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FELLOWSHIP FINAL REPORT

Bio-production of vindoline and catharanthine by recombinant yeast cell factories

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REPORT INFO

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ABSTRACT

The tropical plant Madagascar periwinkle (Catharanthus roseus) is a natural source of anticancer monoterpene indole alkaloids (MIA), such as vinblastine and vincristine, two molecules of major interest and therapeutic values. The MIA biosynthetic pathway in C. roseus is described in the literature as the most complex pathway in all living organisms and shows, in planta, an outstanding compartmentation at both cellular and subcellular levels. Our approach aimed to producing vindoline and catharanthine, two precursors of vinblastine and vincristine, in yeast cell factories. In particular, we developed and optimized yeast cell factories efficiently converting tabersonine to vindoline. First, fine-tuning of heterologous gene copies restrained side metabolites synthesis towards vindoline production. Tabersonine to vindoline bioconversion was further enhanced through a rational medium optimization (pH, composition) and a sequential feeding strategy. Finally, a vindoline titer of 266 mg/L (88% yield) was reached in an optimized fed-batch bioreactor. This precursor-directed synthesis of vindoline thus paves the way towards a future industrial bioproduction through the valorization of abundant tabersonine resources.

1- Introduction

Vinblastine and vincristine are two major anticancer monoterpene indole alkaloids (MIAs) accumulated in the leaves of *Catharanthus roseus*, and extensively used in human chemotherapies (Fig. 1). Due to a constantly growing demand, both vinblastine and vincristine are suffering from recurrent shortages caused by socio-economic (e.g. demand pressure) and/or geopolitical and climatic constraints (Rabin, 2019). Additionally, the intensive culture of *C. roseus* conducted for the supply of these two drugs has a dramatic negative impact on the environment (e.g. water resource, pollution…) driving to the necessity of alternative methods for a more sustainable production according to the Sustainable Development Goals (SDGs) of the United Nations (https://www.un.org/sustainabledevelopment/sustainable-development-goals). In such

a perspective, the bioproduction of MIAs in heterologous hosts constitutes an attractive option to ensure a stable supply to the pharmaceutical industry (Courdavault et al., 2020). Among all generally regarded as safe (GRAS) microorganisms, *Saccharomyces cerevisiae* (baker yeast) displays many advantages such as a well-known sequenced genome, as well as numerous molecular tools for genetic manipulation, and constitutes a highly valuable platform for industrial bio-production of plant natural products (Guirimand et al., 2020). Since both vinblastine and vincristine derive from the condensation of catharanthine and vindoline, a stable supply of these two anticancer compounds could rely on the development of yeast cell factories by metabolic engineering of *S. cerevisiae* to produce vindoline and catharanthine as precursors. In total, the biosynthetic pathway leading to vindoline from tabersonine encounters 7 enzymatic steps, while the catharanthine biosynthesis from tryptamine and secologanine encounters not less than 12 steps (Fig. 2). Conceptually, the vindoline synthesis can be performed either de novo (i.e. from glucose as original carbon source) or through precursor directed synthesis by feeding engineered yeast with tabersonine, extractible at multigram scale from the highly abundant seeds of *Voacanga africana* (Kikura-Hanajiri et al., 2009; Koroch et al., 2009). Although the bioproduction of vindoline by yeast cell factories has been previously reported, the synthesis remains at a scarce level and thus requires optimization to reach industrial scale (Qu et al., 2015).

In the present study, the tabersonine to vindoline, and the [tryptophane + loganine] to catharantine biosynthetic pathways from *C. roseus* were respectively stably integrated within hotspots of the genome of *S. cerevisiae*, using CRISPR-Cas9 molecular tools (Mikkelsen et al., 2012) in order to generate recombinant strains able to produce vindoline and catharantine (Fig. 2 to 4). The fine-tuning of the copy numbers of the pathway genes and the optimization of the fermentation conditions allowed us to synthesize an unprecedented amount of vindoline, as reported in Kulagina et al. (2021), thus paving the way for a complementary production process of anticancer MIAs through metabolic

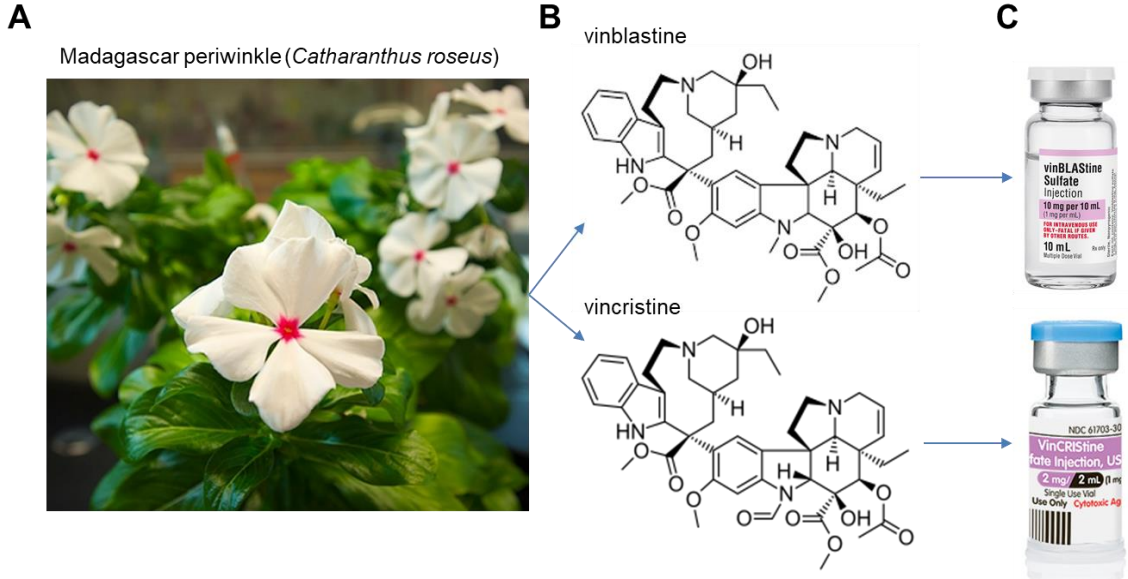

Figure 1. Natural origin of two different monoterpene indole alkaloids molecules with anticancer properties. *Catharanthus roseus* (**A**) is a tropical plant naturally able to synthesize the anticancer molecules vinblastine and vincristine (**B**), both used in chemiotherapy treatments in human medicine (**C**).

engineering combined to the valorization of a renewable natural resource.

Experimental details

Plasmid construction

The vectors used as template donors for the integration of expression cassettes through CRISPR-cas9 method were constructed from a pDONR221 backbone. The Gateway cloning cassette was replaced by a NotI restriction site to insert 500 pb arm sequences upstream and downstream of the different integration loci (Mikkelsen et al., 2012), separated with a SalI restriction site for the cloning of expression cassettes. A selection of promoters and terminators were amplified from yeast gDNA using specific primers, combined via overlap PCR to generate various expression cassettes, and cloned in the SalI restriction site of arms-containing backbone vectors. The expression cassettes are composed of two inverted promoter/terminator couples to produce artificial bidirectional promoters, and SpeI or NheI restriction sites were introduced for further ORF cloning. The sequences of tabersonine 16-hydroxylase 1 and 2 (T16H1, T16H2), tabersonine 16-O-methyltransferase (16OMT), tabersonine 3-oxygenase (T3O), tabersonine 3-reductase (T3R), 16-methoxy-2,3-dihydro-3-hydroxytabersonine N-methyltransferase (NMT), deacetoxyvindoline 4-hydroxylase (D4H) and deacetylvindoline O-acetyltransferase (DAT) from *C. roseus*, as well as NADPH-cytochrome P450 reductase 2 (ATR2) from *Arabidopsis thaliana* were PCR amplified from cDNA, introducing SpeI, NheI or XbaI compatible restriction sites for the cloning into expression cassettes.

Strains

Saccharomyces cerevisiae CEN.PK113-7D (MATa MAL2-8C, SUC2) was used as the starting strain. Single and multiplex CRISPR-Cas9 integration methods were performed to introduce expression cassettes in previously described hotspot yeast loci (Mikkelsen et al., 2012). The CAS9 expression plasmid (pCfB2312) was transformed into *S. cerevisiae* prior the co-transformation with gRNA helper plasmids (pCfB3020, pCfB3041-pCfB3053; Jessop-Fabre et al. 2016) and the appropriate NotI-linearized donor DNA vectors, by using the lithium acetate transformation method (Chen et al. 1992). Yeast transformants were selected on YPD medium (20 $g.L^{-1}$ of peptone, 10 $g.L^{-1}$ of yeast extract, and 20 $g.L^{-1}$ of glucose) supplemented with G418 (200 $mg.L^{-1}$) and nourseothricin (100 $mg.L^{-1}$), and screened for the effective integration of expression cassette by colony PCR with a specific primers couples.

Small-scale culture conditions

Strains pre-cultured in 5mL YPD overnight were diluted 20 times in YPD medium, by default prepared with BovMP peptone or, when stated, with different sources of peptone. Small-scale feedings in final volumes of 200 µL were supplemented with 125 µM of tabersonine and incubated at 28°C, under constant agitation at 200 rpm for 72-120 hours, individually for each strain, replicate and time point. Every 24h glucose was added to the cultures to 20 $g.L^{-1}$ (referred to as standard conditions in this study). When stated, from 48h and every 24h fresh YP made of BovMP peptone and yeast extract was added (referred to as YPt48h). When mentioned, YPD medium was buffered to pH 4.0, pH 5.0 or pH 6.0 at t0 using 50 mM citrate buffers.

Bioreactor culture conditions

For bioreactor cultures, cell stock, cell amplification and bioreactors inoculation were performed as previously described by Marc et al. (2013), with adapted volumes for propagation in YPD. Fed-batch cultures

were performed in 2 L bioreactors (Global Process Control) with a working volume of 1.5 L, managed with the CBio2 software. Temperature was regulated at 30°C and pH at 6.0 by addition of 5 M KOH solution. Air flow and stirring rate were adjusted to maintain fully aerobic conditions, i.e. a dissolved oxygen concentration above 20%

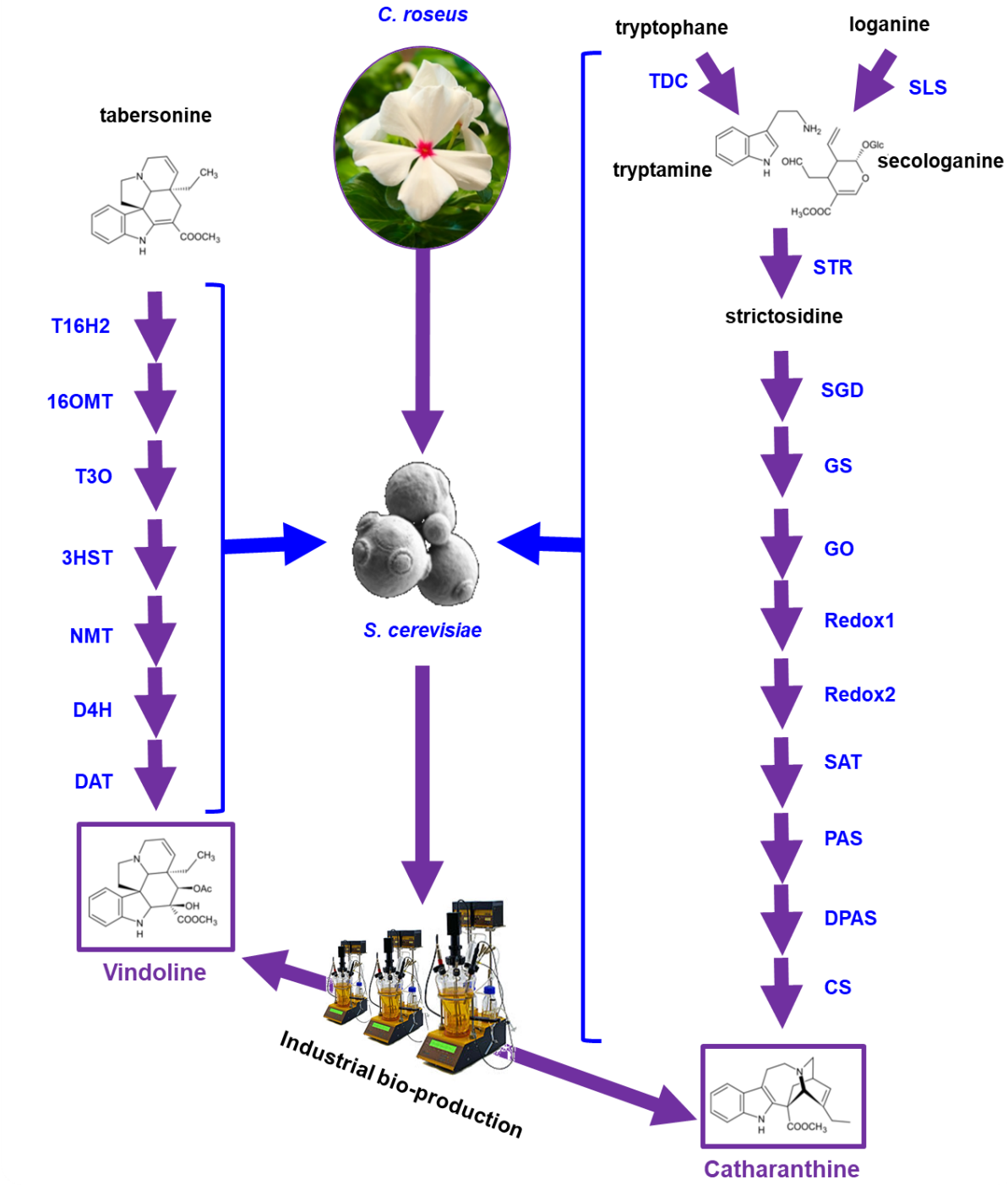

Figure 2. General overview of the strategy used in this project to produce vindoline and catharanthine by recombinant yeast cell factories. The vindoline (left) and catharanthine (right) biosynthetic pathways, encounter 7 and 12 enzymatic steps respectively, are both transferred into the genome of *Saccharomyces cerevisiae* (center) in order to establish cell factories capable to produce these alkaloids in a bio-reactor, at an industrial scale.

of saturation. Initial medium composition contained per liter: 10 g of yeast extract, 20 g of peptone and, when mentioned, 20 g of glucose. Feeding of nutritive elements was ensured by pumping solutions of glucose (200 $g.L^{-1}$) or concentrated YPD (50 $g.L^{-1}$ yeast extract, 100 $g.L^{-1}$ peptone, 200 $g.L^{-1}$ glucose). Feeding of tabersonine was ensured by pulsing a 125 mM solution or pumping a 29 mM one, both prepared in pure ethanol. Four strategies of bioproduction were established, combining pulsed or continuous feeding of glucose, concentrated YPD and tabersonine. Cells did not receive tabersonine in the F0 reference culture, managed as the F1 for nutrients. F1 was designed to mimic the culture conditions at small scale leading to the higher production, by feeding glucose or concentrated YPD by pulses every 24h. F2 was fed continuously at a steady flow rate with an equivalent amount of nutrients than pulsed in the F1 culture, to avoid any starvation. In F1 and F2, tabersonine was fed with an initial 125 µM pulse when starting the culture. In order to enhance the production of vindoline, six more pulses of 125 µM tabersonine were carried out every 12h in F3, based on the F2 nutrients feeding strategy, accordingly. An equivalent amount of tabersonine was continuously fed at a steady flow rate in the F4 culture, from t0 and until the total consumption of the last pulse of tabersonine in F3.

Sample analysis

Determination of tabersonine, vindoline, vindorosine and intermediate metabolites

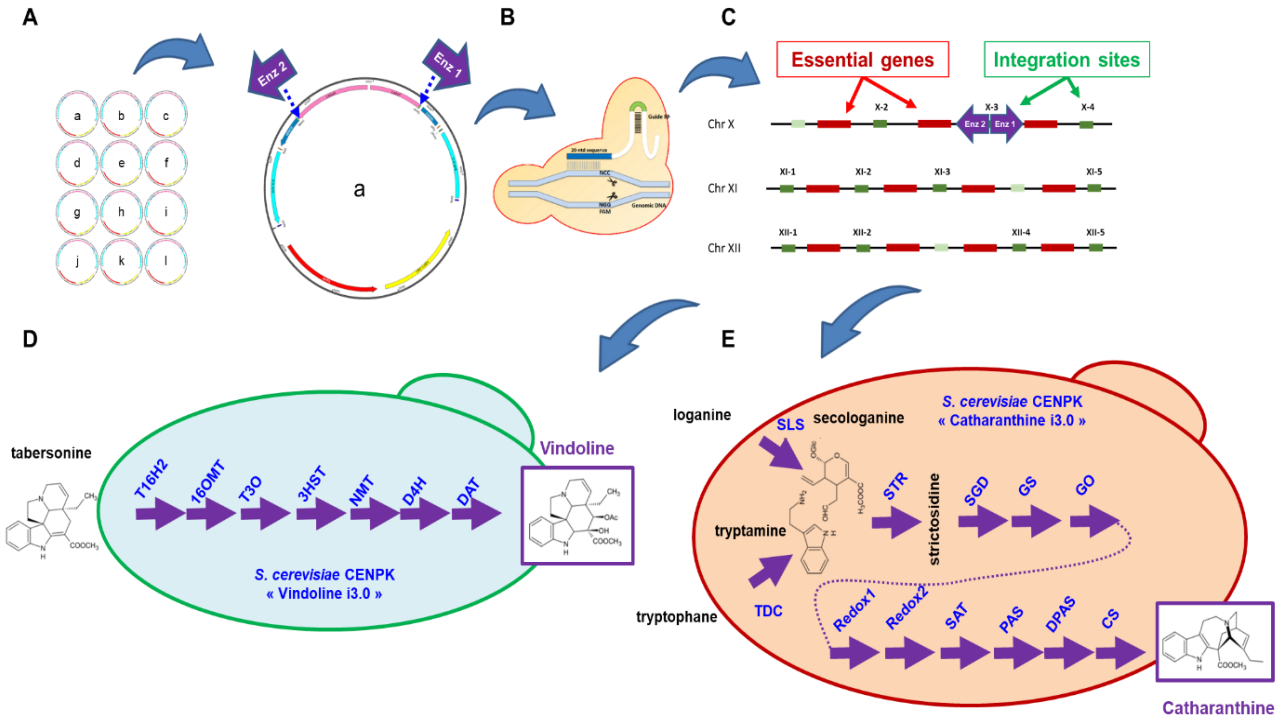

Figure 3. Schematic representation of the strategy implemented in this project to achieve the heterologous bio-production of vindoline and catharanthine by recombinant yeast cell factories. A total of 12 different vectors containing bidirectional strong promoters have been constructed (**A**). Each vector enable us to stably integrate into the genome of *S. cerevisiae* two diferent enzyme genes simultaneously, which means that we are able to integrate up to 22 different genes within the same strain of yeast. The cutting edge molecular tool CRISPR-Cas9 (**B**) is used to integrate these genes at very specific regions ("hot spots") of the genome (**C**), located in close vicinity of essential genes of the yeast cells, ensuring a strong stability and expression levels of the transgenes. This powerful approach allowed to generate the recombinant yeats cell factories *S. cerevisiae* CENPK "Vindoline i3.0" (**D**) and *S. cerevisiae* CENPK "Catharanthine i3.0" (**E**), which will be used for the industrial bio-production of vindoline and catharanthine, respectively.

was performed using an UPLC-MS and parameters as previously described (Parage et al., 2016). Retention time and m/z for each compound are listed in figure 4A. In small-scale experiments prior analysis, supernatants were obtained by centrifugation (10 min, 7 000g), diluted 20 times in MeOH, vortexed and centrifuged (15 min, 20 000g). For bioreactors monitoring, determinations of cell viability and biomass, glucose and fermentation metabolites concentrations were performed as previously described (Marc et al., 2013). Nevertheless, supernatants were obtained by centrifugation (4 min, 4,000g). Off-gas were analyzed by infrared absorption (CO2) and electrochemical measurement (O2) with Tandem analyzers (Magellan BioTech).

2- Results and discussion

2.1. Engineering of a vindoline-producing yeast strain

The bioconversion of tabersonine to vindoline requires seven enzymatic steps starting with T16H hydroxylating tabersonine (1) at carbon 16 to form 16-hydroxytabersonine (2), which is further methylated by 16OMT to 16-methoxytabersonine (3). Downstream conversion to 16-methoxytabersonine epoxide (4) is catalyzed by T3O, which in turn is reduced to 16-methoxy-2,3-dihydro-3-hydroxytabersonine (5) by T3R. In the following steps, the T3R product is converted to desacetoxyvindoline (6) by NMT, then hydroxylated to deacetylvindoline (7) by D4H and finally converted to vindoline (8) by DAT (Fig. 2 and Fig. 4A; (Courdavault et al., 2014)). However, substrate promiscuity enables T3O using tabersonine directly to form tabersonine imine alcohol (9) (Kellner et al., 2015). This marks the onset of a parallel branch pathway leading to the production of undesirable by-products including 2,3-dihydro-3-hydroxytabersonine (10), desacetoxyvindorosine (11), deacetylvindorosine (12) and vindorosine (13), whose in planta amounts depend on T16H expression level (Besseau et al., 2013). Consequently, the heterologous production of vindoline may face suboptimal flux according to the initial decoration of tabersonine by T16H or T3O. In order to create yeast cell factories producing vindoline from tabersonine, we selected the prototrophic S. cerevisiae strain CEN.PK113-7D, commonly used in metabolic engineering for industrial purposes. We initially implemented the five first genes of the tabersonine-to-vindoline biosynthetic pathway to produce desacetoxyvindoline through tabersonine transformation (strain T3110). T16H2, 16OMT, T3O, T3R, NMT coding sequences and ATR2 (required for T16H and T3O reduction) were integrated into three distinct hotspots of the genome of S. cerevisiae, selected for high transcription activity (Mikkelsen et al., 2012), under strong glycolytic promoters. To assess the production of desacetoxyvindoline, T3110 strain was fed with 125 µM of tabersonine and grown during 120h. Culture medium was collected every 24 h and extracellular metabolite content was analyzed by liquid chromatography coupled with mass spectrometry (LC-MS) since the majority of alkaloids are accumulated in the extracellular medium (Qu et al., 2015). Although tabersonine was not fully consumed after 24 h, the T3110 strain produced both desacetoxyvindoline and desacetoxyvindorosine in similar proportions (Fig. 4B). This confirms that the metabolic flux is simultaneously directed towards vindoline and vindorosine pathways as a result of T16H and T3O competition for tabersonine consumption. Therefore, in order to limit the vindorosine accumulation and improve the efficiency of tabersonine bio-conversion, one additional copy of both T16H and 16OMT genes were introduced into the T3110 strain. The resulting daughter strain, named Q7, showed an 11-fold decrease of desacetoxy-

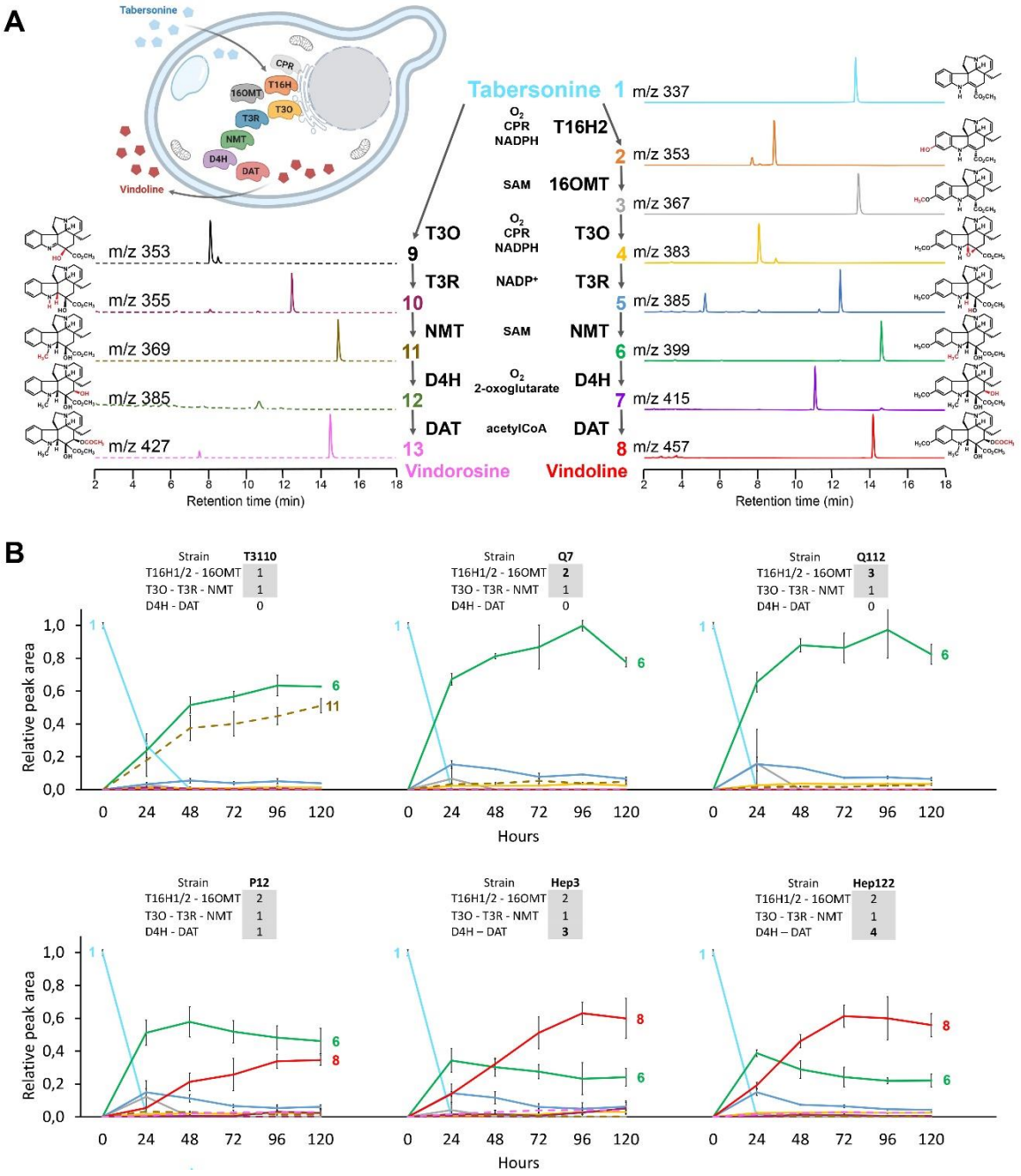

Figure 4. Tailoring yeast cell factories for vindoline bioproduction. (A) Vindoline biosynthetic pathway and parallel branch vindorosine pathway. Each color and number correspond to enzyme product, represented by molecular structure, chromatogram, m/z and retention time : 1 tabersonine, 2 16-hydroxytabersonine, 3 16-methoxytabersonine, 4 16-methoxytabersonine epoxide, 5 16-methoxy-2,3-dihydro-3-hydroxytabersonine, 6 desacetoxyvindoline, 7 deacetylvindoline, 8 vindoline and the by-products 9 tabersonine imine alcohol, 10 2,3-dihydro-3-hydroxytabersonine, 11 desacetoxyvindorosine, 12 deacetylvindorosine and 13 vindorosine. MS/MS fragmentation patterns of compounds are presented in Table S1. Cofactors are indicated for each enzyme. **(B)** Time course monitoring of extracellular metabolite content in generated S. cerevisiae strains. The feedings were performed with 125 µM of tabersonine in the initial 200 µL of YPD/strain/time point. The names and the number of gene copies integrated into each strain are written on the top of each graphic. The curves represent the means of peak areas relative to tabersonine. Error bars : standard deviation (n = 3 biological replicates). By-products are shown in dashed lines.

Table 1. Main kinetic parameters measured during vindoline production in the bioreactor

Strategy	Maximal growth rate (h^{-1})	Max concentrations ± SD		Conversion yield (% of theoretical maximal yield) ($g.g^{-1}$)	Productivity ($mg.l^{-1}.h^{-1}$)	Final fraction of vindoline on end-products ($g.g^{-1}$)
		Vindoline ($mg.l^{-1}$)	Vindorosine ($mg.l^{-1}$)			
F0	0.36	–	–	–	–	–
F1	0.30	19.1 ± 0.3	1.4 ± 0.1	0.42 (31%)	0.32	0.92
F2	0.30	19.2 ± 0.5	1.5 ± 0.1	0.43 (32%)	1.7	0.94
F3	0.29	161 ± 2	51 ± 2	0.62 (45%)	2.1	0.75
F4	0.31	266 ± 8	4.7 ± 0.4	1.20 ± 0.01 (88%)	2.77 ± 0.02	0.98

Growth substrates are fed by pulses (F1) or continuously (F2-F4). Tabersonine is fed by one pulse (F1, F2), seven pulses (F3) or equivalent continuous feeding (F4) (Table S4). Standard deviation : n = 4 independent samples, considering the duplicate experiment for F4. End products are vindoline and vindorosine.

vindorosine accumulation, while desacetoxyvindoline drastically increased and the tabersonine was almost completely consumed after 24 h. Due to this improved metabolism, and given that further increase in T16H/16OMT gene copy number did not impact desacetoxyvindoline production (Q112 strain), the Q7 strain was used as a background for the downstream integration of the final vindoline biosynthesis steps D4H and DAT. The resulting P12 strain, containing a single copy of D4H and DAT, produced 14.7 $mg.L^{-1}$ of vindoline. However, an important accumulation of desacetoxyvindoline was also observed, highlighting a D4H-dependent bottleneck. To circumvent this lack of activity, a total of three copies of both D4H and DAT genes were introduced into the Q7 strain to generate the Hep3 strain. Consequently, the addition of two supplemental copies of D4H and DAT increased vindoline production up to 26.8 $mg.L^{-1}$ after 120 h (i.e. two times higher than P12 strain) and halved the accumulation of desacetoxyvindoline. Interestingly, adding a fourth copy of both D4H and DAT genes did not improve vindoline titer (Hep122 strain), suggesting that other parameters such as culture conditions could be limiting rather than the number of gene copies. Moreover, after 72-96 h the bioconversion was reaching a plateau, which is probably due to the depletion of nutrients in the medium and an increasing competition with native metabolism for cofactors. Thus, Hep3 strain was selected for further optimization of vindoline production.

2.2. Optimization of culture conditions to enhance vindoline production

In order to evaluate the potential effect of culture medium and nutrient supply on the production of vindoline by the vindoline-producing strain Hep3, several sources of peptone nutrients were assayed. Compared to the bovine peptone (BovMP) routinely used, another peptone produced from bovine tissues (BovP), a peptone obtained from a mix of bovine and porcine tissues (BovPrcP), as well as Tryptone and Polypeptone (PolyP) showed drastic negative effects on the activity of NMT and D4H leading to the accumulation of intermediates 5 and 6, along with a poor production of vindoline. Consequently, BovMP was selected for further optimization of culture conditions. Above all, these results demonstrate that growth media composition has an acute effect on

bioproduction underlining the importance of a careful choice of peptone sources.

Similarly, the pH of the culture medium was found to drastically impact the production of vindoline, pH 6.0 being determined as optimal. As an acidification of 2 points of the culture medium pH was observed after 120 h, the importance of maintaining a constant level of optimal pH was investigated. In parallel, to prevent any starvation-related metabolic stress, the supply with fresh YP nutrients (yeast extract and peptone) every 24h after 48h of culture was assessed. The best vindoline production of 34.6 $mg.L^{-1}$ was achieved by adding YP (condition YPt48h), which demonstrated an enhanced activity of D4H after 96h of culture and a 1.5-fold increase of vindoline titer compared to standard conditions (condition BovMP) (Fig. 5A). Interestingly, the sole addition of YP was enough to maintain pH close to 6.0, driving to a slightly higher vindoline production compared to additional buffering of culture medium (condition pH6YPt48h). Hence, using BovMP peptone as the best source of nutrients, and supplementing the culture with YP every 24 h from 48 h post feeding was defined as the optimal conditions for vindoline production.

2.3 Scaling up and engineering of bioreactor feeding to improve vindoline production

In order to scale up the production of vindoline, the Hep3 strain was next cultivated in bioreactors. In control conditions, both wild type and Hep3 strains showed similar maximal growth rates (0.37 and 0.36 h^{-1}, respectively), viability (maintained up to 98 %), as well as biomass and ethanol yields on glucose. This tends to show that overexpression of the vindoline pathway in the recombinant strain was well tolerated and did not constitute any utter metabolic burden for the cells. However, the addition of tabersonine into the culture medium increased the cellular respiration on oxygen, while the maximal growth rate showed a 20% decrease (Table 1). Such phenomenon, routinely observed with the addition of weak acid (Verduyn et al., 1992), reveals an uncoupling effect of tabersonine on energy and biomass productions that did not alter cell viability in all the experiments (up to 98% in all the cultivations).

The F1 feeding experiment in the bioreactor was based on the best production strategy previously defined and relying on 24 h-pulsated supplementations of glucose followed by YPD addition. In these conditions, the conversion yield was found to be two times lower than in small scale experiments (Table 1). However, the speed of production of vindoline was two times higher, with a maximal amount of vindoline produced within only 60 h, while the competing flux leading to the formation of vindorosine remained low (Fig. 5B). In addition, except for the last stage of the starting pulse, a substantial decrease of vindoline cumulated mass was observed when glucose and ethanol were depleted, thus suggesting its consumption. Therefore, in order to improve vindoline production, culture conditions were further optimized. For instance, glucose depletion between pulses was avoided by a continuous feeding of growth substrates (F2 cultivation). Such feeding led to a 3 times faster conversion (22 h) than in the F1 reference culture, and consequently to a higher productivity (Fig. 5B; Table 1). In addition, vindoline was also continuously synthesized and not degraded any longer, even 3 days after the synthesis ended. Moreover, while many biosynthetic intermediates were accumulated in the F1 condition, they were only detected in minute amounts after 22 h of bioconversion in F2, as a result of an extended MIA biosynthetic enzyme activity (Fig. 5B). Above all, this suggests that an active central primary metabolism is critical to provide enough amounts of cofactors required by the heterologous pathway, such as acetyl-coA and NADPH.

In the next step, we investigated the intensification of the vindoline production by numbering-up the pulses of tabersonine

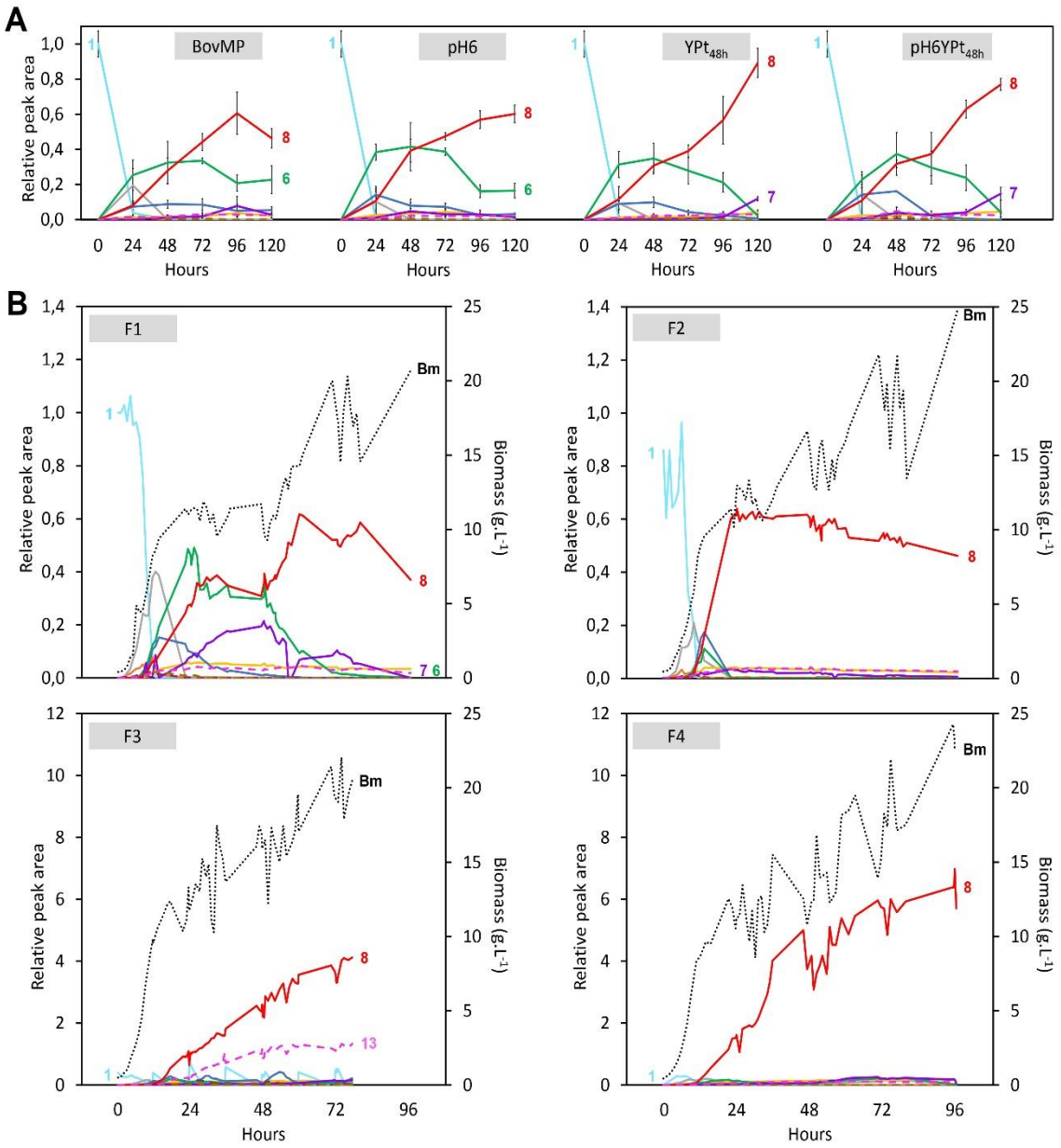

Figure 5. Optimization of vindoline production at small and medium scales. (A) Effect of pH and addition of fresh YP on small-scale vindoline production. The tested condition is notified on the top of each graphic. The curves represent the means of peak areas relative to tabersonine. Error bars : standard deviation (n = 3 biological replicates). The feedings were performed with 125 µM of tabersonine in the initial 200 µL of YPD/strain/time point. **(B)** Effect of growth substrate and tabersonine feeding strategies on vindoline production in fed-batch cultivation. Growth substrates are fed by pulses (F1) or continuously (F2-F4). Tabersonine is fed by one pulse (F1, F2), seven pulses (F3) or equivalent continuous feeding (F4). Bm, biomass.

(F3 cultivation). Interestingly, growth and production were maintained all over the culture, and a circa 50 % increase of the conversion yield was observed compared to F2 culture (Fig. 5B; Table 1). This marked increase probably resulted from cell environment management, reducing the impact of tabersonine uncoupling effect that induces huge pH and pO2 drops. Nevertheless, the tabersonine flux towards vindorosine highly increased at the expanse of the vindoline pathway resulting in a 3:1 vindoline/vindorosine ratio (Table 1; Fig. 5B). In addition, a higher accumulation of

biosynthetic intermediates was also monitored during the whole experiment. To prevent the formation of undesired products and reduce heterologous pathway bottlenecks, we combined an uninterrupted active central metabolism (continuous glucose feeding under the Crabtree effect's critical uptake rate, avoiding ethanol production) with a restrained consumption rate of tabersonine. Accordingly, we limited the specific uptake rate of tabersonine under its maximal value (1.03 +/- 0.04 $mg.g^{-1}.h^{-1}$) through a continuous feeding in the F4 cultivation. This strategy prevented tabersonine accumulation in the medium after 10 h and led to a 88% tabersonine bioconversion into vindoline, which was equivalently replicated. Consequently, very low levels of vindorosine and vindoline biosynthetic intermediates were observed. (Table 1; Fig. 5B). Thus, the control of central and heterologous metabolic fluxes by rational process design appeared as a relevant strategy to further improve vindoline production. In these conditions, vindoline reached a final titer of 266 $mg.L^{-1}$ after 96 h of culture and thus a 20-fold higher productivity than the original biosynthesis reported by Qu and colleagues (2015) with an episomal strain. To date, it constitutes the highest vindoline production ever reported.

3- Conclusion

Through a fine-tuned integration of heterologous biosynthetic genes combined to the optimization of culture conditions, we generated a yeast strain synthesizing high amounts of vindoline via the bioconversion of tabersonine (88% yield). Based on the quantity of tabersonine accumulated in the seeds of *V. Africana*, and an annual exportation of 1600 tons of seeds, a total production of 22.5 tons of vindoline can be expected using this newly developed yeast strain (Kikura-Hanajiri et al., 2009; Koroch et al., 2009). A similar approach was applied to integrate the catharanthine biosynthetic pathway within hotspots of the genome of *S. cerevisiae* to produce catharanthine from tryptophane and loganine (data not shown). Such advances open new perspectives towards the development of a potential alternative supply of vindoline and catharanthine to avoid vinblastine and vincristine shortage in the future.

4- Perspectives of future collaborations with the host laboratory

Guirimand and his colleagues at the BBV Laboratory eagerly look forward to the next phase of our work together. Technical development will continue through our efforts to establish new cell factories able to bio-produce a wide palette of valuable natural compounds, such as anti-cancer alkaloids used in human health.

5- Articles published in the framework of the fellowship

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