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Project title: Unrevealing the anti-aging and anti-obesity properties of the cyanobacterium *Spirulina* (*Arthrospira*) *platensis*.

Abstract

In recent years, marine natural products have become one of the most important resources of novel lead compounds for critical diseases associated with age and obesity, such as cancer, neurodegenerative diseases, metabolic syndrome and many others. Spirulina, a dietary supplement made from blue-green algae (cyanobacteria: *Arthrospira platensis*), is a source of many, but only partially identified and purified compounds. Spirulina contains high levels of macro and micro nutrient. It is particularly rich in proteins (65% to 70%) as well as phycocyanin (PC), vitamins, beta-carotene, polyunsaturated fatty acids, minerals, especially iron, polysaccharides and others. Although the anti-aging and the anti-obesity activity of PC has been investigated, how exactly this compound works against aging and lipotoxicity remains elusive. We will use the yeast *Saccharomyces cerevisiae* as a model organism to unravel the anti-aging and anti-obesity properties of PC and possibly of other compounds purified from this cyanobacterium.

Background, aims and significance of the proposed work

The major health problems observed all over the world are often associated to age since people are living longer and obesity. The loss of lipid homeostasis can lead to lipid overload and cellular lipotoxicity, which in higher eukaryotes can trigger many diseases, including metabolic syndrome, type II diabetes mellitus, cardiovascular disorders, cancer and others. The lipid species that are most relevant for lipotoxicity include free fatty acids (FFA), ceramide, cholesterol, and diacylglycerol (DG). Therefore, identifying natural compounds for the treatment of these diseases might be of great benefit for public health.
S. platensis is a source of various and only partially identified and purified compounds with potential health benefits and activities. Phycocyanin (PC), a phycobiliprotein, accounts for up to 20% of this cyanobacterium's dry weight and is considered responsible for its anti-cancer, anti-inflammatory and antioxidant activities. However, its molecular mechanism of action has not been identified [1]. Moreover, although several mechanisms have been proposed to explain the anti-obesity effects of *S. platensis* (and microalgae in general), this issue has not yet been completely clarified. In particular, it is not yet completely known which bioactive compounds are responsible for the anti-obesity effects and the potential synergies among compounds still has to be investigated [2, 3].
Aim of this research project is to use *S. cerevisiae* as a model organism to unravel the molecular mechanisms at the basis of the anti-aging and anti-obesity effect of *S. platensis*. To reach this aim, well-established models for aging studies and lipotoxicity will be used. In particular, to study the anti-obesity effect, we will make use of a mutant strain completely lacking neutral lipids in combination with the exogenous addition of fatty acids, a mutant which accumulates endogenous DG and a mutant unable to degrade triacylglycerols (TAG), which consequently accumulate within the cell.

Experimental plan

Phycobiliproteins (PBPs) have been widely characterized for their *in vivo* and *in vitro* antioxidant activity. They have been shown to reduce oxidative stress in both yeast and mammals. Data in literature show that PBPs express diverse antioxidant activity (free radical scavenging or metal ion chelating activity), depending on the type of PBPs and on the different cyanobacteria the molecules have been purified. Since, ROS are considered important factors causing aging, the anti-aging activity of PBPs has been investigated. However, the question how exactly the PBPs works against aging remains elusive. We will investigate the anti-aging effect of PBPs, in particular phycocyanin (PC) from *S. platensis* (eventually the total extract and other putative bioactive compounds and peptides isolated from this organism will also be tested), using *S. cerevisiae* as a model organism. Yeast is widely used as a model to study aging as the principal pathways

are conserved during evolution. We will study the ability of PC to extend chronological life span of yeast cells. In particular, we will cultivate both a wt and a *Kllsm4*Δ mutant showing a premature aging and cell death in the presence and absence of PC and we will measure their ability to form colonies over the time. An increase in cell viability in treated cells compared to the control will be a clear indicator of the effectiveness of such substance on the aging process. Cell apoptosis and necrosis, ROS levels, expression of genes and enzyme involved in oxidative stress response, glutathione metabolism will be measured. The ability to modulate proteostasis will also be investigated, since proteostasis disruption has been proposed as a possible mechanism leading to aging. We will also study the effect of PC on cell survival following an oxidative stress such as the treatment of cultures with hydrogen peroxide. Importantly, in order to find new physiologically relevant PC targets, we will perform the above-mentioned experiments using mutants affecting longevity, such as *ras2*Δ, *sch9*Δ, *tor1*Δ, *snf1*Δ mutants and others.

The loss of lipid homeostasis can lead to lipid overload and is associated with many diseases. Data in literature show evidences supporting an anti-obesity effect of several microalgae, including *S. platensis*. To study the putative beneficial effects of PC from *S. platensis* (possibly the beneficial effects of total extract and other putative bioactive compounds and peptides isolated from this organism will also be tested) on obesity, we will make use of yeast models for lipotoxicity.
A model for acute lipotoxicity is represented by the use of a yeast quadruple mutant (QKO) completely lacking neutral lipids in combination with the exogenous addition of fatty acids [4], since it is known that excess dietary free fatty acids (FFAs) contribute to obesity and linked metabolic disorders. The QKO mutant lacks all four acyltransferases necessary for triacylglycerols (TAG) and steryl esters (SE) synthesis, namely Are1p, Are2p, Dga1p, and Lro1p and consequently it is unable to detoxify FFAs. Using this model for acute lipotoxicity, Rochenfeller et al. showed that yeast cell death induced by FFAs is mediated by a necrotic pathway, depending on functional mitochondria and leading to accumulation of ROS. We will cultivate both wt and QKO strains with different FFAs, in the presence and absence of PC and we will measure their ability to form colonies over the time. An increase in cell viability in PC treated cells compared to the control will be a clear indicator of the effectiveness of such substance on lipotoxicity. Cell apoptosis and necrosis, ROS levels, expression of genes and enzymes involved in oxidative stress response, glutathione metabolism will be measured. Since it has been shown [4] that various cooking oils displays differential grades of cytotoxicity in yeast upon concomitant addition of TAG lipase to externally hydrolyze TAG and as such to mimic the microenvironment of the mammalian intestine, we will test the effect of PC administration on oils cytotoxicity.
Another model for lipotoxicity is represented by the triple knockout strain *dga1*Δ *lro1*Δ *dgk1*Δ (TKO) deleted in genes of three Diacylglycerol (DG)-metabolizing enzymes which accumulates endogenous DG, a metabolite suspected to mediate lipotoxicity [5]. The authors showed that DG accumulation induces glucose-dependent and ROS-associated necrotic cell death in yeast, providing evidence for the existence of a lipotoxic cell death pathway that can be triggered by excess DG. The toxic effects of DG are linked to glucose metabolism and require a functional Rim101 signaling cascade, an established pathway that triggers a transcriptional response to alkaline or lipid stress. They propose that the Rim101 pathway senses DG-induced lipid perturbation and conducts a signaling response that either facilitates cellular adaptation or triggers lipotoxic cell death. We will investigate whether administration of PC to the TKO stain alleviates the cell death phenotype. In particular, cell growth, survival, cell death and oxidative stress will be determined. The effect of different carbon sources and the impact on the Rim101 signaling pathway will also be investigated. Moreover, since increased DG levels and necrotic cell death can also be achieved by either externally supplementation of lipids such as palmitoleic acid or administration of the small, cell permeable DG analog 1,2-dioctanoyl-sn-glycerol, we will perform the above mentioned study in the presence of these compounds.
Finally, another experimental model is represented by the double mutant lacking Tgl3p and Tgl4p lipases, of which orthologues exist in mammalian cells, which is characterized by an “obesity” phenotype. This fat

yeast is unable to degrade TAG, which accumulate within the cell, and shows defective growth initiation after quiescence, underlying the importance of TAG pools and lipolysis for supporting growth. Massive accumulation of TAG has also been reported in the *snf1*Δ mutant. The key enzyme of fatty acid *de novo* synthesis, acetyl-CoA carboxylase, Acc1p, is a direct target of Snf1. Mutants lacking this kinase display hyperactive Acc1p and, consequently, massive accumulation of TAG. The effect of PC on these experimental models will also be investigated. Finally, metabolomics, transcriptomic and lipidomics analysis might also be performed with the aim to highlight changes eventually caused by addition of PC and/or other components purified from *S. platensis* to our experimental models.

Feasibility and financial support

I declare the economic sustainability of the project.

Literature references

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