Microbial Cell Factories

CORRECTION

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Correction to: Engineering the synthetic β-alanine pathway in *Komagataella phaffii* for conversion of methanol into 3-hydroxypropionic acid

Sílvia Àvila-Cabré[1], Míriam Pérez-Trujillo[2], Joan Albiol[1] and Pau Ferrer[1*]

Correction to: Microb Cell Fact (2023) 22:237
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In the original version of this article [1], the promoters used to control the expression of the *ydfG* gene (P_{POR1} and P_{PDC1}) in strains PpCβ10 and PpCβ20 were inadvertently swapped. Consequently, the correct genetic constructs are as follows: The *ydfG* gene was expressed under the control of the weak P_{PDC1} in strain PpCβ10 (and thereby in all PpCβ10-derived strains), whereas the medium strength P_{POR1} was used in strain PpCβ20 (and thereby in all PpCβ20-derived strains).

While the data from experiments performed with each of these strains are not affected by this correction and the fundamental conclusions of the study remain valid, the following statements, as well as Fig. 3; Table 3, have been corrected in this erratum.

Page no. 1 of the original publication under section **Abstract**, subsection **Results**, fifth sentence should be replaced by "The addition of a second copy of the *panD* gene and selection of a medium strength promoter to drive expression of the *ydfG* gene in the PpCβ21 strain resulted in an additional increase in the final 3-HP titer ($1.2\ g\ l^{-1}$)".

Page no. 4 of the original publication under section **The expression of *panD*, *yhxA* and *ydfG* genes in *K. phaffii* generates a 3-HP-producing strain**, the end of the last sentence of the first paragraph should read as follows "…, namely the moderate strength mitochondrial porin (*POR1*) promoter, obtaining the PpCβ20 strain, and the weak pyruvate decarboxylase (*PDC1*) promoter, obtaining the PpCβ10 strain".

Similarly, in the second paragraph of the same section (Page 4), the third sentence should be "The heterologous expression of *panD*, *yhxA* and *ydfG* genes resulted in $0.93 \pm 0.03\ g\ l^{-1}$ of 3-HP after 48 h of cultivation when the weak P_{PDC1} was used to control the expression of the *ydfG* gene (PpCβ10 strain), whereas a slightly but significantly higher titer (p-value < 0.05), $1.04 \pm 0.01\ g\ l^{-1}$ 3-HP, was obtained with the P_{POR1} driving the expression of this gene (PpCβ20 strain)".

Page no. 5 of the original publication under section **Improving 3-HP production by optimizing the flux through the β-alanine synthetic pathway**, the last three sentences need to be rephrased as follows: "These results indicate that higher expression levels of the *ydfG* gene (i.e., under the control of P_{POR1}) seem to be beneficial in terms of 3-HP production. These results point at the final step of the pathway, which catalyzes the NADPH-dependent reduction of the malonic semialdehyde to 3-HP, as a critical rate-limiting reaction. Notably, the increase in the

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*Correspondence:
Pau Ferrer
Pau.Ferrer@uab.cat
[1]Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Catalonia, Spain
[2]Servei de Ressonància Magnètica Nuclear, Facultat de Ciències i Biociències, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Catalonia, Spain

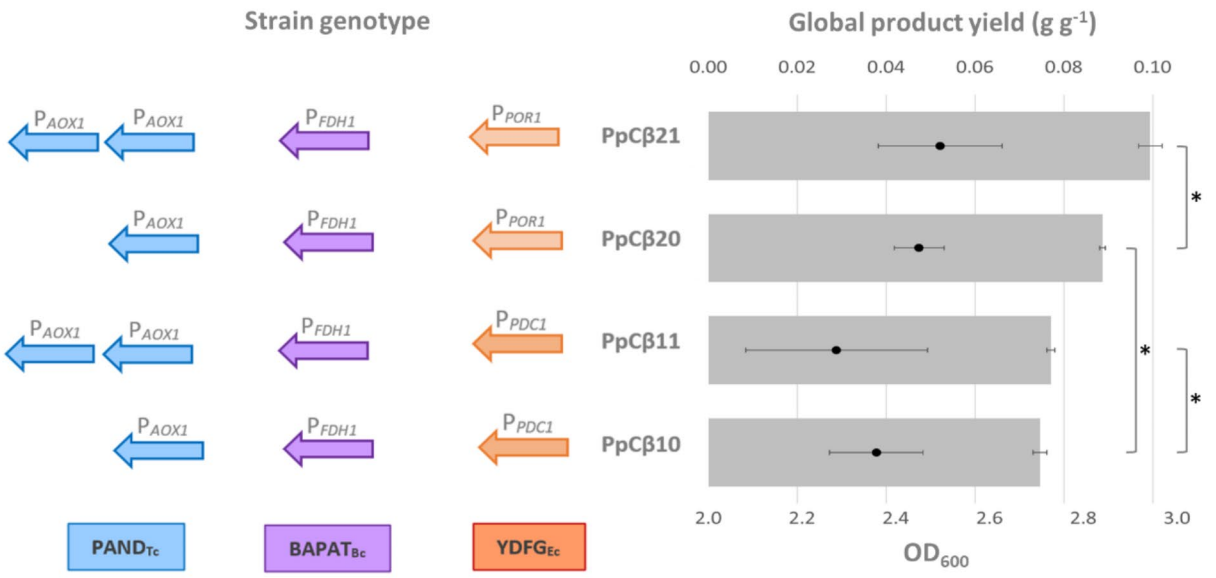

Fig. 3 Global product yields ($Y_{P/S}$) calculated for the base strains constructed in this study. The key heterologous enzymes expressed in each strain are depicted in the left side of the graph. One colored arrow corresponds to one copy of the related expression cassette (blue for $PAND_{Tc}$, purple for $BAPAT_{Bc}$, and orange for $YDFG_{Ec}$). Promoters selected to drive expression of every transcriptional unit are mentioned above the arrows. On the right side, the grey bars show the average product yield (g g^{-1}), and the black dots represent the averaged endpoint OD_{600}. The error bars show the standard deviation, and the asterisk sign indicates a significant difference in global 3-HP yields between the strains indicated in brackets (p-values < 0.05)

Table 3 List of plasmids and strains used in this study

Plasmids/Strains	Modules/Genotype	Reference
***K. phaffii* strains**		
CBS7435	Wild-type	CBS, Centraalbureau voor Schimmelcultures
PpCβ10	P_{AOX1}_$PAND_{Tc}$ + P_{FDH1}_$BAPAT_{Bc}$ + P_{PDC1}_$YDFG_{Ec}$	This work
PpCβ20	P_{AOX1}_$PAND_{Tc}$ + P_{FDH1}_$BAPAT_{Bc}$ + P_{POR1}_$YDFG_{Ec}$	
PpCβ11	2x(P_{AOX1}_$PAND_{Tc}$) + P_{FDH1}_$BAPAT_{Bc}$ + P_{PDC1}_$YDFG_{Ec}$	
PpCβ21	2x(P_{AOX1}_$PAND_{Tc}$) + P_{FDH1}_$BAPAT_{Bc}$ + P_{POR1}_$YDFG_{Ec}$	
PpCβ20-P	PpCβ20 + P_{FDH1}_$FDH(V9)_{Pse}$	
PpCβ21-P	PpCβ21 + P_{FDH1}_$FDH(V9)_{Pse}$	

flux through the β-alanine pathway enabled by the insertion of an additional copy of the *panD* gene under the strong *AOX1* promoter combined with the expression of the *ydfG* gene under the control of the medium-strength *POR1* promoter did not further exacerbate the limitation of NADPH equivalents for anabolism/cell growth on methanol in small scale cultures (Fig. 3)."

Page 5 of the original publication Fig. 3 should be corrected to the following Fig. 3, in which the promoters used for *ydfG* expression (in orange) are correctly assigned to the corresponding strain.

Page no. 6 of the original publication under section **Evaluating the impact of the β-alanine pathway on cell growth kinetics**, the fourth sentence needs to be changed as follows: "Interestingly, higher levels of the NADPH-consuming $YDFG_{Ec}$ enzyme of the β-alanine pathway (i.e., *ydfG* gene expressed under the control of P_{POR1}, strain PpCβ20) proved to be beneficial, both in terms of 3-HP production and in μ_{max} reduction (referred to the μ_{max} of the wild type strain), which was less pronounced (μ_{max} reduction from 0.14 h^{-1} to 0.11 h^{-1}) than that observed for strain PpCβ10 (μ_{max} reduction from 0.14 h^{-1} to 0.09 h^{-1})."

Table S1 Stoichiometric analysis of the main metabolic pathways towards 3-HP using different carbon sources

ATP Synthase: NADH + H^+ → NAD^+ + 1.5 ATP		
Redox balancing mechanisms: NADPH → NADH		
GLUCOSE	Malonyl-CoA pathway	0.5 Glucose + 2 ATP → 3-HP
	β-alanine pathway	0.5 Glucose → 3-HP
METHANOL	Malonyl-CoA pathway	3 Methanol + 4 ATP → 3-HP
	β-alanine pathway	3 Methanol + 2 ATP → 3-HP
GLYCEROL	Malonyl-CoA pathway	Glycerol + 0.5 ATP → 3-HP
	β-alanine pathway	Glycerol → 3-HP + 1.5 ATP

Page no. 11 of the original publication, Table 3 should be modified as follows:

Finally, upon publication of this article [1], it was brought to our attention that in Table S1 from Additional file 1, we utilized a mechanistic P/O value of 2.5 for mitochondrial NADH oxidation in our stoichiometric analysis of both the Malonyl-CoA and the β-alanine pathways towards 3-HP using different carbon sources, indicating

that 2.5 ATP are obtained per oxygen atom reduced by the respiratory chain. This value was chosen based on historical estimations found in several earlier reports [2]. However, other studies suggest a lower theoretical P/O ratio of 1.5 for *Saccharomyces cerevisiae* as more realistic [3], which has also been used in *K. phaffii* genome-scale models for validation of phenotypic predictions [4, 5]. Accordingly, Table S1 from Additional file 1 should be modified as follows:

Importantly, the stoichiometric calculations considering a theoretical P/O value of 1.5 do not alter the resulting stoichiometries for 3-HP production from glucose and methanol. Consequently, the original publication's conclusion that the β-alanine pathway is more energetically efficient than the malonyl-CoA pathway for producing one molecule of 3-HP from three molecules of methanol still holds true.

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Author contributions
SAC and PF wrote the corrections to the original article. SAC introduced the corrections to figures and tables. JA contributed to the correction of Table S1 and to its corresponding justification. All authors (SAC, PF, JA and MPT) reviewed and approved the corrections to the original article.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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