Poster Number 63

Effects of Spirulina platensis-derived polysaccharides on the promotion of extracellular matrix production and inhibition of hydrogen peroxide-induced fibroblast injury

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Introduction:

Polysaccharides from *Spirulina platensis* (PSP) are water-soluble biological macromolecules that possess various bioactivities [1]. Currently, the outdoor cultivation of *S. platensis* is associated with a series of problems, such as yield reduction, difficulty in harvesting and low content of active substances[2]. In our previous studies, a genetically stable mutant of *S. platensis* was screened and obtained by space breeding. It was named H11 and it showed higher polysaccharide yield, this laid the foundation for large-scale outdoor cultivation and industrial applications of H11 mutant strains.

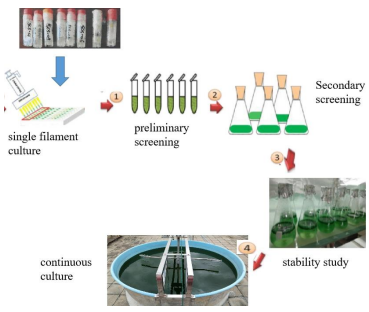
Skin aging is accompanied by a decrease in tissue structure, moisture content, smoothness, elasticity, compliance, and fibroblast damage [3]. Decreased collagen and hyaluronic acid (HA) levels results in gradually reduced water retention ability and reduction of skin elasticity. Eventually, these changes led to the emergence of wrinkles [4]. The free-radical theory of aging indicates that oxidative damage caused by the accumulation of free radicals is the main cause of skin aging, and fibroblasts play an important role in this process[5-6].

In this study, exogenous H_2O_2 was used to induce cell damage by disrupting the dynamic balance between reactive oxygen species (ROS) levels and antioxidant enzymes. We examined the protective effect of PSP on oxidative stress in human skin fibroblasts (HSFs) via MTT assay. Biochemical methods were used to determine the levels of oxidative stress-related molecules.

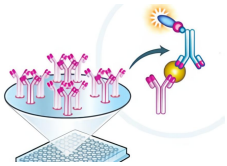
Dichlorodihydrofluorescein (DCF) was used to label ROS. The apoptotic rate of H_2O_2-treated fibroblasts was observed using Hoechst 33342 dye. Enzyme-linked immunosorbent assay (ELISA) was used to measure the HA and type I collagen (ColI) levels in the culture of skin fibroblasts. This study aimed to study the protective effects of PSP against H_2O_2-induced oxidative stress injury in HSFs.

Materials & Methods:

S. platensis culture and water-soluble polysaccharides extraction



Quantification of hyaluronic acid and type I collagen levels via ELISA



Determination of ROS, MDA, SOD, GSH, and GSH-Px

After discarding the medium, HSFs were incubated in FBS-free DMEM containing 10 μM 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) at 37°C for 30 min in the dark. Subsequently, the cells were washed three times with PBS, and ROS levels were measured using a fluorescent microplate reader (Ex/Em = 504/529 nm).

The supernatant was used to detect malondialdehyde (MDA) content according to the manufacturer's instructions. HSFs were harvested and used to determine the levels of GSH along with the activities of superoxide dismutase (SOD) and GSH-Px according to previously described protocols. Protein concentrations were determined using the bicinchoninic acid (BCA) method.

Results & Discussion:

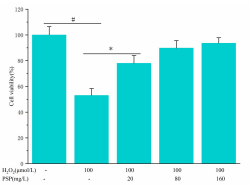


Fig.-1 Inhibition of PSP on H_2O_2-induced viability decrease of HSFs.

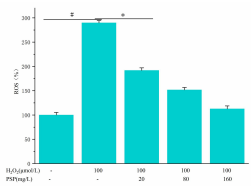


Fig.-2 Effects of PSP pre-treatment on H_2O_2-induced ROS level.

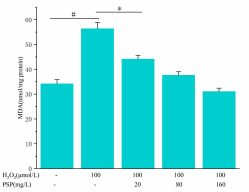


Fig.-3 Effects of PSP pre-treatment on H_2O_2-induced MDA level.

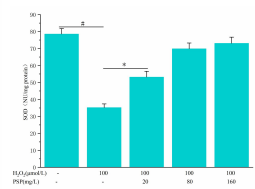


Fig.-4 Protective effects of PSP pre-treatment on SOD activity.

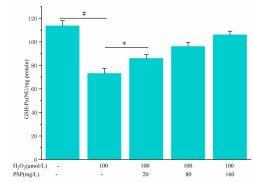


Fig.-5 Protective effects of PSP pre-treatment on GSH-Px activity.

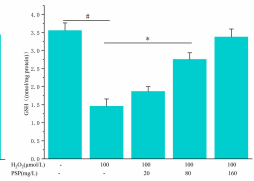


Fig.-6 Protective effects of PSP pre-treatment on GSH level.

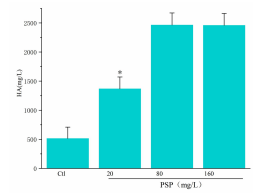


Fig.-7 Effects of PSP treatment on hyaluronic acid level.

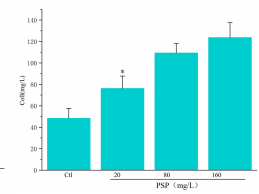


Fig.-8 Effects of PSP treatment on type I collagen level.

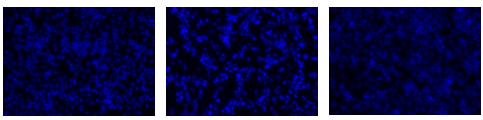


Fig.-9 Effects of PSP on H_2O_2-induced apoptosis of HSFs. (Left) HSFs without the incubation of PSP and H_2O_2; (Middle) HSFs with the incubation of 100μmol/L H_2O_2 for 4 hours; (Right) HSFs with the pre-treatment of 160mg/L PSP for 24 hours prior to the incubation of 100μmol/L H_2O_2 for 4 hours.

Conclusions:

This study investigated the protective effects of PSP against H_2O_2-induced oxidative stress injury in HSFs. The results demonstrated that PSP improved the cellular antioxidant system and extracellular matrix production. Our study demonstrated that PSP has the potential to be used as a component of anti-aging cosmetics.

References:

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