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# Deciphering the melatonin metabolism in *Saccharomyces cerevisiae* by the bioconversion of related metabolites

**Running title:** Melatonin synthesis in yeasts

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## Abstract

Melatonin (Mel), originally considered a neurohormone, has been detected in beverages and food-fermented products in which yeast metabolism is highly important. This indolamine is synthesized from serotonin, with L-tryptophan being the initial substrate of both. Regarding Mel metabolism, the biosynthetic pathway in mammals consists in four-step reactions. However, six genes are implicated in the synthesis of Mel in plants, which suggest the presence of many pathways. The aim of this study is to provide new empirical data on the production of Mel and other indole-related compounds in the yeast *Saccharomyces cerevisiae*

*(S. cerevisiae)*. To this end, we performed the addition of the pathway intermediates in *S. cerevisiae* cells in different growth stages (exponential and arrested cells) to follow the bioconversion and new indolic compound production from them. The different bioconverted indolic compounds tested (L-tryptophan, 5-hydroxytryptophan, tryptamine, serotonin, N-acetylserotonin, 5-metoxytryptamine and Mel) were analyzed by UHPLC-MS/MS from the extra- and intracellular contents. Our results showed that serotonin, in yeast, was prevalently formed via tryptophan decarboxylation, followed by tryptamine hydroxylation as in plants. Mel production from serotonin can be achieved by either N-acetylation, followed by O-methylation or O-methylation, in turn followed by N-acetylation. Accordingly, the classic pathway of Mel synthesis in vertebrates does not seems prevalent in yeast.

**Keywords:** Indolic compounds, bioconversion, melatonin metabolism, yeast, substrate pulses, indole biosynthesis pathway

## 1. Introduction

Lerner *et al*. in 1958 described melatonin (Mel) to be a neurohormone in the bovine pineal gland (1). It was subsequently, demonstrated that this indoleamine was also present in multiple extrapineal tissues (2,3). In the last two decades, Mel has been well accepted as a ubiquitous molecule present in many organisms belonging to different biological kingdoms (4–6).

In 1999, Sprenger et al. (7) conducted the first study about Mel synthesis in yeast. In this work, the authors reported that baker´s yeast was able to synthesize Mel from L-tryptophan (L-Trp), serotonin (Ser), N-acetylserotonin (NAS) and 5-methoxytryptamine (5MT) as potential precursors. Since then, very few studies have been carried out on this topic since most research has focused on demonstrating the presence of Mel or biosynthesis by yeasts in a fermentative context (8–12). Moreover, the biosynthetic pathway of Mel and other ICs is

completely unknown in yeasts. The only gene described and characterized as being involved in Mel production is *PAA1*, a polyamine acetyltransferase identified as the homolog of aralkylamine *N*-acetyltransferase (AANAT) (13), while the remaining genes and enzymes of the route are unknown to date.

Another issue to find out in the coming future is the physiological sense of Mel synthesis in yeasts. As for its properties, it is clear that Mel has a multiple effect on different organisms, such as the regulation of circadian rhythms and seasonal reproduction, immune system modulator, inhibitor of tumor growth and protection against UV light, among others (14–18). Mel's antioxidant action is well established, and it can act as a radical scavenger, by regulating antioxidant enzymes or contributing to the homeostasis of mitochondria (19–22). Recently, three independent works have also proved the role of Mel as an antioxidant and as UV protector in *S. cerevisiae* (23–25). Mel is also hypothesized to act as a growth signal molecule, (26) but the role of produced Mel is not very well known.

This work aims to provide new empirical data to unveil Mel production in *S. cerevisiae*. To this end, we carried out several pulses with the intermediates of the pathway (L-Trp, 5-hydroxytryptophan (5-OH-Trp), Ser, NAS, tryptamine and 5-MT) in cells in different growth stages. We evaluated the bioconversion of several indolic compounds (ICs) related with Mel in exponentially growing yeast cells using yeast minimal media (SD) in the batch-mode and synthetic must (SM) in a continuous culture. We also performed the experiment in arrested cells in salt medium (SLT) by following, but extending, the study conducted by Sprenger et al. (7). The intracellular content of cells and extracellular media, were analyzed by ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS).

## 2. Material and Methods

### 2.1. Chemicals and reagents

All the ICs used were: L-Trp, 5-OH-Trp, tryptamine, Ser, 5-MT, NAS, Mel purchased from Sigma-Aldrich (Madrid, Spain). Stock solutions were prepared and filtered through a 0.22 µm nylon filter. The calibration curve for HPLC-MS/MS was freshly prepared every time by diluting the solutions of 1 mg/mL of each IC in MilliQ-water to a final concentrations of 0.1, 1, 10, 100 and 1000 ng/ml. Stock solutions were stored at -20 ºC for no longer than 1 month. HPLC grade ethanol and acetonitrile were obtained from Panreac (Panreac AppliChem, Spain).

### 2.2.Yeast strains and inoculum preparation

The *S. cerevisiae* yeast strain used in this study was wine commercial strain QA23, provided by Lallemand S.A. (Canada) as active dry yeasts (ADY). For ADY QA23, the inoculum was prepared by rehydrating cells in sterile distilled water during 30 min at 37 ºC, and it was inoculated in the same media used for the indicated experiment.

### 2.3.Batch cultivations

For batch cultivations, two different growth modes were used. The first consisted of arresting cells in the depletion/restoration experiments described by Sprenger et al. (7) with some modifications. Yeast cells were suspended at cell densities of $\sim 10^8$ cells/mL in SLT (50 mM $Na_2HPO_4$, 0.5% NaCl, adjusted to pH 5.8 using citric acid) and incubated in Erlenmeyer flasks with orbital agitation (150 rpm) at 28 °C for 4 h. After the incubation period in SLT, ICs were added at a final concentration of 1 mM. Sampling was done 15 min after the indolic pulse. For harvesting, 10 mL were centrifuged at 4000 rpm for 3 min at 4 ºC. The obtained pellet ($\sim 10^9$ cells) was washed twice with sterile distilled water. Pellets were frozen with

liquid nitrogen and stored at -80 ºC and supernatants were stored at -20 ºC until further extractions.

The second batch-mode was performed in SD (0.17% (w/v) yeast nitrogen base, 0.5% (w/v) ammonium sulfate, 2% (w/v) glucose). Strain QA23, from the overnight preculture in SD, was inoculated in 20 ml SD at an optical density of 600 nm ($OD_{600}$) of 0.1 and incubated at 28 ºC until the $OD_{600}$ reached ~0.8. When cells were in the exponential growth stage, ICs at the 1mM final concentration were added. A volume of ~ 10 units of $OD_{600}$ was sampled at 15 min and centrifuged at 4000 rpm for 3 min at 4 ºC. After centrifugation, the cell suspension was washed twice with sterile distilled water. Finally the pellet was frozen with liquid nitrogen and stored at -80 ºC until intracellular extraction and the supernatant was stored at -20 ºC until further extraction.

### 2.4. Chemostat cultivation

The synthetic fermentation medium used for the continuous culture was a modified SM described by Bely et al. (27) with some modifications, such as 200 g/L of reducing sugars (100 g/L glucose and 100 g/L fructose), no anaerobic factors, and the total assimilable nitrogen source (YAN) 100 mg N/L came in the ammonium form ($NH_4Cl$).

Chemostat cultivation was performed in a 500 mL mini bioreactor (My Control miniBio, Applikon biotechnology, The Netherlands) with a working volume of 300 mL. The dilution rate (D) was 0.15 $h^{-1}$, the stirrer employed worked at 300 rpm and temperature was kept at 28 ºC. The medium pH remained at 3.3 using a pH sensor, and was controlled by an alkali pump by adding 1N NaOH. Before starting the continuous culture, strain QA23 was inoculated at ~$2x10^6$ cells/mL and grown in the batch mode to achieve enough biomass. The continuous culture was connected when the batch culture entered the stationary phase. The IC addition pulse in the culture was for a 0.5mM final concentration, and was performed only after all the

continuous cultures had been running for at least five residence times and the biomass values were constant. Once addition was done, a volume of ~ 35 units of $OD_{600}$ was sampled at 15 min and centrifuged at 4000 rpm for 3 min at 4 ºC. After centrifugation, the cell suspension was washed twice with sterile distilled water, and the pellet was frozen with liquid nitrogen and stored at -80 ºC until intracellular extractions. Finally, the supernatant was stored at -20 ºC until further extractions.

### 2.5. Sample preparation

For the determination of the intra and extracellular ICs, culture supernatants and the intracellular content were extracted and analyzed by UHPLC-MS/MS. The intracellular metabolite content was obtained by adding 1 mL of cold mixture ethanol/water 50% (v/v) to the cell pellets and 0.3 mL of glass beads. Cells were ruptured by a Tehtnica MillMix 20 homogenizer (Tehtnica, Slovenia) with three shaking cycles at 30 $s^{-1}$ for 30 s at 4 °C. Lysed cells were centrifuged at 5000 × g and 4 °C for 10 min and supernatant was filtered by a 0.22-µm nylon filter, collected and placed inside a new tube and stored at -20 °C until further use.

For the extracellular extraction, an equal amount of absolute ethanol was added to the supernatant. The mixture was filtered by a 0.22-µm nylon filter (Phenomenex, USA) and stored at -20 ºC until analysis.

### 2.6. Indolic compound analysis by UHPLC-MS/MS

The indolic studied metabolites were quantified by UHPLC-MS/MS as described previously (28). The MRM transitions used for quantification were *m/z* 205 → 118.03 and *m/z* 205 → 145.85 for L-Trp, *m/z* 177 → 132.21 and 177 → 160.03 for Ser, *m/z* 221 → 162.19 and 221 → 203.99 for 5-OH-Trp, *m/z* 219 → 159.96 and *m/z* 219 → 202.00 for NAS, *m/z* 233 →

174.10 and 233 → 216.10 for Mel, *m/z* 161> 144 for tryptamine and *m/z* 191.2> 174 for 5-MT.

## 3. Results and Discussion

It is known that Mel is produced by yeasts during alcoholic fermentation and this production depends on the medium, mainly the L-Trp and sugar concentrations, and also the growth phase (9,11,29–31), among others conditions. However, the biosynthetic pathway of Mel and other ICs is completely unknown in yeasts. Conversely, this metabolic route has been extensively studied and well established in other organisms. Although differences in the intermediates and branches of the route have been found in different organisms (32), there is a high degree of conservation in the enzymatic reactions which leads to the synthesis of Mel. To unveil the putative biosynthetic route and the order of the enzymatic steps in yeasts, we studied the production of ICs, both intra- and extracellularly, after pulses of different intermediates of the route in different growth systems: exponential growth conditions in SD and continuous culture with SM. Chemostat cultures help to accurately control many variables, such as the specific growth rate, temperature and pH, among others. This steady state is only disrupted by the pulses of different intermediates, to produce an over-flow of its metabolic conversion.

### 3.1. Pulses of melatonin intermediates to yeast cells: a general overview

L-Trp, 5-OH-Trp, Ser, NAS, tryptamine and 5-MT were pulsed to the cells grown in SD (the batch culture) or SM (the continuous culture), and to the cells incubated in a non-proliferative medium SLT (arrested-cells). After 15 min of the pulses, the intracellular and extracellular metabolites were analyzed by UHPLC/HRMS (Table S1). As many data were collected, and to provide a better understanding, we represented the increases in the different ICs on a heatmap (Fig. 1).

Although different growth systems provide considerable similarities in the different indole bioconversions after each pulse, we also detected differences in the production of the indolic metabolites among growth media, which could be explained by either a higher permeability of cells to the pulsed compounds or a different bioconversion rate in the various growth states. Most of the produced metabolites were detected either intracellularly or extracellularly, but a direct correlation was not always found between the presence in the medium and in the cellular compartment. This could also be due to the fact that, under specific conditions, certain compounds, are released to the medium or are rapidly converted into other metabolites (33). Previous publications have also reported the rapid turnover of Mel, as well as other 5-methoxylated related indoles, as a result of different mechanisms, such as photooxidation (34), hydroxylation by free radicals (35) or metabolization by different enzymes (36), which can also contribute to these differences observed among the various growth systems and cellular states.

The conversion of L-Trp into Ser involves two reactions: hydroxylation and decarboxylation. However, the order of these reactions described for distinct organisms differs. On the one hand, L-Trp is hydroxylated to 5-OH-Trp, by the tryptophan hydroxylase (TPH) enzyme, and is then decarboxylated, by aromatic L-amino acid decarboxylase (AADC), which gives rise to Ser. On the other hand, and alternative way, AADC can decarboxylate L-Trp into tryptamine, and then tryptamine is hydroxylated by tryptamine 5-hydroxylase (T5H), to result in Ser. The second part of the biosynthetic route consists in the conversion of Ser into Mel. Once again, this conversion can also occur in two different ways and the enzymes described as being involved are: AANAT or acetylserotonin O-methyltransferase (ASMT)/caffeic acid O-methyltransferase (COMT). If Ser is acetylated by AANAT, it produces NAS, which can then be O-methylated by ASMT/COMT to produce Mel. However, Ser can first be O-methylated and then the resulting 5-MT is acetylated by AANAT to generate Mel.

### 3.1.1. Hydroxylation activity

As explained above, there are two hydroxylation activities involved in the synthesis of Ser. The hydroxylation of L-Trp to 5-OH-Trp is carried out by the TPH enzyme. This reaction is known as the first step in the classic Mel biosynthetic pathway described for animals. Conversely, in plants, hydroxylation is a second step in the biosynthetic pathway, and occurs by T5H converting tryptamine into Ser. In order to determine which hydroxylation reaction preferentially occurs in *S. cerevisiae*, we used L-Trp and tryptamine as substrates in feeding experiments. When L-Trp was added as a precursor, we were not able to detect 5-OH-Trp either intra or extracellularly for any of the growth systems (Fig. 1). Nonetheless, when we supplemented the media with tryptamine, we mostly detected Ser both extra- and intracellularly in all the growth systems (Table S1 and Fig. 2A). We highlight the vast amount of intracellular Ser observed in SLT (more than 300 ng/mL), which was by far the highest concentration detected for any analyzed conditions. In light of these results, the conversion of tryptamine into Ser seemed the preferential hydroxylation reaction, whereas L-Trp hydroxylation must be a very scarce or a null enzymatic reaction in *S. cerevisiae*.

Aromatic amino acid hydroxylases (AAAH) are non-heme iron family of enzymes in which TPH is encompassed together with phenylalananine and tyrosine hydroxylase (PAH and TH) (37). Moreover, AAAH are pterin-dependent enzymes that require the presence of special cofactors, such as tetrahydrobiopterin or tetrahydromonapterin (38), except for a TPH from dinoflagellate *Gonyaulax polyedra*, which doesn't require tetrahydropterin cofactors (39). In fact the heterologous expression of AAAH in *S. cerevisiae* has involved the engineering of the biosynthetic and recycling pathways for these essential cofactors in order to avoid continuous and expensive exogenous additions (38,40,41). Notwithstanding, in these works some native fungi genes, such as dihydrofolate reductase (*DFR1*) and GTP cyclohydrolase I

(*FOL2*), involved in tetrahydrofolate and folic acid biosynthesis, have been shown to play a positive role in pterin-based cofactors regeneration (40,41).

Despite our data pointing out the lack of genes and enzymes for the biosynthesis of 5-OH-Trp from L-Trp, we observed the transformation of this compound into other metabolites when it is present in the medium. The 5-OH-Trp pulse resulted mainly in the synthesis of L-Trp, but also in that of tryptamine, Ser and NAS. This compound has been detected in both grape must (31,42) and a synthetic lab medium (43). Thus the presence of 5-OH-Trp in natural or synthetic media could be used by yeasts to produce different indolic metabolites. In our case, the different media that we used (SLT, SD and SM) were modified and did not contain amino acids as a nitrogen source. Thus we avoided the presence of L-Trp or 5-OH-Trp, which could interfere with bioconversion assay.

Further evidence for the hydroxylation of tryptamine to Ser in *S. cerevisiae* relies on the direct correlation between an effective shikimate pathway for the synthesis of L-Trp and the presence of tryptamine-hydroxylation activity, as described in many organisms (32). The shikimate pathway is the central core of the aromatic amino acid biosynthetic pathway (AAP) in bacteria, fungi and plants (44), but is not operative in animals.

### 3.1.2. Decarboxylation step

A lyase enzyme that belongs to the group of AADC is responsible for carrying out the two possible alternative decarboxylation reactions involved in the synthesis of Ser: either the conversion of L-Trp into tryptamine or 5-OH-Trp into Ser. We detected both metabolites after the pulse of L-Trp and the pulse of 5-OH-Trp, respectively, and with differences in the concentration and location depending on the growth medium (Fig. 1 and Table S1). Tryptamine was detected mainly extracellularly after the pulses in SD and SLT. As mentioned above, the decarboxylation of L-Trp into the biogenic amine is the first step in

most organisms with an active shikimate pathway (32). Our data also support this reaction as being the most probabilistic step in the synthesis of Mel in *S. cerevisiae*. Yet despite being less abundant, the presence of 5-OH-Trp has also been evidenced in natural media (31,42). In these cases 5-OH-Trp can also be decarboxylated to directly produce Ser. In fact, we detected intracellular and extracellular Ser after the 5-OH-Trp pulse, with good accumulation in the cells incubated in SLT (Fig. 2B).

However, question marks appear with these results: which enzyme carries out this decarboxylation? Are both decarboxylation activities performed by the same decarboxylase? In *S. cerevisiae*, the decarboxylation of the alpha-ketoacid from L-Trp (indolpyruvate) could be performed by any of the different broad substrate specificity decarboxylases *PDC1*, *PDC5*, *PDC6* and *ARO10* (45,46). Yet despite the presence of several decarboxylases in *S. cerevisiae*, none has been described with L-Trp decarboxylase activity, but they have been reported in other fungi (47,48).

The mammalian AADC enzyme (DDC) catalyzes the decarboxylation of different types of aromatic amino acids, whereas several decarboxylases with different activity and substrate specificity have been described in plants (49). The tryptophan decarboxylase (TDC) of plants can use L-Trp and 5-OH-Trp as substrates (50,51). This TDC of plants can be functional in *S. cerevisiae* because Ser was produced, at the expense of the 5-OH-Trp levels (52).

One interesting result, which has not been reported to date, was the significant 5-OH-Trp production that took place when Ser was used as a precursor under all the conditions, mainly extracellularly (Fig. 2C). This production involved a reversible reaction of the decarboxylation step that is, the carboxylation of Ser into 5-OH-Trp.

This way that 5-OH-Trp formation occurs has not been taken into account to date. Nonetheless, more studies are needed to understand the mechanism and the physiological relevance of this 5-OH-Trp production.

### 3.1.3. N-acetylation step

In the second part of the Mel pathway, N-acetylation can occupy the first step with the conversion of Ser into NAS or with an alternative step with the acetylation of 5MT into Mel. Both N-acetylations are led by AANAT and homologous yeast gene *PAA1* has been described (13). Once again, we observed the production of both metabolites NAS and Mel under most of the conditions tested after the respective pulses (Fig. 1 and Table S1). The highest NAS concentration was observed intracellularly in the cells incubated in SLT, but the most striking result was the high Mel concentration detected in the growth medium after the 5MT pulses (Fig. 2D). According to our experience, feeding cells with 5MT was the most reproducible and effective method to synthesize Mel. The fact that Mel in *S. cerevisiae* can be preferentially synthesized from 5MT, and at higher concentrations, instead of from NAS, supports the alternative pathway hypothesized by Tan et al. (32), and highlights that the last enzyme in this pathway must be *N*-acetyl transferase instead of *O*-methyltransferase. This hypothesis has also been supported by the results obtained by Ganguly et al. (13), in which the characterization of enzyme encoded by *PAA1* was able to acetylate 5MT to Mel.

### 3.1.4. O-methylation step

Axelrod and Weissbach (53) described enzyme ASMT as being responsible for performing the conversion of N-acetyl Ser into Mel at the expense of SAM. This enzyme, along with other enzymes that possess N-acetylserotonin O-methyltransferase activity, such as COMT, has been regarded as the last enzyme of the conventional Mel biosynthesis pathway. This activity in plants, is the penultimate one as it has been found that both recombinant *Arabidopsis thaliana* ASMT and COMT are able to use Ser as a substrate to produce 5MT (54,55). To elucidate what could take place in *S. cerevisiae*, we carried out pulses with both substrates Ser and NAS. However, we cannot rule out any of these metabolic steps because

we detected the production of both metabolites under different growth conditions and at distinct locations. For NAS used as a substrate, we observed Mel production in both intra- and extracellular compartments during growth in SM, but with wide variability. As regards Ser as a substrate, we observed an increase in 5MT under extracellular SLT and SM conditions and only under intracellular conditions for SLT (Fig. 1 and Table S1). Likewise, the key point in future research is to discover which enzyme in yeasts is able to methylate both substrates.

### 3.2.Catabolism

Regarding Mel catabolism, hydroxylation, demethylation, deacetylation, and deoxygenation reactions can lead to the synthesis of different metabolites, such as N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), N1-acetyl-5-methoxykynuramine (AMK), 2-hydroxymelatonin or 6-hydroxymelatonin in distinct organisms (56,57). Subsequent studies on yeast Mel metabolism should include the catabolites downstream of Mel synthesis, to further enrich the comprehension of this metabolic pathway. Tan et al. (58) reported that some of these Mel-catabolites can reach up to 1000-fold Mel levels. Thus Mel should be considered as an intermediate rather than a final product in the synthesis of ICs in yeasts. In fact, the catalytic efficiency ($V_{max}/K_m$) of melatonin 2-hydroxylase (M2H) from plants, which converts Mel into 2-hydroxymelatonin, is 2000-fold higher than that of ASMT (59,60).

The conversion of Mel into NAS by O-demethylation has been described for humans, and implies subforms of cytochrome P(450) (56,61). To find out if this reaction also occurrs in yeast, we performed a pulse of Mel to a wine *S. cerevisiae* strain incubated in SLT medium. Interestingly, we observed the production of large amounts of NAS and 5MT in the extracellular fraction (Fig. 3). The catabolic deacetylation pathway leading to 5MT formation was previously observed in *S. cerevisiae* (7). To date however, the O-demethylation of Mel

into NAS had not been reported in *S. cerevisiae*. In humans, Mel is metabolized principally to 6-hydroxymelatonin, which is further conjugated with sulfate and excreted in urine, whereas Mel O-demethylation represents a minor reaction (62). In our case, 5MT formation was greater than that of NAS, but both catabolites were found in significant amounts in the growth medium. In future experiments, we should broaden the metabolytes analyzed to other Mel catabolism products such as 6-hydroxymelatonin.

### 3.3.Melatonin synthesis in yeast: a putative biosynthetic pathway

Based on the results obtained from the different bioconversion experiments, the putative Mel biosynthetic pathway is proposed in Fig. 4. Unlike plants and animals, it seems that the synthesis of 5-OH-Trp by the hydroxylation of L-Trp does not occur in yeasts. Instead L-Trp can be decarboxylated into tryptamine as the first step of the route. Later this tryptamine is used as a precursor for Ser synthesis by hydroxylation. Yet despite us not detecting the 5-OH-Trp formation from L-Trp, *S. cerevisiae* is able to transform this compound into Ser, and its presence in the medium can serve to produce different ICs. Interestingly, we also observed the 5-OH-Trp production in larger amounts in a reversible reaction from Ser. This Ser carboxylation has not yet been reported and it is worth studying in forthcoming experiments to gain insights into not only the physiological and metabolic conditions under which this transformation happens in yeasts, but also into the putative genes and enzymes involved in it. From Ser, the two converted products were NAS (*N*-acetylation) and 5MT (*O*-methylation), with the latter being the preferred substrate to produce Mel. Finally, the yeast was able to convert Mel into its two precursors, NAS and 5MT, by N-deacetylation or O-demethylation activities, respectively, in reversible steps to most described Mel pathways.

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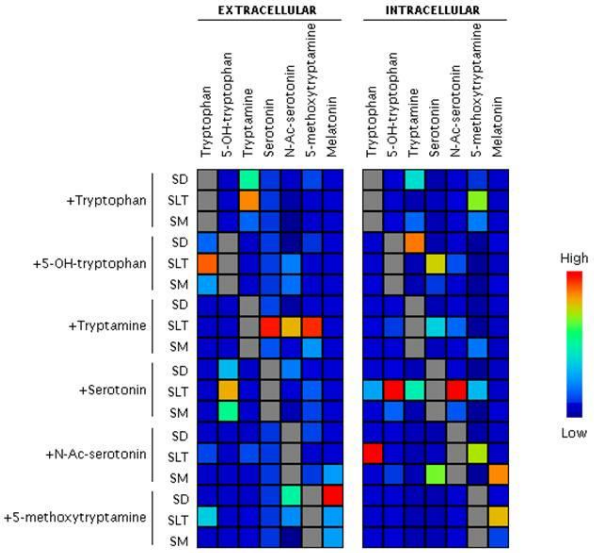
**Figure legends**

**Fig. 1** Effect of the addition of different potential melatonin precursors (rows) on the increase of its related products (columns) extra- and intracellularly in *Saccharomyces cerevisiae*. Differences in concentrations were normalized and the increases of the compound used in each pulse are not considered (grey boxes). Defined synthetic (SD), salt medium (SLT) and synthetic must (SM).

**Fig. 2** Metabolite production in yeast after addition of exogenous tryptamine (A), 5-hydroxytryptophan (B), serotonin (C) and 5-methoxytryptamine (D) in the extra- and intracellular production (dotted and stripped bars respectively). Different growth systems are defined synthetic (SD), salt medium (SLT) and synthetic must (SM). The error bars represent SD.

**Fig. 3** Melatonin catabolites produced extracellularly after 1 mM exogenous melatonin addition to arrested yeast cells in salt medium. N-acetylserotonin (NAS), 5-methoxytryptamine (5MT). Error bars represent SD.

**Fig. 4** The hypothesized melatonin biosynthetic pathway in yeast. L-Trp (tryptophan), Tryp (tryptamine), 5OH-Trp (5-hydroxytryptophan), Ser (serotonin), NAS (N-acetylserotonin), 5MT (5 methoxytryptamine). Enzymatic steps are shown in numbers: 1 (tryptophan decarboxylation), 2 (tryptamine hydroxylation), 3 (5-hydroxytryptophan decarboxylation), 4 (5-hydroxytryptophan carboxylation), 5 (serotonin N-acetyltation), 6 (N-acetylserotonin O-methylation), 7 (N-acetylserotonin O-demethylation), 8 (serotonin O-methylation), 9 (5-methoxytriptamine N-acetylation), 10 (melatonin deacetylation).

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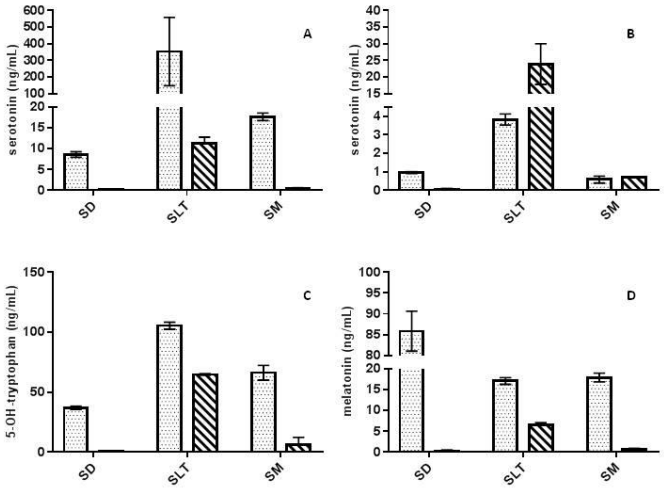


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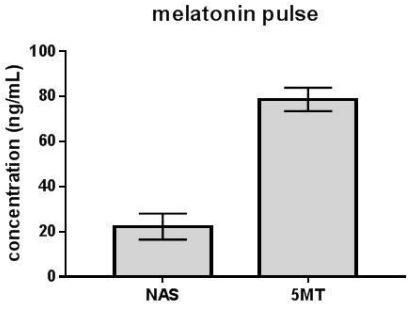


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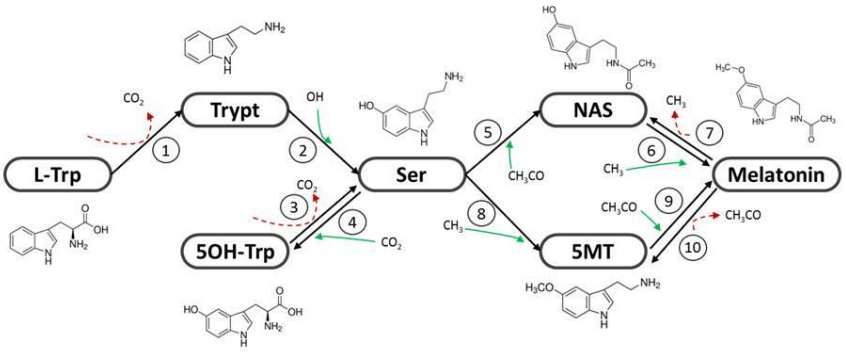


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