. 2015).

Currently, little is known about the genetic background involved in the microbial biosynthetic and catabolic pathways of serotonin and melatonin. Nevertheless, it has been suggested that the expression of the genes encoding the biosynthetic enzymes tryptophan 5-hydroxylase and serotonin *N*-acetyltransferase is one of the most relevant factors during melatonin production (Tan *et al*. 2015; Luo *et al.* 2020). Recently, several genes from bacterial origin were tested to construct a melatonin-producing *Escherichia coli* strain (Luo *et al.* 2020). For example,

the *ddc* gene –encoding the aromatic L-amino acid decarboxylase- from *Candidatus Koribacter versatilis Ellin* 345 and *Draconibacterium orientale*, and *aanat* gene -encoding the aralkylamine *N*-acetyltransferase- from *Streptomyces griseofuscus* were assayed (Luo *et al.* 2020). More studies are required to elucidate the complete synthetic and catabolic pathways (including genes, enzymes and other implied compounds) of serotonin and melatonin in different prokaryotic and eukaryotic microbes.

**Physiological role of microbial serotonin and melatonin in microorganisms**

In microorganisms, the main role of biomediators is related to growth, intercellular communication, interaction with enemies, morphogenesis, and protection against environmental changes. However, in some cases, their production at high doses acts as a defense mechanism, a response to a stress conditions, or a source of nutrients (Roshchina 2016; Bisquert *et al*. 2018).

The essential biomediator serotonin can participate as an intercellular communication agent between microorganisms and has a relevant function in the growth regulation and the development of other microbial cells (Roshchina 2016). Millimolar to micromolar concentrations of serotonin stimulated the growth and acted as an intercellular communicator to synchronize cell aggregation in the yeast *Candida guilliermondii*, Gram-positive bacteria, including *Enterococcus faecalis* (Strakhovskaya *et al*. 1993), and Gram-negative bacteria, including *E. coli* and *Rhodospirillum rubrum* (Oleskin *et al*. 1998). Moreover, there is evidence that exogenous serotonin favors biofilm formation in *Pseudomonas aeruginosa* strains via quorum sensing (Knecht *et al*. 2016). Other reported functions for this compound are related to its protector role under stressful conditions such as UV radiation by transforming 5-hydroxy-L-tryptophan into serotonin (Fraikin *et al*. 1989).

In unicellular organisms, the original and primary function of melatonin is to act as an antioxidant and free radical scavenger (Tan *et al*. 2015). As an antioxidant, this compound exhibits various unique characteristics that differ from classic antioxidants, including its cascade reaction with free radicals and its ability to induce itself under moderate oxidative stress. Such characteristics make melatonin a powerful antioxidant with an endogenous origin that protects organisms from catastrophic oxidative stress (Hardeland *et al*. 1995; Bisquert *et al*. 2018). Another function that has been related to this compound is its participation in the regulation of circadian rhythms in some microorganisms such as human gut bacteria and *Lingulodinium polyedra,* in which higher levels of melatonin were detected in dark conditions (Hardeland *et al*. 1995; Paulose *et al.* 2016).

In summary, serotonin and melatonin exert crucial effects on microorganisms. Consequently, from an evolutionary perspective, all microorganisms should have the capacity to produce these indoleamines or other biomediators that serve them for perpetuation. Further studies on this subject are necessary to determine the generality of microbial ability to synthesize biomediators.

## Effects of serotonin and melatonin on human health: current and potential uses as therapeutic and/or preventive agents

### The role of serotonin and melatonin in mental and neurological disorders

Serotonin has been extensively associated with human well-being (Li *et al*. 2016). In the central nervous system, serotonin plays a vital role as a neurotransmitter and regulates several reactions and responses to environmental changes, such as mood, nociception, cognition, impulsivity, aggressiveness, eating behavior, and body temperature (O'Mahony *et al*. 2015).

Serotonin exhibits a satisfactory antioxidant activity (Purushothaman *et al*. 2020). Moreover, this neurochemical plays a significant role in the modulation of behavior in a wide range of psychiatric disorders including depression, anxiety, post-traumatic stress, schizophrenia, anorexia nervosa, among others (O'Leary *et al.* 2020). In some psychiatric diseases, defects in synaptic molecular signaling involving serotonergic transmission have been implied (Purves *et al.* 2018). In this sense, due primarily to its function as a neurotransmitter, serotonin is a relevant pharmacological target in the treatment of certain disorders such as depression and certain anxiety disorders (O'Leary *et al*. 2020). Drugs modifying serotonergic transmission and/or selective serotonin receptors are effective (Sharma 2004). For example, some pharmacological agents widely recognized for the treatment of several mental disorders are the selective serotonin reuptake inhibitors, which could block the reabsorption of this substance in neurons increasing its levels in brain (Stein and Stahl 2000). Serotonin is reduced with age, especially in the brain in cases of Alzheimer's disease (Rosales-Corral *et al*. 2012). Serotonin depletion has been also related to neuroinflammation since inflammation process contributes to the depletion of tryptophan, which reduces serotonin in the brain (Birner *et al*. 2017). However, serotonin is slightly soluble in aqueous solutions and cannot cross the blood-brain barrier; thus, oral administration of serotonin is not capable of regulating its levels in the central nervous system. In this sense, other strategies are used to modify the serotonin metabolism in the central nervous system. These strategies primarily involve the intake of serotonin precursors, such as tryptophan

or 5-hydroxy-L-tryptophan, which increase plasma and brain serotonin levels (Ravindran and da Silva 2013; Dome *et al*. 2019).

Another relevant indoleamine with positive effects on human health is melatonin, which is mainly produced in the pineal gland and has the ability to regulate the circadian sleep-wake rhythm (Castaño *et al*. 2018). Melatonin is soluble in both lipid and aqueous solutions; thus, when orally administered, it can pass through all cell barriers after its absorption from the gut (Erland and Saxena 2017). The oral administration of is compound has shown positive effects on people with sleep problems or jet lag (Castaño *et al*. 2018).

On the other hand, numerous studies have evidenced the ability of melatonin to prevent the development and progression of neurodegeneration and/or mitigate some of the symptoms of neurodegenerative disorders such as Alzheimer, Parkinson and Huntington (Cardinali, 2019; Genario *et al*. 2019; Tamtaji *et al*. 2020). Moreover, *Genario et al*. (2019) summarized the critical role of melatonin in these and other central nervous system disorders like insomnia, epilepsy, autism, depression, and anxiety. In nervous pathologies, there are differential alterations of melatonin in body fluids. For example, melatonin levels significantly decreased in cerebrospinal fluid in bipolar disorder, whereas a reduction in major depressive disorder was found in serum (Bumb *et al*. 2016).

The modulation of the inflammatory response plays an essential role in the prevention, mitigation, and treatment of several mental and neurological disorders (Rhie *et al*. 2020). In this sense, melatonin combines chronobiotic and cytoprotective properties by modifying the biological rhythm and reversing the inflammatory and oxidative damages seen in central nervous system pathogenesis (Cardinali, 2019; Genario *et al.* 2019). Melatonin can remove numerous reactive oxygen species or reactive nitrogen species compared to classic antioxidants. The melatonin molecule and its secondary and tertiary metabolites are able to neutralize various toxic oxygen derivatives through the free radical scavenging cascade (Tan *et al*. 2015). Besides its scavenging activity, melatonin has been shown to elevate the antioxidant enzymes superoxide dismutase, glutathione reductase, and catalase (Purushothaman *et al*. 2020). In this sense, this substance is relevant in the treatment of neurodegenerative diseases associated with age since they are related to an oxidative damage in the central nervous system (Corpas *et al.* 2018).

**The role of serotonin and melatonin in other physiological or pathological processes**

Serotonin is a bioactive substance that also acts at the peripheral level, modulating intestinal function, immune response, differentiation of blood cells, and hemodynamic function (Yabut *et al.* 2019). In peripheral sites like gastrointestinal tract, pancreas or blood, serotonin can act as a hormone, auto- and/or paracrine factor, or intracellular signaling molecule to regulate, for example, the metabolic homeostasis (El-Merahbi *et al.* 2015). Also, extracellular serotonin could be degraded by various cell types like osteoclasts or pancreatic cells, and intracellular metabolites of serotonin could also act as signaling compounds (Waku *et al*. 2010). Due to its beneficial functions, serotonin could be employed in different products in order to regulate its normal levels in peripheral sites. In addition, the antimycotic effect of serotonin against *Aspergillus* sp. strains, which are the primary cause of fungal infections in immunosuppressed patients, was described (Perkhofer *et al*. 2007). Serotonin negatively affects ergosterol synthesis of *Aspergillus* sp. strains fungal cell membrane integrity and hyphal growth (Perkhofer *et al*. 2007).

Likewise, serotonin derivatives have a wide range of biological activities. They have been associated with an aging inhibitor mechanism due to their potent antioxidant activity (Kang *et al.* 2009). For example, the antioxidant activities of feruloylserotonin and 4-coumaroylserotonin are stronger than α-tocopherol, a natural antioxidant, and similar to butylhydroxyanisole, a synthetic antioxidant (Kang *et al*. 2009).

With respect to melatonin, in several peripheral sites, it regulates the circadian rhythm of different physiological like cardiovascular regulation (Imenshahidi *et al.* 2020), and also acts as a free radical scavenger and a broad-spectrum antioxidant (Manchester *et al*. 2015). Melatonin can also decrease blood pressure in patients with hypertension (Grossman et al., 2006), and procoagulant levels in plasma in patients with coronary artery disease (Wirtz *et al.* 2008).

Moreover, several clinical studies demonstrated that the ingestion of melatonin produces a reduction in the toxicity of chemotherapeutic agents in cancer patients (Palmer *et al*. 2020). On the other hand, experimental models on rats showed that melatonin delays tumorigenesis initiation since interactions with DNA-bound carcinogens prevent the accumulation of mutations inside cells and the initiation of cancer (Kumar *et al*. 2017). Melatonin has also the ability to repair cellular damage and protect healthy cells (Kumar *et al*. 2017). Tamtaji *et al*. (2019) reviewed the results of several *in vivo* and *in vitro* studies claiming that supplementation with melatonin showed a therapeutic alternative for pancreatic cancer. This effect could be associated with the ability of melatonin to induce the apoptosis of cancer cells, which involves a broad spectrum of molecular mechanisms such as oxidative stress.

On the other hand, melatonin has been well-described as a coadjuvant treatment in several gut diseases by increasing the proliferation of immune cells and the antimicrobial activity of intestinal microorganisms (Xu *et al*. 2019; Ma *et al*. 2020). Besides, some authors have also reported the antibacterial and antifungal effects of melatonin (Kilinçel *et al*. 2019; Xu *et al*. 2019). The antifungal property of melatonin, which was tested in experimental models using rats infected with *Candida albicans,* showed a reduction in the expression of interleukin-6 (Kilinçel *et al*. 2019). Daryani *et al*. (2018) described melatonin therapeutic effect in the treatment of parasitic infections mainly caused by parasitic protozoa, suggesting that melatonin enhances the host immune response in infections such as Chagas disease, amoebiasis, giardiasis, and malarial infections, among others.

In addition, the use of melatonin as a potential therapeutic or preventive drug against various antiviral infections has been proposed (García *et al*. 2020; Reiter *et al*. 2020). In particular, the multifunctional properties of melatonin (e.g., as an antioxidant, anti-inflammatory, and antiviral against some viruses) allow suggesting its use –alone or as complement of other drugs- in patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Reiter *et al*. 2020; Zhou *et al*. 2020). SARS-CoV-2 is the virus responsible for the current complex coronavirus disease (COVID-19) pandemic (Guo *et al.* 2020). COVID-19 can cause a cytokine storm that negatively influences on the adaptive immunity against SARS-CoV-2 infection (Reiter *et al*. 2020). In many cases, a severe septic response occurs, and the death of patients is due to an excessive inflammatory reaction (Acuña-Castroviejo *et al*. 2020). Considering that COVID-19 could also negatively affect melatonin synthesis (Grunewald *et al.* 2020), several studies have proposed the use of melatonin as a therapeutic agent. In addition to its powerful anti-inflammatory and antioxidant activities, melatonin also shows immunomodulatory properties, and it is able to repair the oxidative damage caused in mitochondria as a result of an exaggerated inflammatory response (Acuña-Castroviejo *et al*. 2020; Reiter *et al.* 2020). However, more studies on the therapeutic or preventive action of this substance are required to warrant their safety and efficacy in several viral infections, including COVID-19. Figure 3 summarizes the beneficial effects of melatonin on human health.

**Microbial production of beneficial indoleamines as bioactive compounds in biotechnological products**

In this century, medical and economic interest in neuroactive compounds produced by microorganisms has increased due to their benefits on human health (Lyte, 2013; Oleskin *et al*. 2017). The industrial production of beneficial indoleamines is relevant for their potential inclusion in food and pharmaceutical products, such as regulating serotonin and melatonin levels, and in this way contribute to the treatment and / or prophylaxis of certain physical and mental disorders (O'Mahony *et al*. 2015; O'Leary *et al*. 2020).

Currently, melatonin is used in several products to treat and prevent sleep-related problems and jet lag (Erland and Saxena, 2017). In fact, this substance is commonly available as commercial dietary and pharmaceutical supplements in many countries (Erland and Saxena, 2017). At an industrial level, melatonin is commonly produced by chemical synthesis, using solvents and toxic catalysts to avoid the viral contamination related to extraction in animal tissues (Sun *et al*. 2016). In contrast, microbial production can be an "eco-friendly" and more sustainable alternative to conventional chemical processes (Germann *et al.* 2016; Luo and Foster, 2019; Luo *et al*. 2020).

Respecting to the biological production of indoleamines of interest, Germann *et al*. (2016) proposed a novel alternative using a "microbial cell factory" to synthesize melatonin. These authors reported the construction and expression of a *de novo* biosynthesis pathway for melatonin using a *Saccharomyces cerevisiae* strain. After the addition of foreign genes involved in melatonin biosynthesis as well as other cofactors that support the pathway, and the modification and optimization of fermentation and extraction proceeds, the melatonin production achieved was 14.5 mg $L^{-1}$. This production was obtained using a simulated fed-batch system with glucose as the sole carbon source. Although this strategy could be a possibility to produce melatonin on an industrial scale in future, further studies are still necessary, especially those related to a green and effective purification process in order to obtain high melatonin purity.

Likewise, Luo and Foster (2019) developed and patented a process to optimize a "microbial cell factory" for melatonin production and other related compounds. This invention is based on recombinant microbial cells of *E. coli* with heterologous nucleic acid sequences encoding a monoxygenase for producing the oxidation of an amino acid in order to obtain melatonin and related compounds. Recently, Luo *et al*. (2020) described a bioprocess to improve the microbial production of melatonin using *E. coli* recombinant cells. After some engineering modifications and optimization of fermentation conditions, the maximum melatonin titer achieved after fed-batch fermentations was 2000 mg $L^{-1}$ (20 fold-times improvement in comparison with the initial

strain) using external supplementation of tryptophan, and ~1000 mg $L^{-1}$ with only glucose as carbon source for tryptophan supply. These findings constitute the basis for the commercial production of melatonin using *E. coli* recombinant strains as a microbial factory.

On the other hand, probiotic microorganisms are currently employed in the United States of America (Quantum Nutrition Labs (http://healthline.cc)) for the production of melatonin at industrial scale. Nevertheless, detailed information on microbial strains and production processes is not readily available, possibly due to industrial property rights. As described above (Table 1), the natural ability to synthesize serotonin and melatonin has been evidenced in several microorganisms from environments and foods. However, wild-type microorganisms have not been yet evaluated as candidates for industrial production of the indoleamines of interest. This is an innovative research subject that deserves further studies.

**Quantification methods for melatonin and serotonin**

As already mentioned, the production of beneficial indoleamines by microorganisms has gained special attention in the recent years. However, sometimes their detection turns difficult due to the low quantities produced and the sample characteristics. Thus, the development of rapid and effective quantification methods for their detection is crucial.

Some of the first techniques described for indoleamine detection, especially for melatonin, are thin layer chromatography (Costantini and Paoli 1998), gas chromatography and mass spectroscopy (Best *et al*. 1993), radioimmunoassay (Van Tassel *et al*. 2001) and enzyme-linked immunosorbent assay (Maldonado *et al*. 2009) (Table 2). Although enzyme-linked immunosorbent assays are simple, rapid, and low cost, they have the disadvantage of the cross-reactivity between other compounds with a similar structure, which traduces in low specificity (Kennaway 2019). In the last decade, different cutting-edge methods of analytical chemistry were developed. Most of them focus on quantifying indoleamines in human serum, fruits, or fermented products (Table 2).

Currently, two of the most analytical methods employed to detect melatonin are based on liquid chromatography separation coupled with fluorescence (Yin *et al*. 2016; Albu and Radu 2018) and mass spectrometry (Wolrab *et al*. 2016; Fernández-Cruz *et al*. 2017; Jiang *et al*. 2019). The liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been extensively described in different studies to determine the amount of melatonin and serotonin in plants and food samples (Özen and Ekşi 2016). Recently, the development of an analytical method allowed the simultaneous quantification of melatonin and related compounds in wines by using solid phase

extraction to pre-concentrate the sample followed by Ultra high performance liquid chromatography (UHPLC) analysis with either fluorescence or mass spectrometer detectors (Fracassetti *et al*. 2019). UHPLC, coupled with mass spectrometry, is commonly employed for metabolomics analysis in various biological samples, including those from microbial cultures, since it provides higher peak capacity and improved resolution and sensibility in complex samples (Fernández-Pachón *et al*. 2014; Ma *et al*. 2017; Fernandez-Cruz *et al*. 2019; Fracassetti *et al*. 2020).

On the other hand, Muñiz-Calvo *et al*. (2017) have also proposed a voltammetric technique directly applied to yeasts for the quantification of indoleamines. This method is based on the different electroactive characteristics of the compounds involved which allow distinguishing between melatonin, thryptophol, and indole-3-acetic acid. The procedure has shown some advantages compared with HPLC methods (Azizoğlu *et al*. 2017), which are sometimes more technically complex and expensive, and provides the analyses of the samples with minimal pre-treatment and within a short time. However, the main problem is the lower discriminability of the method since some voltammetric signals showed coincidence among them (Muñiz-Calvo *et al*. 2017).

Morcillo-Parra *et al*. (2019) proposed using a fluorescent bioassay based on the β-lactamase enzyme reporter system for detecting melatonin in grape must and wine samples comparing the results obtained with LC-MS/MS analysis. The study revealed that this technique is suitable for the determination of melatonin content in food samples due to the decrease in LOD and quantification limit (LOQ) (LOD and LOQ 0.12 and 0.41 ng $mL^{-1}$ for wine samples). However, the authors highlighted the importance of the extraction method given the interference observed in complex matrices such as grape must.

**Conclusions**

Various prokaryotic and eukaryotic microbes from different origins have the natural ability to synthesize beneficial indoleamines such as serotonin and melatonin. In some microorganisms, these substances have been associated with functions such as intercellular communication, growth, and protection against environmental changes. Since these compounds exert crucial functions in microbial cells, the following questions arise: are all microorganisms able to produce these compounds? What are the suitable growth conditions to favor their production? Considering these

questions, it is possible that beneficial indoleamines production has not been yet evidenced in numerous microorganisms.

Even though many aspects of serotonin and melatonin produced by microorganisms have already been studied, there are many others that still need further investigation. More omics analyses (e.g., metagenomics, metatranscriptomics, metaproteomics and metabolomics) are required to identify pathways and key enzymes related to serotonin and melatonin biosynthesis in non-genetically modified microbes. Wild-type microorganisms producing high levels of beneficial indoleamine should be selected, and the bioprocess conditions should be optimized for potential industrial applications. On the other hand, suitable microbial strains providing precursors for indoleamines synthesis can be used to construct and/or redesign microbial biosynthetic pathways, through the introduction and/or overexpression of biosynthetic genes of serotonin and melatonin production (Figure 4). In this sense, several strategies of metabolic engineering and synthetic biology, such as homologous recombination and CRISPR-Cas9 technologies, can be applied to improve serotonin and melatonin biosynthesis in microorganisms. Microbial cell factories should be optimized through successive design-build-test-learn cycles (Luo *et al.* 2020). These cycles can include enzyme and promoter engineering, metabolic flux control, pathway optimization, and genome-scale metabolic models, among other strategies (Guan *et al*. 2020). Moreover, the selection of the accurate analytical methodology, taking into account the characteristics of the sample analyzed and the limitations of the instruments used, is relevant in the study of these biomolecules. In view of all the positive effects of these substances on human health, developing a suitable and economically profitable biological process to produce them in higher amounts becomes essential.

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**Conflict of Interest**

The authors declare no conflicts of interest.

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**Figure legends**

**Figure 1** Serotonin and melatonin biosynthesis pathways in humans (modified from Kumar *et al*. (2017) and Yılmaz and Gökmen (2020)).

**Figure 2** Serotonin and melatonin synthesis pathways in plants (modified from Kumar et al. (2017) and Yılmaz and Gökmen (2020)).

**Figure 3** Positive effects of melatonin on human health: current and potential uses as a therapeutic or preventive agent.

**Figure 4** Strategies of metabolic engineering for serotonin and melatonin biosynthesis. Once the biosynthetic pathway is elucidated, the design-build-test-learn cycle can be applied to construct and optimize microbial cell factories for serotonin and melatonin biosynthesis.

**Table 1** Serotonin- and/or melatonin-producing microorganisms from different origins

| Origin of microorganism | Microorganism | | Indoleamine produced (maximum concentration)* | Reference |
|---|---|---|---|---|
| Marine environment | Protists | *Lingulodinium polyedra* | Melatonin (7.26 ± 0.21 ng $mg^{-1}$ protein) | Hardeland *et al*. (1995) |
| | Gram-negative bacteria | *Erythrobacter longus* | Melatonin (29.20 ng $mg^{-1}$ protein) | Tilden *et al*. (1997) |
| Oil polluted soil | Gram-positive bacteria | *Geobacillus stearothermophilus* | Serotonin (NA) and melatonin (NA) | Al-Hassan *et al*. (2011) |
| Grapevine roots | Gram-positive bacteria | *Bacillus amyloliquefaciens* SB-9 | Serotonin (3.81 ± 0.46 ng $mL^{-1}$); melatonin (1.19 ± 0.12 ng $mL^{-1}$) | Jiao *et al*. (2016) |
| | | *Bacillus thuringiensis* CS-9 | Melatonin (0.53 ng $mL^{-1}$) | Jiao *et al.* (2016) |
| | Gram-negative bacteria | *Agrobacterium tumefaciens* CS-30 | Melatonin (0.22 ng $mL^{-1}$) | Jiao *et al.* (2016) |
| | | *Variovorax* sp. VA-10 | Melatonin (NA) | Jiao *et al.* (2016) |
| | | *Pseudomonas* sp. VA-7 | Melatonin (NA) | Jiao *et al.* (2016) |
| | | *Pseudomonas fluorescens* RG11 | Serotonin (8.28 ± 0.65 ng $mL^{-1}$); melatonin (1.32 ± 0.12 ng $mL^{-1}$) | Ma *et al.* (2017) |
| Orange fruit | Yeasts | *Pichia kluyveri* | Melatonin (20.00 ± 2.02 ng $mL^{-1}$) | Fernández-Pachón *et al.* (2014) |
| Wine | Yeasts | *Saccharomyces cerevisiae* ARM | Melatonin (NA) | Rodriguez-Naranjo *et al.* (2012) |
| | | *Saccharomyces uvarum* S6U | Melatonin (NA) | Rodriguez-Naranjo *et al.* (2012) |
| | | *S. cerevisiae var. bayanus* BC | Melatonin (NA) | Rodriguez-Naranjo *et al.* (2012) |
| | | *S. cerevisiae* QA23 | Serotonin and melatonin (different concentrations)† | Muñiz-Calvo *et al.* (2019) |
| | | *S. cerevisiae* (AROMA WHITE) | Serotonin (4.5 ng $mL^{-1}$); melatonin (0.14 ng $mL^{-1}$) | Fernández-Cruz *et al.* (2016) |
| | Gram-positive bacteria | *Oenococcus oeni* UMB434 | Melatonin (0.0036 ± 0.0007 $ng_T$ $mL^{-1}$)‡ | Fracassetti *et al.* (2020) |
| | | *O. oeni* UMB436 | Melatonin (0.0022 ± 0.0008 $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |
| | | *O. oeni* UMB438 | Melatonin (0.0027 ± 0.0004 $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |
| | | *O. oeni* UMB462 (DMS20252T) | Melatonin (0.0049 ± 0.0010 $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |
| | | *O. oeni* UMB471 | Melatonin (0.0026 ± 0.0009 $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |

| | | |
|---|---|---|
| *O. oeni* UMB472 | Melatonin ($0.0078 \pm 0.0023$ $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |
| *O. oeni* UMB473 | Melatonin ($0.0013 \pm 0.0002$ $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |
| *O. oeni* UMB474 | Melatonin ($0.0021 \pm 0.0005$ $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |
| *O. oeni* UMB475 | Melatonin ($0.0025 \pm 0.0013$ $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |
| *O. oeni* UMB477 | Melatonin ($0.0049 \pm 0.0007$ $ng_T$ $mL^{-1}$) | Fracassetti *et al.* (2020) |

*Maximal concentration measured of serotonin and/or melatonin (extracellular production). †Different concentrations depending on culture media and added precursors (Muñiz-Calvo *et al.* 2019). ‡Theoretical volumetric production per $10^8$ CFU $mL^{-1}$ (CFU: colony-forming units). NA: data not available.

**Table 2** Different quantification methods for indoleamines detection

| Quantification method* | LOD† and LOQ‡ | Indoleamine measured/Sample analyzed | Reference |
|---|---|---|---|
| HPLC-FL | NA | Melatonin/Lupin primary roots | Arnao and Hernández-Ruiz (2007) |
| | LOD: 0.01 ng $mL^{-1}$, LOQ: 0.03 ng $mL^{-1}$ | Wines | Mercolini *et al*. (2008) |
| | LOD: 1.1 ng $mL^{-1}$, LOQ: 2.5 ng $mL^{-1}$ | Melatonin/Infant formula | Albu and Radu (2018) |
| | LOD: 1.2 ng $mL^{-1}$, LOQ: 1.4 ng $mL^{-1}$ | Serotonin/Infant formula | Albu and Radu (2018) |
| MEPS-HPLC-FL | LOD: 0.02 ng $mL^{-1}$, LOQ: 0.05 ng $mL^{-1}$ | Melatonin/Grapes, must, wine, grappa | Mercolini *et al*. (2012) |
| UHPLC-FL | LOD: 0.06 to 0.08 ng $mL^{-1}$, LOQ: NA | Melatonin/Plasma, eggs, milk | Yin *et al*. (2016) |
| HPLC-MS/MS | LOD: 0.1 ng $mL^{-1}$, LOQ: 0.5 ng $mL^{-1}$ | Melatonin/Human serum | Yang *et al*. (2002) |
| | NA | Melatonin and serotonin/Cherry fruits and juices | Özen and Ekşi (2016) |
| UHPLC-MS/MS | LOD: 0.006 ng mL-1, LOQ: 0.017 ng $mL^{-1}$ | Melatonin/Human serum | Wolrab *et al*. (2016) |
| | LOD: 0.028 ng $mL^{-1}$, LOQ: 0.1 ng $mL^{-1}$ | *N*-acetylserotonin/Human serum | Wolrab *et al*. (2016) |
| | LOD: 0.0047 ng $mL^{-1}$, LOQ: 0.0144 ng $mL^{-1}$ | Melatonin /Wines | Fernández-Cruz *et al*. (2016) |
| | LOD: 0.006 ng $mL^{-1}$; LOQ: 0.0204 ng $mL^{-1}$ | Serotonin/Wines | Fernández-Cruz *et al.* (2016) |
| | LOD: 0.0023 ng $mL^{-1}$; LOQ: 0.018 ng $mL^{-1}$ | Melatonin/Wines | Fracassetti *et al.* (2019) |
| | LOD: 0.030 ng $mL^{-1}$, LOQ: 0.085 ng $mL^{-1}$ | Melatonin/Wines | Gomez *et al.* (2012) |
| SCF-MS/MS | LOD: 0.017 ng $mL^{-1}$, LOQ: 0.05 ng $mL^{-1}$ | Melatonin/ Human serum | Wolrab *et al.* (2016) |
| | LOD: 0.006 ng $mL^{-1}$, LOQ: 0.018 ng $mL^{-1}$ | *N*-acetylserotonin/Human serum | Wolrab *et al.* (2016) |
| GC-MS | NA | Melatonin/Tomatoes fruits | Van Tassel *et al.* (2001) |
| Immunoprecipitation | NA | Melatonin/Tomatoes, ginger, marine green macroalga *Ulva Lactuca* | Pape and Luning (2006) |
| Radioimmunoassay | NA | Melatonin/Tomato fruits | Van Tassel *et al.* (2001) |
| Enzyme-linked | LOD: 0.0025 ng $mL^{-1}$, LOQ: NA | Melatonin/Beer | Maldonado *et al.* (2009) |

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|---|---|---|---|
| immunosorbent assay | | | |
| Cell-based fluorescent | LOD: 0.21 ng $mL^{-1}$ , LOQ: 0.67 ng $mL^{-1}$ | Melatonin/Grape must | Morcillo-Parra *et al.* (2019) |
| bioassay using β-lactamase reporter | LOD: 0.12 ng $mL^{-1}$, LOQ: 0.41 ng $mL^{-1}$ | Melatonin/Wines | Morcillo-Parra *et al.* (2019) |
| UHPLC-EC | NA | Melatonin/Intracellular *Saccharomyces* strains production | Muñiz-Calvo *et al.* (2017) |
| DSI-MS | LOD: 920 ng $mL^{-1}$, LOQ: 3940 ng $mL^{-1}$ | Melatonin/Commercial melatonin products | Jiang *et al.* (2019) |
| Real-time detection by FSCV | LOD: 5.56 ng $mL^{-1}$, LOQ: NA | Melatonin/Lymph node slices | Hensley *et al.* (2018) |

*HPLC: high performance liquid chromatography. UHPLC: ultra high performance liquid chromatography. GC: gas chromatography. FL: fluorescence detection. MS: mass spectrometry. EC: electrochemical detection. DSI: droplet spray ionization. SFC: supercritical fluid chromatography. FSCV: fast-scan cyclic voltammetry. MEPS: miniaturized microextraction by packed sorbent. LOD†: limit of detection. LOQ‡: limit of quantification. NA: data not available.