Metabolic engineering of curcumin production in *Saccharomyces cerevisiae*

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Background

Curcumin is the main active compound found in the rhizomes of turmeric (*Curcuma longa*) [1]. For centuries, turmeric rhizomes have been used in culinary and as natural yellow colouring agent. In addition, the curcumin rich rhizome extract has been used in Asian traditional medicine for the treatment of several disorders. Curcumin is composed by two phenolic groups connected by two α,β-unsaturated carbonyl groups and is chemically categorized as diarylheptanoid. The reported biological activities of curcumin include anti-inflammatory, antioxidant, anti-cancer among others [2]. Attributable to those potential therapeutic properties, the curcumin market size reached 58.2 million dollars in 2020 and is expected to triplicate in 2028 [3]. Nowadays, curcumin emerges as one of the most studied natural therapeutic products. However, plant-derived curcumin extracted from turmeric rhizomes has several associated problems. These limitations include the restricted production due to the plant seasonality and the geographical conditions [4]. Additionally, curcumin extraction and purification is challenging and expensive [5]. The chemical synthesis could be used to obtain curcumin, however, it applies harsh chemicals and extreme conditions [5]. Therefore, the microbial production could be solution to produce high amounts of curcumin. Microorganisms can grow inexpensively and have rapid production cycles. Additionally, the downstream processes are easier to implement comparatively to plant extraction. The biosynthesis of curcumin in *C. longa* starts with the phenylpropanoid pathway where tyrosine/phenylalanine are converted to the hydroxycinnamic acid and curcumin precursor ferulic acid by the action of several enzymes. Ferulic acid is then activated through condensation with a CoA molecule under the catalysis of 4-coumarate-CoA ligase (4CL). Afterwards, type III polyketide synthases (PKSs), diketide-CoA synthase (DCS) and curcumin synthase (CURS), synthesize curcumin from two molecules of activated ferulic acid (feruloyl-CoA) with the involvement of one malonyl-CoA molecule [6, 7]. Until the date, the metabolic engineering of microorganisms to produce curcumin was mainly achieved in *Escherichia coli* [8–10]. Herein, we intend to understand if the GRAS yeast *Saccharomyces cerevisiae* can be a suitable platform to genetically engineer towards curcumin biosynthesis. For that purpose, curcumin biosynthetic genes were expressed in *S. cerevisiae* (**Figure 1**) and curcumin production evaluated from supplemented ferulic acid. Moreover, a *fdc1* (encoding for ferulic acid descarboxylase) knock-out strain was developed to prevent ferulic acid consumption and increase the curcumin titers [11].

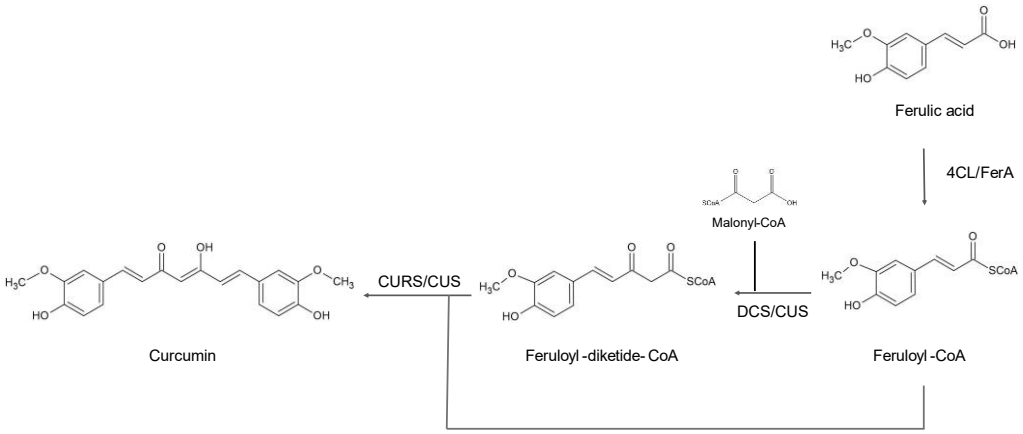

Figure 1: Curcumin biosynthetic genes expressed in *Saccharomyces cerevisiae.* 4CL: 4-coumarate-CoA ligase; CURS: curcumin synthase; CUS: curcuminoid synthase; DCS: diketide-CoA synthase; FerA: feruloyl-CoA synthetase.

Methods

To synthesize curcumin in yeast different enzymatic combinations were tested. The enzymes 4CL1 from *Arabidopsis thaliana* or feruloyl-CoA synthetase (FerA) from *P. paucimobilis* and the PKSs from *O. sativa* (CUS) or *C. longa* (DCS and CURS1) were cloned in a 2micron plasmid (pSP-GM1) under control of the strong constitutive promoters. The constructed plasmids were transformed in *S. cerevisiae* BY4741 wild-type strain. Curcumin production experiments were performed in 250 mL flasks containing 50 mL of Yeast Nitrogen Base (YNB) media of five individual transformants for each mutant strain. Ferulic acid was supplemented at a concentration of 16 mg/L at 24 h of fermentation. Fermentations were carried for 72 h. Produced curcumin was extracted from cells using methanol and the extracts analysed by UHPLC. To construct the *fdc1* knock-out strain, a CRISPR-Cas9 plasmid (pCRCT) was constructed and transformed into the wild-type strain. The *fdc1* deletion was confirmed by sequencing. After curing the CRISPR-Cas9 plasmid, the curcumin biosynthetic pathway was inserted in this strain and curcumin production evaluated.

Results

The biosynthesis of curcumin by engineered *S. cerevisiae* was first attempted by introducing two enzymes responsible for the conversion of phenylpropanoid ferulic acid into curcumin, namely the 4CL1 from *A. thaliana* and curcuminoid synthase (CUS) from *Oryza sativa*. CUS is also type III PKS that is capable of catalysing the same reactions carried by DCS and CURS in a single step. The quantification revealed that this strain produced a maximum curcumin titer of 88.8 µg/L. Afterwards, a pathway harbouring DCS and CURS from *C. longa* was also tested to evaluate if the curcumin production could be further increased. These transformants showed a higher capacity to produce curcumin from supplemented ferulic acid reaching 1067.0 µg/L (13.3 mol% yield) of curcumin. Overall, the production of curcumin using the DCS/CURS system

was much higher than when CUS was used. Subsequently, it was tested if the use of feruloyl-CoA synthetase (FerA) from *Pseudomonas paucimobilis* instead of 4CL could further improve the curcumin titers. Using this set of enzymes, the curcumin production increased 1.3-fold relatively to 4CL pathway. FerA was expressed for the first time in *S. cerevisiae* to efficiently convert ferulic acid to feruloyl-CoA. Moreover, FerA/DCS/CURS pathway was transformed in *S. cerevisiae* Δ*fdc1*. Using *S. cerevisiae* Δ*fdc1* the curcumin titers reached 2728.1 µg/L (yielding 34.1% mol/mol) (**Table 1**).

Table 1: Curcumin production by the different *Saccharomyces cerevisiae* strains harboring the curcumin biosynthetic pathway. Ferulic acid (16 mg/L) was added to the culture medium at 24 h of fermentation. Colonies 1 to 5 were picked from the transformation plate.

	Curcumin colony titer (µg/L)					
Phenotype	**1**	**2**	**3**	**4**	**5**	**Average (Standard deviation)**
(↑4CL↑CUS)	78.2	88.8	73.9	87.3	23.0	70.2 (22.1)
(↑4CL↑DCS↑CURS)	609.1	1067.0	704.3	356.1	173.6	582.5 (279.5)
(↑FerA↑DCS↑CURS)	1127.3	1413.3	1014.0	1321.3	1248.8	1224.9 (128.7)
(Δ*fdc1*↑FerA↑DCS↑CURS)	2728.1	1789.1	2036.2	1585.5	1275.9	1883.0 (548.5)

Conclusion

This study demonstrates that *S. cerevisiae* is a suitable biological chassis to produce the polyphenol curcumin. Curcuminoid biosynthetic enzymes were successfully expressed in *S. cerevisiae* and the recombinant yeast was able to produce pure curcumin from supplemented ferulic acid using 4CL1 from *A. thaliana* or FerA from *P. paucimobilis* combined with the type III PKS from *O. sativa* (CUS), or from *C. longa* (CURS and DCS). The combination of the bacterial FerA and type III PKS from *C. longa* resulted in higher curcumin titers relatively to the other combinations tested. In addition, the deletion of *fdc1* improved curcumin production. This work represents the first report addressing the production of curcumin in *S. cerevisiae*

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