

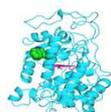
Skin whitening effects by melanin decolorization of recombinant lignin peroxidase

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Introduction:

Melanin, which protects the skin cells from ultraviolet (UV) radiation, is synthesized from melanocytes in the basal layer of epidermis and transferred to the surrounding keratinocytes. The melanin produced under normal conditions is mostly degraded, but abnormally overproduced melanin is not decomposed and remains in keratinocytes, causing pigmentation. Lignin peroxidase (LiP) has a high potential as a skin whitening agent due to its high oxidative ability to break the binding of melanin. However, the lignin peroxidase has been commonly used as an unpurified crude enzyme so far because it is difficult to express and purify the lignin peroxidase since lignin peroxidase is composed of 17 different isozymes. In this study, we demonstrated the effects of recombinant lignin peroxidase isozyme (LiPH8) encoded by the genome of *Phanerochaete chrysosporium* on the decolorization of melanin. In order to optimize the maximal LiPH8 activity on the melanin decolorization efficiency, in-situ experimental conditions were established with regards to the control of pH and the concentration of hydrogen peroxide. The results showed that the melanin decolorization efficiency was dramatically increased at the following conditions, pH 4.0 and 250 μM concentration of hydrogen peroxide. Furthermore, LiPH8 was typically applied to the 3D skin model and the significant reduction of melanin in the stratum corneum was observed using Fontana-Masson (FM) staining.

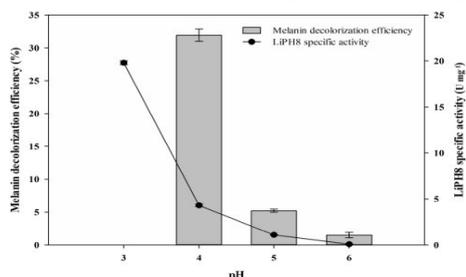
Materials & Methods:



- ▶ **Recombinant LiPH8**
 - LiPH8 from *Phanerochaete chrysosporium* (Bioneer Company).
- ▶ **3D skin information**
 - Reconstructed Human Pigmented Epidermis Phototype VI (SkinEthic)
- ▶ **Histological Analysis**
 - Fontana Masson staining

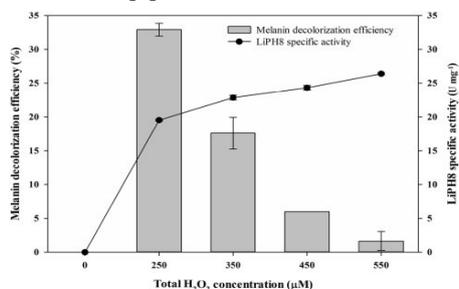
Results & Discussion:

▶ Decolorization efficiency of melanin on varying pH and ionic strength



The melanin decolorization efficiency was 33% at pH 4.0, but decreased to less than 10% at pH 5.0 and pH 6.0.

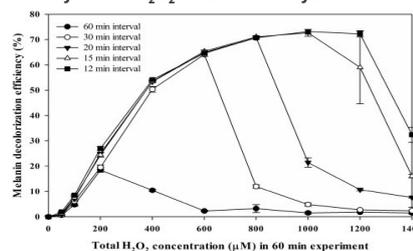
▶ Inhibition effect of excess H₂O₂ concentration on melanin decolorization



The melanin decolorization efficiency was approached to the highest level of 31.9% at 250 μM H₂O₂, it decreased gradually with increase in H₂O₂ concentration.

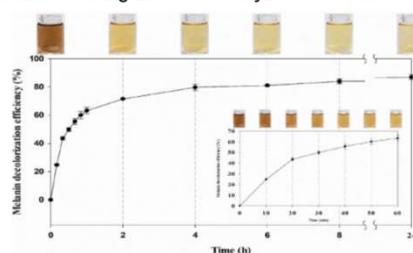
Results & Discussion:

▶ Effect of intermittently added H₂O₂ on LiPH8-catalyzed melanin decolorization



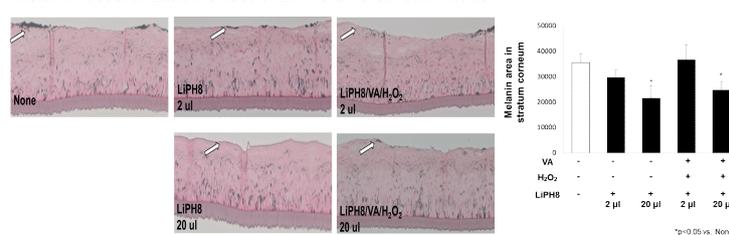
Intermittent feeding of H₂O₂ over certain time intervals is effective for improved melanin decolorization by repressing inactivation of LiPH8 caused by excessive H₂O₂.

▶ Melanin decolorization using LiPH8 co-catalyzed with GOx



A total of 43.5% melanin decolorization efficiency was achieved within 20 min and the overall efficiency approached up to 63.3% in 1 h.

▶ Melanin decolorization effect of LiPH8 on a 3D skin model



LiPH8 was typically treated for 3 days on 3D skin that transferred melanin to the outer layer while stimulating melanogenesis with α -MSH. The area of melanin (black) contained in the stratum corneum of the 3D skin reduced with the application of LiPH8 compared to the control.

Conclusions:

- Recombinant LiPH8 expressed in *E. coli* can decolorize melanin efficiently and effectively.
- Co-catalysis of LiPH8 and GOx was performed the improved decolorization efficiency of melanin.
- Melanin decolorization efficiency up to 63.3% was achieved within 1 h and later to 84.0% in 8 h.
- LiPH8 effectively increased the melanin decolorization in the stratum corneum of the 3D skin.
- Taken together, LiPH8 can be used as an effective agent for melanin decolorization ingredient in brightening cosmetics.

Acknowledgments:

We gratefully acknowledge the Korea NRF (Project No. 20177M1A2A2087630) and MOTIE/KEIT (Project No. 20002810) for their support of this research work

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