

# ***Study on the High-Activity Superoxide Dismutase of the Polar Yeast *Cryptococcus albidosimilis*: From Extreme Survival to Functional Skincare***

**Jake F. Wang<sup>1,a</sup>, Doris Dai<sup>1,b,\*</sup>, Bella Song<sup>1,c</sup>, Waifun Mok<sup>1,d</sup>**

<sup>1</sup>Biowell R&D Center, Eternal Grace Pte. Ltd, Singapore

<sup>a</sup>naizhuangwang@Gmail.com, <sup>b</sup>djj9@163.com, <sup>c</sup>blingbin10@Gmail.com,

<sup>d</sup>jakewang0815@Gmail.com

\*Corresponding author

**Keywords:** Superoxide Dismutase, Polar Yeast, Antioxidant, Skincare

**Abstract:** Superoxide dismutase (SOD) is an essential antioxidant enzyme that catalyzes the conversion of superoxide radicals into oxygen and hydrogen peroxide, playing a crucial role in reducing oxidative stress. This study focuses on *Cryptococcus albidosimilis*, a polar yeast with high SOD activity, isolated from Antarctic soil. This yeast exhibits impressive resistance to cold, ultraviolet radiation, and oxidative stress, positioning it as a promising source of stable SOD. Our research characterizes *C. albidosimilis* SOD, revealing activity levels of about 3000 U/mg, significantly higher than those of conventional yeasts. Notable features include enhanced structural stability, retaining 80% activity under simulated gastric conditions and sustaining 90% activity at 45°C for up to 30 hours. Additionally, the unique molecular structure of this enzyme enhances its resilience against environmental stressors. In conjunction with the SOD analysis, we conducted an exclusive experiment at Biowell's laboratory to evaluate the potential of our proprietary Pure-Glo whitening composition, which incorporates *Cryptococcus albidosimilis* SOD along with glutathione, glacial water extract, and white tomato extract. This formulation demonstrated a significant ability to inhibit MITF (Microphthalmia-Associated Transcription Factor) gene signaling, a critical regulatory factor in melanin production, achieving a 67% inhibition rate. This breakthrough further confirms the effectiveness of Pure-Glo as a targeted skin whitening agent that operates at the genetic level.

## **1. Introduction**

Superoxide dismutase (SOD) is a crucial antioxidant enzyme that efficiently catalyzes the conversion of superoxide radicals ( $O_2^-$ ) into oxygen and hydrogen peroxide, aiding organisms in coping with various oxidative stresses. Given the close association between oxidative damage, aging, and chronic diseases, SOD has garnered widespread attention in medical, nutritional, and cosmetic fields<sup>[1]</sup>. However, traditional sources of SOD (such as animal-derived, conventional yeast, or recombinant SOD) exhibit limitations concerning stability, gastrointestinal tolerance, production costs, and yields. To address these challenges, our research team isolated various yeast strains with

"extreme survival capabilities" from soil samples collected in Antarctica in 1992<sup>[2]</sup>. Through systematic screening and evaluation of antioxidant enzyme activities, we identified *Cryptococcus albidosimilis*, which maintains high SOD activity under extreme cold, intense ultraviolet radiation, and oxidative stress<sup>[3]</sup>. This study's objectives include:

- Presenting the key characteristics of *Cryptococcus albidosimilis* SOD and the potential reasons for its high activity and stability.

- Exploring its potential applications in dietary supplements and skincare products while discussing consumer needs.

## 2. Discovery of the Polar Yeast *Cryptococcus albidosimilis*

During the collection of soil, mosses, and glacial matrix samples in Antarctica, researchers isolated dozens of yeast strains. Morphological observations and genomic sequencing allowed us to identify several strains exhibiting strong resistance to cold and radiation. Subsequent antioxidant enzyme activity assays revealed that *Cryptococcus albidosimilis* maintains high growth rates even at 4 °C or lower, with SOD activity measured at approximately 3000 U/mg, significantly higher than that of typical yeasts (around 1000 U/mg)<sup>[4]</sup>.

This high activity and environmental adaptability suggest that the SOD produced by this yeast exhibits exceptional stability under various stressors, laying the groundwork for its extensive application in food, health, and cosmetic industries.<sup>[5]</sup>.

## 3. Method

### 3.1 SOD Extraction Process

To extract the enzyme, the yeast slurry was first incubated in isopropanol for 2 hours to eliminate impurities. Subsequently, the isopropanol was removed by filtration to enhance the purity of the slurry. Following this, 50 mmol/L potassium phosphate buffer (pH 7.0) was added, and the mixture was stirred before being allowed to stand for 2 hours to facilitate enzyme release. The resulting mixture was then subjected to centrifugation to separate the yeast cells, and the pH of the supernatant was adjusted using hydrochloric acid. Lastly, the enzyme was extracted using acetone, yielding a crude enzyme solution suitable for further analysis and applications<sup>[6]</sup>.

### 3.2 SOD Activity Determination Method

Reagent A: Prepare a Tris-HCl buffer (pH 8.20) with 0.1 mol/L Tris and 1 mmol/L EDTA 2Na by dissolving 1.2114 g of Tris and 37.2 mg of EDTA 2Na in 62.4 mL of 0.1 mol/L hydrochloric acid, then dilute to 100 mL with distilled water.

Reagent B: Prepare a 4.5 mmol/L nitroblue tetrazolium chloride (NBT) solution by dissolving 56.7 mg of NBT in a small volume of 10 mmol/L hydrochloric acid and diluting to 100 mL.

Take 0.0896 g of the SOD extract, dilute to 10 mL with distilled water, and centrifuge at 4000 r/min. Collect 1.0 mL of the supernatant and dilute it to 250 mL<sup>[7]</sup>.

Measure absorbance at 320 nm using a UV-visible spectrophotometer. Record the absorbance change of the blank after 1 minute as  $\Delta A_{320} (\text{min}^{-1})$ . The  $\Delta A_{320} (\text{min}^{-1})$  for the blank should be around 0.060. For example, if the blank's absorbance is 0.0894 and becomes 0.1502 after one minute, then  $\Delta A_{320} (\text{min}^{-1})$  is 0.0608, meeting assay requirements.

### 3.3 MITF Gene Signal Inhibition Detection

To evaluate the effect of Pure-Glo on MITF gene signaling, we conducted quantitative polymerase chain reaction (qPCR) to measure MITF expression in treated skin cells by extracting total RNA, reverse transcribing it into cDNA, amplifying the MITF gene with specific primers, and analyzing the fluorescence signals relative to an internal reference gene (e.g.,  $\beta$ -actin) to assess the inhibitory effect at various concentrations<sup>[8]</sup>.

### 3.4 Tyrosinase Inhibition Assay

To evaluate the tyrosinase inhibition activity of Pure-Glo, a standard dopachrome assay was performed. Tyrosinase enzyme (100 U/mL) and L-tyrosine (0.5 mM) were incubated with Pure-Glo in phosphate buffer (pH 6.8) at 25 ° C for 20 minutes. The formation of dopachrome was measured at 475 nm using a spectrophotometer, and the inhibition percentage was calculated relative to a control sample without Pure-Glo.

## 4. Key Features

### 4.1 Emphasizing the Uniqueness of "Polar Survival"

Microorganisms in polar regions must withstand extreme cold, hypoxia, intense ultraviolet radiation, and nutrient scarcity.

- Extremesurvival conditions: *Cryptococcus albidosimilis* thrives in Antarctic soils and glaciers, enabling survival in sub-zero temperatures.

- Evolutionary advantages: Over time, this yeast has developed robust protective mechanisms, such as high levels of SOD, polysaccharides, and peptides, ensuring cellular vitality under extreme cold, high radiation, and oxidative pressure.

- Polar genetic characteristics: Consequently, the SOD from *Cryptococcus albidosimilis* is believed to possess a unique molecular structure and folding pattern that allows it to maintain high activity even under harsh conditions<sup>[9]</sup>.

### 4.2 Exploring the Scientific Logic behind "Enhanced Enzyme Stability"

Compared to conventional microorganisms, polar yeasts exhibit advantages:

- Optimized protein structure: Evidence suggests that *Cryptococcus albidosimilis* SOD has greater resistance to variations in temperature, pH, and mechanical forces, attributed to its amino acid sequence and three-dimensional conformation. This stability is crucial for maintaining enzyme activity during storage and processing, ensuring that it remains effective in its application<sup>[10]</sup>.

- Holistic protective mechanisms: Associated polysaccharides, peptides, and minerals can work in concert to minimize damage to the enzyme. These components not only support the structural integrity of SOD but also bolster its functional efficacy in diverse environments<sup>[11]</sup>.

- Tolerance data: Table 1 illustrates that *Cryptococcus albidosimilis* Superoxide Dismutase (SOD) retains approximately 80% of its enzymatic activity after 2 hours in simulated gastric acid (pH 2.0), significantly outperforming conventional yeast SOD, which shows only about 50% activity retention under the same conditions. Furthermore, at 45 °C for 30 hours, *Cryptococcus albidosimilis* SOD maintains an impressive 90% activity, while conventional yeast SOD retains only 11%. These findings underscore the superior stability of *Cryptococcus albidosimilis* SOD, indicating its strong potential for use in dietary supplements and skincare formulations where maintaining enzyme functionality is essential.

Table 1: Comparison of Tolerance between *Cryptococcus albidosimilis* SOD and Conventional Yeast SOD under Different Conditions

Experimental Conditions	<i>Cryptococcus albidosimilis</i> SOD Activity Retention	Conventional Yeast SOD Activity Retention	Improvement
Simulated Gastric Acid (pH 2.0, 2h)	80%	50%	30%
Thermal Stability (45 °C, 30h)	90%	11%	79%

### 4.3 Aligning with Consumer Needs

In modern society, factors such as work stress, pollution, and irregular eating habits lead to an excess of free radicals in the body. A high-stability and high-activity SOD precisely caters to people's demands for a "potent antioxidant" that is both safe and reliable. Busy consumers often seek a "polar" style robust shield against internal and external oxidative pressures<sup>[12]</sup>.

### 4.4 Targeting MITF Gene Signaling with Pure-Glo

To substantiate the claim that Pure-Glo is the world's first whitening agent targeting and inhibiting MITF gene signaling, we conducted a controlled experiment at Biowell's laboratory. The formulation of Pure-Glo consists of a combination of *Cryptococcus albidosimilis* SOD, Glutathione, Glacial Water Extract, and White Tomato Extract. These bioactive components were systematically evaluated for their capacity to inhibit MITF gene expression, a pivotal regulator of melanin biosynthesis<sup>[13]</sup>. The results, detailed in Table 2, demonstrate that Pure-Glo treatment yields a statistically significant inhibition of MITF gene expression in a dose-dependent manner. Notably, the high-dose treatment group exhibited a 67% reduction in MITF expression, thereby confirming the formulation's efficacy in modulating skin pigmentation on a genetic basis.

Table 2: Inhibition of MITF Gene Expression by Pure-Glo Treatment at Varying Dosages

Treatment Group	MITF Inhibition (%)
Control (No Treatment)	0%
Pure-Glo (Low Dose)	15%
Pure-Glo (Medium Dose)	49%
Pure-Glo (High Dose)	67%

These findings position Pure-Glo as a novel intervention in the field of skin whitening agents, effectively targeting the MITF signaling pathway to facilitate a significant decrease in melanin production. With an inhibition rate of 67%, Pure-Glo represents a scientifically advanced formulation that operates at the genetic level, marking a substantial advancement in the skincare industry. This innovative approach could provide a more effective strategy for addressing hyperpigmentation and achieving a more uniform skin tone.

In an in vitro study, the tyrosinase inhibition activity of Pure-Glo was evaluated using a standard dopachrome assay. Tyrosinase, a key enzyme in melanin biosynthesis, catalyzes the oxidation of L-tyrosine to L-DOPA and subsequently to dopachrome, leading to melanin production. The assay measured the enzymatic activity by monitoring the absorbance at 475 nm. Results demonstrated that Pure-Glo effectively inhibited tyrosinase activity by 81%, indicating its strong potential in reducing melanin synthesis and skin pigmentation. This finding further supports Pure-Glo as an advanced skin-brightening formulation.

## 5. Future Applications: From Dietary Supplements to Functional Skincare

### 5.1 Advantages in Dietary Supplements

Due to occupational and life pressures, unbalanced diets, and environmental pollution, modern individuals often accumulate excess free radicals in their bodies, increasing the risk of chronic diseases and accelerating aging. Highly active and stable SOD supplements can provide the body with additional antioxidant support, but their effectiveness largely depends on the enzyme's survival rate during the oral route. *Cryptococcus albidosimilis*-derived SOD, with its outstanding tolerance, is more likely to pass through the gastrointestinal barrier and reach the small intestine, effectively scavenging free radicals.

### 5.2 Potential in Functional Skincare

As the body's exposed organ, the skin is susceptible to oxidative damage from UV radiation, pollution, and other external factors. Incorporating polar yeast SOD into skincare formulations not only enhances antioxidant capacity, but also leverages accompanying polysaccharides and peptide components to provide moisturizing and reparative effects. Previous literature shows that yeast extracts play a positive role in repairing skin barriers and soothing sensitive skin. High-stability SOD remains active over extended periods during product storage and use, adding competitive benefits to end products.

### 5.3 Connecting with Consumer Demands

For modern individuals who lead hectic lifestyles, “polar survival power” and “high enzyme stability” go beyond scientific concepts, linking directly to everyday health management and skincare needs. When pressure, pollution, and an irregular diet exacerbate free radical levels, offering an SOD sourced from extreme environments—one proven safe and effective under harsh conditions—carries enormous market appeal.

## 6. Conclusion

Overall, *Cryptococcus albidosimilis* not only boasts high-activity SOD but also exhibits exceptional structural stability, making it a crucial breakthrough for finding ideal antioxidant enzyme sources. Its polar origin endows it with the ability to maintain robust enzymatic activity even in low-temperature, high-UV environments, and to tolerate temperature, pH, and gastrointestinal challenges. For those seeking solutions to oxidative stress and health management in today's society, this yeast SOD shows potential for more effective free radical clearance in dietary supplements and sustained antioxidant and repair effects in skincare products. In addition, the exclusive Pure-Glo whitening composition, which includes Yeast SOD, Glutathione, Glacial Water Extract, and White Tomato Extract, has proven to be an innovative solution for targeting melanin production. The 67% inhibition of MITF gene signaling demonstrates the efficacy of Pure-Glo as a scientifically advanced skin brightening agent that works at the genetic level. This further solidifies its position as a cutting-edge product in the skincare industry, offering a safe and effective solution for consumers seeking to improve skin tone and pigmentation. By combining “polar survival power” and “high enzyme stability,” *Cryptococcus albidosimilis* SOD, along with the Pure-Glo formula, meets the modern consumer's desire for safe, efficient, and long-lasting functional products. With further developments in fermentation techniques and industrial applications, *Cryptococcus albidosimilis* SOD and the Pure-Glo formula are expected to find increasingly broad use in medical, nutritional, and cosmetic fields.

## References

- [1] Rosa, L. H., Vieira, M. L., Santiago, I. F., & Rosa, C. A. (2010). Endophytic fungi from Antarctic vascular plants: diversity, enzymes and bioprospecting for bioactive compounds. *Polar Biology*, 33(8), 997–1006.
- [2] Buzzini, P., Branda, E., Goretti, M., & Turchetti, B. (2012). Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. *FEMS Microbiology Ecology*, 82(2), 217–241.
- [3] Margesin, R., & Zhang, D. C. (2013). Genomics of extremophiles for sustainable bioproduction. *Trends in Biotechnology*, 31(1), 2–8.
- [4] Pryor, W. A. (1986). Oxy-radicals and related species: their formation, lifetimes, and reactions. *Annual Review of Physiology*, 48, 657–667.
- [5] Scandalios, J. G. (2005). Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Brazilian Journal of Medical and Biological Research*, 38(7), 995–1014.
- [6] Ryu, S., Lee, S., Cho, E. J., & Kim, Y. S. (2014). High-level production of superoxide dismutase in recombinant *Saccharomyces cerevisiae* and its stability enhancement. *Biotechnology and Bioprocess Engineering*, 19(6), 1065–1072.
- [7] Dei, H. K. (2011). Dietary inclusion of exogenous superoxide dismutase in poultry diets: benefits and potential applications. *World's Poultry Science Journal*, 67(3), 447–458.
- [8] Ryu, A., Arakane, K., & Koide, C. (2008). Protective effects of superoxide dismutase on oxidative stress in the skin: in vitro and in vivo studies. *Journal of Dermatological Science*, 50(2), 161–169.
- [9] Villarreal-Soto, S. A., Beaufort, S., & Bouajila, J., et al. (2018). Yeast-derived bioactive compounds in skincare: their roles in antioxidant, anti-inflammatory and barrier function. *International Journal of Cosmetic Science*, 40(3), 199–210.
- [10] Kim, H. K., & Yang, S. H. (2020). Application of microbial-sourced antioxidants in cosmetics. *Critical Reviews in Food Science and Nutrition*, 60(23), 3971–3985.
- [11] Chen, B., Zeng, H., & Liu, J. (2021). Cold-active enzymes from psychrophilic microorganisms: a case study of industrial applications of polyextremophiles. *Extremophiles*, 25(1), 1–11.
- [12] Triloknath, T., Chandel, A. K., & Singh, S. P. (2022). Exploring extremophiles for novel enzymes with industrial applications: status and prospects. *Bioresource Technology*, 359, 127512.
- [13] Wang, Q., Li, P., & Dai, Y. (2023). Industrial production of robust antioxidant enzymes from cold-adapted yeasts: advances in bioprocess and product development. *Applied Microbiology and Biotechnology*, 107(4), 1457–1470.