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USE OF *SACCHAROMYCES CEREVISIAE* AS AN ANTI-AGING STUDY TOOL

Mircea-Cosmin PRISTAVU[1, 2], Camelia Filofteia DIGUŢĂ[1], Ionuţ MORARU[2], Florentina MATEI[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]SC Laboratoarele Medica SRL, 11 Frasinului Street, Otopeni, Ilfov, Romania

Corresponding author email: cosmin_pristavu96@yahoo.com

Abstract

The paper aims to present the review of the recent research on anti-aging effect of bioactive plant compounds studied by the use of Saccharomyces cerevisiae as a model organism. Numerous features, such as the chronological lifespan (CLS) and / or the replicative lifespan (RLS) or the measurement of the yeast telomere length, associated with the aging process have been the targets of many studies around the world. The yeast, S. cerevisiae has emerged as a powerful genetic and chemical screening platform because it combines a fast workflow with experimental facilities and the availability of a wide range of mutants. Given these aspects and the mechanisms of aging between yeast and humans, the testing of candidate anti-aging substances has proven to be very successful in finding potential molecular targets. The mentioned methods are very difficult to apply in humans and therefore the side effects are unpredictable and because of this it is very important to quickly discover biologically active plant compounds that can mimic the anti-aging action as close to the truth as possible.

Key words*: anti-aging, yeast, chronological lifespan, replicative lifespan, telomere.*

INTRODUCTION

Older people tend to have costly chronic diseases that negatively impact their quality of life and functional output. In fact, aging itself is the leading risk factor for an array of diseases that increasingly plague the world population. If researchers can understand aging and modify its rate, the consequences are likely to be a reduced incidence or progression of disease leading to increased health span, allowing older people to keep working and avoid high health care costs.

The potential of interventional approaches targeted at aging has yet to be realized in part because aging is a complicated multisystem process that has remained enigmatic. However, research over the last two decades has led to significant excitement.

Model organisms are key species, used by researchers to investigate and unravel various biological processes which lead understanding certain human diseases mechanisms. The most studied and cited organisms in numerous reviews and research articles within Google scholar comprise yeast (*Saccharomyces cerevisiae*) (see reviews Karathia et al., 2011; Sudiyani et al., 2021), fruit fly (*Drosophila melanogaster*) (see review Lee and Min, 2019), nematode worm (*Caenorhabditis elegans*) (see review Mack et al., 2018), and mouse (*Mus musculus*) (see review Vanhooren and Libert, 2013). A great deal of attention has been focused to *Saccharomyces cerevisiae* as useful model organism to investigate anti-aging compounds and understand the aging mechanisms (Longo et al., 2012; Zimmermann et al., 2018; Sudiyani et al., 2021). *S. cerevisiae* is easy of and safety to manipulate and breed in a laboratory conditions, are relatively inexpensive, have short generation periods, does not have any ethical constraints, has the whole genome sequenced (≈12-Mb bp, 16 chromosomes and 6000 genes) and an appreciable homologous of yeast genes with human genes associated with aging and/or longevity (see http://genomics.senescence.info/genes/models.html), making them ideal for experimentation (Goffeau et al., 1997; Sherman, 1998; Goffeau, 2000; Longo et al., 1996; Longo et al., 1997; Fabrizio et al., 2003; Fabrizio et al., 2004; Fabrizio et al., 2005; Fabrizio et al., 2007;

Burtner et al., 2009; Karathia et al., 2011; Murakami et al., 2011; Longo et al., 2012).

S. cerevisiae is a flexible and stable model system of eukaryotic genetics. Mutant screening and study of division is simpler and easier than in multicellular species in the yeast and is well preserved across eukaryotic taxa for basic eukaryotic biodiversity, such as cell cycling regulation and generating alternation. Yeast has high endogenous recombination rates, and a variety of extrachromosomal DNA elements can turn yeast cells steadily. The study and cloning of genes are therefore considerably simpler than in more complex eukaryotes in this organism (Madia et al., 2007; Greig et al., 2009; Hanson, 2018).

People have been cultivating yeast to produce beer, bread and wine since the dawn of agriculture. The budding yeast *S. cerevisiae* has grown into an outstandingly tractable eukaryotic model structure, as a domesticated microorganism and as a reproductive eukaryote.

S. cerevisiae contributed actively and implicitly to the discovery of supposedly more aging genes than other models (Longo et al., 2012). The lifespan in yeast is classified as replicative lifespan (RLS) and chronological lifespan (CLS). RLS is defined as the number of daughter cells an individual yeast mother can produce before cell enters senescence and eventually dies (Mortimer and Johnston, 1959). CLS is defined as the period of time that a cell can stay alive without dividing in a stationary phase (Longo et al., 2012). The reality is that the scientific community share the opinion that both yeast assays have good models for understanding aging and will continue to be strong. In this respect, we have therefore tried to construct a review describing the consensus opinion of the employed tools, but also not shying away from the points of disagreement and the technical issues that should be considered when studying aging in yeast.

In search of new anti-aging agents, several extracts have been reported that possess multiple anti-aging activities relevant on *S. cerevisiae* as model organisms. Lutchman et al. (2016) reported 6 plant extracts induced a dramatic delay yeast chronological aging through different signalling pathways. Hesperidin has been reported to produce anti-aging effects in yeast via inhibition of ROS and the *UTH1* gene expression, and increase in *SIR2* (Silent information regulator 2) activity. Apple extract (Stirpe et al., 2017), herb plant *Gastrodia elata* (Orchidaceae) (Lin et al., 2016), Roselle petal extract (Sarima et al., 2019), and clover bud extract (Astuti et al., 2019), rice bran extract (Sunthonkun et al., 2019) were reported also to increase the life span in yeast (Table 1).

Table 1. Plants or plant derived extracts used as anti-aging agents on the yeast *S. cerevisiae,* mentioned in the paper

Plant subjects	References
Curcumin isolated from rhizomes of Turmeric (*Curcuma longa*)	Stępień et al., 2020
Roselle Petal Hydroethanolic Crude Extract (*Hibiscus sabdariffa* L.)	Sarima et al., 2019
Ethanol derived clove bud extract	Astuti et al., 2019
Rice bran extract (*Oryza sativa*)	Sunthonkun et al., 2019
Annurca apple (*M. pumila* Miller cv Annurca) extract	Stirpe et al., 2017
Parishin extracted from *Gastrodia elata*	Lin et al., 2016
Galinsoga parviflora and *Fumaria officinalis* extracts	Chanaj-Kaczmarek et al., 2015
Hesperidin derived from *Citrus* genus	Sun et al., 2012

Hesperidin derived from *Citrus* genus increased the lifespan of yeast by inhibition ROS (Reactive Oxygen Species) level and extention of *UTH1* gene expression (Sun et al., 2012). Stępień et al. (2020) reported the anti-oxidative and anti-aging effects of curcumin and can delays aging process in the *S. cerevisiae* through hormesis effect. Aging studies such as, testing the antioxidant power or the H_2O_2 sensitivity of a plant subject, are becoming increasingly prominent in biomedical research (Astuti et al., 2016; Stirpe et al., 2017). The reasons for this are obvious. The demographics of the world are rapidly changing, leaving a population with an increasing number of elders and a declining number of working-age individuals to support them.

It is our hope that readers interested in aging will be able to gain a strong understanding of the state of play in one article, and that clearly

articulated points of lack of consensus will serve to stimulate further experimentation leading to clarification as other authors have performed before (Longo et al., 2012). There are more advantages than disadvantages in using yeast *S. cerevisiae* as a model organism in demonstrating the anti-aging effects of certain plants (Figure 1).

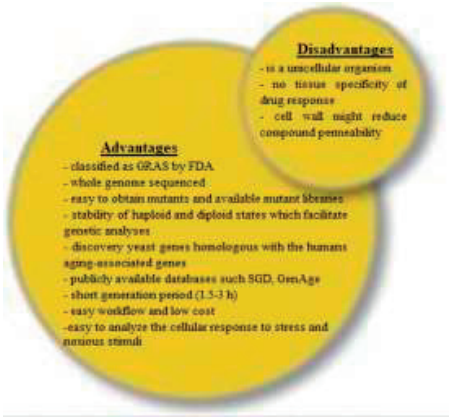

Figure 1. Advantages and disadvantages of using *Saccharomyces cerevisiae* as a model organism (adapted after Zimmermann et al., 2018) GRAS - Generally Recognized as Safe; FDA - Food and Drug Administration; SGD- Saccharomyces Genome database

MATERIALS AND METHODS

The materials used are represented by scientific publications from around the world.
The methods used are in according with the paper aims, respectively: to present the review of the recent research on anti-aging effect of bioactive plant compounds studied by the use of *Saccharomyces cerevisiae* as a model organism.

RESULTS AND DISCUSSIONS

METHODS USED IN ANTI-AGING STUDIES BASED ON *S. CEREVISIAE* CELL MODEL

To determine the potential anti-aging effect of certain plant extracts using the yeast *S. cerevisiae*, many methods have been tested some with good results others less but are of interest in the future (Table 1). In the following will be grouped and described the methods used in the anti-aging studies on *S. cerevisiae*, respectively (1) culture dependent methods; (2) microscopy methods and (3) genetic and molecular tools.

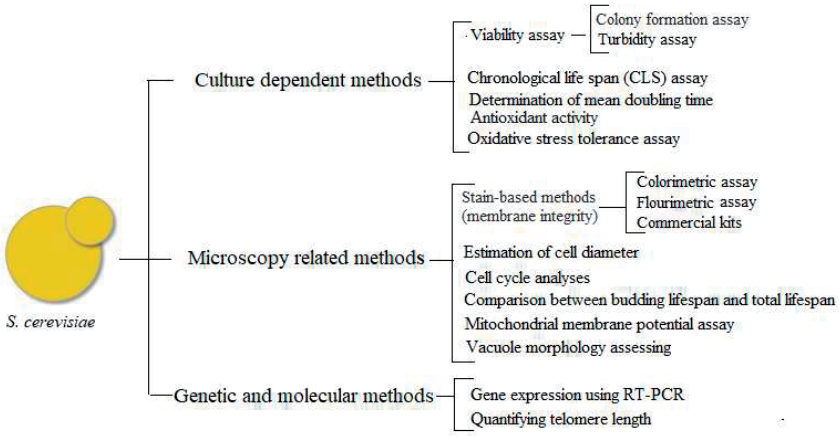

Figure 2. Methods to study anti-aging of *S. cerevisiae*

Culture dependent methods

Viability (defined as percentage of viable cells in a whole population) and vitality (defined as physiological capabilities of a cell) represent two different aspects of yeast cell. In the following paragraph will be described the culture dependent methods, respectively: cell viability assay, chronological life span (CLS) assay, the phenotypic analysis, mean doubling time, antioxidant activity and oxidative stress assays.

Cell viability assay

Cell viability is the most commonly method to investigate the response of *Saccharomyces cerevisiae* yeast cell after exposure to various

stimuli (e.g plant extract). Kwolek-Mirek and Zadrag-Tecza (2014) have proposed a classification of available methods to determine viability, namely: (i) methods based on the yeast cells ability to grow on solid or liquid media and (ii) stain-based methods, respectively (see below). Cell viability can be determined by different methods such colony formation assay (colony forming unit/mL or CFU/ml) (Chanaj-Kaczmarek et al., 2015) or spot dilution assays (Astuti et al., 2019; Sunthonkun et al., 2019); cells culture in liquid medium (turbidity assay) (Kwolek-Mirek and Zadrag-Tecza, 2014). These methods are the easy way of use at a reasonable cost and reliably give accurate results, but it is required longer time to obtain the results and fails to provide an accurate estimation of viable cells or of the ability of cells to reproduce (bud). The most classical method to determine growth curves is based by cultivation the cells in a standard liquid with or without stimuli treatment and counting the proliferating cells over at different time intervals. The turbidity assay was the main method used to measure the absolute number of *S. cerevisiae* cells at optical densities at 600 nm (OD_{600}) by performing measurements at 1 h intervals for 12 - 24 h. Curcumin supplementation led to a direct inhibition of the growth rate in all the analyzed strains (Stępień et al., 2020). The growth curve study should be highlighted as showing the behaviour of the whole population of the cells (Molon et al., 2016). Also, Lin et al. (2016) performed kinetics of growth assay of yeast under the influence of parishin which is a phenolic glucoside isolated from *Gastrodia elata*, an important traditional Chinese medicine plant. Unfortunately, significant changes were not observed in resveratrol treatment group and parishin treatment group.

Chanaj-Kaczmarek et al. (2015) reported that the extracts of *G. parviflora* and *F. officinalis* that contained phenolic compounds improved the viability of *Δsod1* mutant cells, using colony formation assay. The yeast's cells viability clearly differed from the isogenic wild type and was distinctly affected by pretreatment with the studied extracts in a concentration dependent manner. Moreover, the observed effects of both extracts were analogous to the reference substances used in the experiment, i.e., gallic acid, rutin and Trolox (vitamin E analogue). It appears that the changes in the cell energy status caused by the tested extracts affected the cell viability (Chanaj-Kaczmarek et al., 2015).

Stirpe et al. (2017) tested the *S. cerevisiae*'s cells viability treated with extract of Annurca apple variety. They reported that the viability of untreated cells significantly reduced during cultivation and the presence of the extract at the concentration of 10 mg/ml clearly prevented cell death while lower doses of the extract, such as 1 mg/ml, were almost ineffective.

Chronological life span (CLS) assay

The period that the non-dividing yeast cell lasts is the Chronological Life Cycle (CLS). CLS is usually determined by growing a culture of yeast cells that exit most cells from the cell cycle into the postdiauxic state (Longo et al., 1996). The postdiauxic process begins approximately 24 hours after initial inoculation, when cells deplete extracellular glucose, reduce growth dramatically, and turn to an ethanol-dependent mitochondrial respiratory model (Werner-Washburne et al., 1996). The stationary phase is characterized by lower metabolism rates and stress-resistance up regulation at the end of the postdiauxic phase between days 2-7 depending on the medium used in their experiment. In addition to the RLS test, the yeast CLS assay was designed to provide the ability to model the aging of non-splitting organism cells (Longo et al., 1996; Longo et al., 1997; Fabrizio et al., 2003; Fabrizio et al., 2004; Fabrizio et al., 2007; Burtner et al., 2009; Murakami et al., 2011). The extract of Hom Dang rice bran or antioxidants quercetin or protocatechuic acid prolonged life-span of pre-treated wild-yeast cells (Sunthonkun et al., 2019).

The studies of Stępień et al. (2020) indicate that curcumin isolated from *Curcuma longa* rhizome can affect chronological and replicative aging of the yeast. Curcumin accelerates without anti-oxidant protection and without pathways the DNA repair. They also showed that hypertrophic cells are likely to enable yeast to live during chronological aging because of high tolerance to environmental stress factors.

Dakik (2020) discovered fifteen new geroprotective plant extracts that extend the chronological lifespan of budding *Saccharomyces cerevisiae.*

Determination of mean doubling time

For each examined cell as stated before, the mean doubling time can be calculated. When assessing the reproductive capacity, the doubling time can be also determined. The periods of the first two developmental stages have not been considered (the first and second doubling times are longer than those of older cells) in the case of Molon et al. (2016). Curcumin was added to cultures and the development of cell cycle, according to iron availability, was extended and delayed, as the concomitant enrichment of iron and curcumin had a detrimental effect on growth delay (Minear et al., 2011).

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays have been widely used to assess the antioxidant activity of plant extracts. The IC_{50}, known as the concentration of substance that reduces the free radicals by 50% should be determined.

Anti-oxidant activity of ethanol-derived roselle petal extract based on DPPH and ABTS assays were 367.6 ppm and 921.4 ppm, respectively, which indicates that the extract is promising as antioxidant agent (Re et al., 1999; Sarima et al., 2019)

The results revealed that the DPPH-based IC_{50} value and the ABTS value were different. This proved that each sample would apply different pathways of scavenging the free radicals. The raw extract of roselle petals shows the highest activity of antioxidants for scavenging DPPH radicals based on the in-vitro analysis of antioxidants. This shows that the extract as an antioxidant is promising (Sarima et al., 2019). Lin et al. (2016) focused to measure the parameters related to antioxidation in yeast using parishin extracted from *Gastrodia elata*. Parishin significantly increased the number of colonies of yeast. Moreover, the viability of yeast after treatment with parishin was notably increased compared with positive control group under oxidative stress condition. These results suggested that antioxidation played an important role in the antiaging effect of parishin.

Sunthonkun et al. (2019) reported that rice bran extract, antioxidants quercetin or protocatechuic acid delays chronological aging and extend the life-span of *S. cerevisiae* wild-type BY4742 via reduction of the ROS accumulation.

Oxidative stress assay

Environmental/abiotic stress conditions are contributing in inducing oxidative stress through overaccumulation of reactive oxygen species (ROS), causing potential damage to the biomolecules (DNA, proteins and lipids). To verify the anti-oxidative properties of plant extracts, in order to evaluate the impact of oxidative stress provoked by plant extract, *S. cerevisiae* cells were cultured on specific medium with addition various concentrations of H_2O_2. Xiang et al. (2011) evidenced that phloridzin (or phlorizin), major phenolic compound present in apple and apple juice, increased the viability of the yeast dose-dependently under oxidative stress by H_2O_2 (7.5 mM), and a low dose of phloridzin reduced the reactive oxygen species (ROS) level in cells exposed to oxidative stress. Sarima et al. (2019) reported that the roselle petals extracts at 300 ppm concentration increased the life span in yeast against oxidative stress with a high concentration of H_2O_2 (9 mM). In another study, the ethanol-derived clove bud extract (100 ppm) improved *S. cerevisiae* viability following H_2O_2-induced oxidative stress (Astuti et al., 2019).

Stępień et al. (2020) verified the sensitivity of the *S. cerevisiae* wild-type strain BY4741 and isogenic mutant strains *sod1Δ*, *sod2Δ*, *rad52Δ* treated with curcumin under stressed factors such as Congo red, methyl methanesulfonate, sodium chloride (NaCl), hydrogen peroxide (H_2O_2), dodecyl sodium sulfate (SDS), acetic acid and subjected to thermal shock. The authors demonstrated the growth on the YPD medium with addition of curcumin after cell incubation with H_2O_2 (5 mM), which is a strong oxidant. In all analyzed strains, it would also seem that curcumin increases cell resistance to Congo Red, especially in the highest concentration that shows the effect of

biogenesis in the yeast cell walls. The tolerance of yeast cells to acetic acid decreases even with curcumin. Curcumin has a minor or no effect at all on osmotic and thermal stress. One or more MOO (Multi Objective optimization) affect-tability is normal in yeast, since growth barriers at high concentrations have been achieved, as shown (Stępień et al., 2020).

Microscopy related methods

Different assays may use the microscopy tools. For example, it can be studied the yeast budding lifespan or vacuole morphology by the use of different types of microscopes, or the visualisation of nuclei fragmentation or mitochondrial activity by fluorescence microscopy.

Stain-based methods

Stain-based methods can provide the differential identification of dead cells in a culture as well as the percentage of viable cells. Dyes penetrate yeast with damaged cell membrane. Commonly used dyes includes colorimetric dyes such as methylene blue, trypan blue (Krzepiłko, 2009; Sunthonkun et al., 2019) or the fluorescent nucleic acid dyes such as dihydrorhodamine 123, fluorescein diacetate (FDA)/propidium iodide (PI) (Madeo et al., 1999; Stirpe et al., 2017; Stępień et al., 2020). These methods are easy to perform, reliable, efficient, cost-effective and rapid (from a few minutes to a few hours). Fluorometric assays have been extensively used to assess the viability of yeast cells by fluorescence microscopy or flow cytometry, and they offer many advantages compared with colorimetric assay (Kwolek-Mirek and Zadrag-Tecza, 2014). By co-staining the cells with PI/FDA for 15-20 minute in the dark at room temperature, viable yeast cells were green fluorescent, and dead cells were red fluorescent, when was examined under a fluorescence microscope using a selective wavelength (Sun et al., 2012; Lin et al., 2016; Sunthonkun et al., 2019; Stępień et al., 2020). Commercial kits have been developed by several companies, which provide simple and sensitive assays for distinction viable yeast cells in mixtures cultures or in pure cultures (Krzepiłko, 2009).

Estimation of cell diameter

Measuring the cell dimensions is a standard test conducted across several disciplines by researchers. Stępień et al. (2020) analyzed the samples by optical microscopy and the images obtained during the routine lifespan determination procedure used to estimate cell diameter (treated with curcumin) within 2, 5, 8, 12 days. It was observed that during the chronological lifespan, only hypertrophic cells were able to survive. Cell size is a physical parameter that, by the surface to volume ratio, determines the size of the cell.

The studies conducted by Zadrag-Tecza et al. (2018) demonstrate the existence of two size thresholds: the first leads to cessation of reproduction, but cells are still alive; the second leads to cell death. So, cell size may affect not only the reproductive potential but also the total lifespan of cells by shortening the postreproductive phase.

Cell cycle analyses

The cell lifetime is known as the number of daughters that are manufactured by the mother cell until the cells irrevocably stop in the traditional replication aging model. Analyzes based on this model can be carried out using a microscope with a micromanipulator to separate daughter cells from the mother cell (Kaeberlein, 2010). As example, can be provided the testing of curcumin (Minear et al., 2011); it was shown in the culture extends and slows development in the cell cycle based on supply of iron, as simultaneous supplementing iron and curcumin alleviates the detrimental effects of the growth delay.

Curcumin against a range of tumour forms has been also shown to be biologically active. The G2/M cell cycle stops the sprays of the head and neck and in colorectal cancer carcinoma (Jaiswal et al., 2002; Hu et al., 2017).

Comparison between budding lifespan and total lifespan

A routine micromanipulatory procedure discussed above specified the budding period of individual mother yeast cells as the number of mitotic cycles (budding) during the cell life (Molon et al., 2018). Each mother's number of buds indicates its reproductive capability (budding lifespan) (Kaeberlein et al., 2006; Lee et al., 2006; Wei et al., 2008; Wei et al., 2009).

The total lifetime of the single mother cell was described as the life cycle of a single mother cell. The cumulative longevity was measured as the total of all maternal and reproductive lives. The reproductive lifespan was described as the period from the first to the last budding, and the lifespan from the last budding to the cell death. The lifespan of the *S. cerevisiae* yeast was determined as previously described by Minois et al. (2005) with small modification (Molon et al., 2018).

Therefore, a new unit, namely the total lifespan (TLS), may be divided into two phases: the reproductive phase, during which the cell undergoes mitosis cycles to produce successive buds, and the postreproductive phase, which extends from the last division to cell death or can be described as the amount of asexual reproductor's lifetime and post-reproductory lifespan and lifetime passing from the last one to the death of the cell, was suggested with a view to determining the actual life period of mitotically active cells (Zadrag-Tecza et al., 2008). In this experiment, they used a wild-type strain (BY4741) and some mutants *sod1Δ* and *rad52Δ.*

In the case of wild strains only the curcumin supplementation has an effect on the reproductive capacity, while in the case of the mutants, the cell reproduction potential of curcumin decreased statistically substantially. Addition of curcumin had a positive influence on the wild-type *S. cerevisiae* BY4741 cells' mean and full life spans. For other strains, no major effect on the average lifetime of curcumin was noted. Moreover, the length of the time of the cells' ability to bud (reproductive life) and the period of their life but unable to bud was determined (post-reproductive lifespan). The supplementation of curcumin greatly prolonged the reproductive life for the wild-type strain BY4741. In the case of the mutant strain *rad52Δ*, the addition of curcumin will reduce reproductive life significantly (Stępień et al., 2020).

The experimental data obtained by Zadrag-Tecza et al. (2018) for cells with higher ploidy do not indicate that the reproductive potential increases proportionally to the number of the genome copies. Moreover, in this case, the genetic background may also be an important factor. When the reproductive phase of cell life was expressed in units of time differences in the reproductive potential between cells differring in ploidy were significantly lower than when the reproductive phase was expressed in the number of daughters produced by these cells.

Mitochondrial membrane potential assay

Mitochondria is deeply implicated in aging and age-related diseases (Bratic & Larsson, 2013) by generation reactive oxygen species (ROS) as a consequence of the electron transport that drives oxidative ATP synthesis. Rhodamine B (red-fluorescent stain), rhodamine 123 (green fluorescent stain), DAPI (4′,6-diamidino-2-phenylindole) 2′,7′-dichlorodihydrofluorescein diacetate are the widely used mitochondrial fluorescent markers to assess the mitochondria-dependent oxidative stress response following extract treatment (Xiang et al, 2011; Sun et al., 2012; Lin et al., 2016; Kwolek-Mirek and Zadrag-Tecza, 2014; Sarima et al., 2019; Sunthonkun et al., 2019; Stępień et al., 2020). The mitochondrial activity is observed using fluorescence microscope.

A low clove extract therapy level concentration was found not to cause activity of mitochondrial yeasts, like with no extract. On the other hand, high extract concentrations contributed to heavy fluorescence intensity and their effect on mitochondrial activity induced. Clove extract is therefore susceptible to mediating antioxidant cell reaction independently, as indicated by calory-reduction-mediated antioxidant stress response, from the adaptive ROS signalling. Bioactive compounds such as quercetin, resveratrol and curcumin, previously studied could specifically scavenge reactive oxygen species (Metodiewa et al., 1996; Guha et al., 2010; Barzegar et al., 2011; Pan et al., 2011).

Thus, the findings from Astuti et al. (2019) clearly indicate that large dose of clove extract has prooxidant behaviour and probably interferes with adaptive mitochondrial ROS signalling in cellular yeast systems, which results in an oxidative stress-sensitive phenotype. In the previous research, quercetin, a compound within antioxidants, was involved in promoting activity in mitochondria (Ortega et al., 2009). By inducing fluorescence from rhodamine B showed that the addition of

roselle petal extract at concentration of 300 ppm could enhance mitochondrial activity (Sarima et al., 2019).

Intracellular reactive oxygen species in yeast cultures were determined using fluorescence assays with 2′,7′-dichlorodihydrofluorescein diacetate (Wang et al., 1999). Intracellular damage (oxidation) caused by ROS formation during breathing in mitochondria can accumulate over time and inhibit cell re-entry into the cell cycle (Kaeberlein, 2010). The result is attributed to the anti-oxidant effects of curcumin since the intracellular level of ROS and lipofuscin was greatly decreased (Stępień et al., 2020).

Vacuole morphology assessing

A recent review illustrated the importance of vacuoles as regulators of cellular pH homeostasis, multiple metabolic pathways and lifespan (Aufschnaiter & Büttner, 2019). Usually, a wild type cell harbours between one and four vacuoles, depending on the growth and surface conditions (Banta et al., 1988). Vacuoles are highly dynamic organelles which, in response to the diverse environmental conditions and during aging, may undergo distinct morphological changes. Sophisticated molecular machinery regulating vacuolar fusion and fission governs the adaption of vacuolar morphology to the respective cellular requirements. The fission of the vacuole permits an organelle heritage in cells that proliferate (Weisman, 2003).

The nutrient limitation causes vacuolar fusion and causes an enlarged vacuole, whereas the hyperosmotic shock causes vacuolar fission which leads to many cells with small vacuoles (Baba et al., 1994; Bonangelino et al., 2002). Vacuoles grow in size with an advanced age. The proportion of the volume of the cell during aging does not increase but rather increases with age, as the cell volume itself expands.

Vacuolar morphology was studied just prior to age-related death of the replicative old mother cells using a microfluidic dissection platform. Thereby, tubular, large, fused as well as fragmented vacuoles seem to be prominent organelle structures in mother cells destined to die. The authors suggest that a tubular shape of the vacuole right before death is the product of a failure of cytokinesis associated with a failure of vacuole segregation (Lee et al., 2012).

The vacuoles of the *S. cerevisiae* are the largest compartments in yeast which can be detected by microscopy techniques using vital staining with fluorescent dyes such as MDY-64 (Krzepiłko, 2009). Various proteins have been shown to differentially affect vacuolar shape and size, among them the vacuolar protein sorting (VPS) proteins that are essentially involved in proper targeting of proteins to the vacuole and processing of vacuolar hydrolases (Rothman et al., 1989).

Over the years, the *vps* mutants have been divided and classified into different classes based on the distinct vacuolar morphology observed via microscopy tools to understand vacuolar functions and morphology (Banta et al., 1988; Raymond et al., 1992). Because by the successful delivery of proteins into the vacuole, defects in this direction inevitably impact aging to retain organelle identity and function. Using a competitive genome-wide screen was performed on viable deletion mutants to identify genes implicated in protein targeting to the vacuole, involved in the regulation of yeast chronological life span. (Fabrizio et al., 2010).

Genetic and molecular methods

On genetic and molecular side different techniques were already used in the anti-aging yeast studies, anti-aging genes expression by RT-PCR or the quantification of the telomere length. Databases such as Saccharomyces Genome Database (SGD; http://www.yeastgenome.org/), GenAge give freely available information to the scientific community in experimental design to facilitate research into the biology of budding yeast *S. cerevisiae* as model organism. SGD collects and organizes scientific information for the budding yeast *Saccharomyces cerevisiae* as model organism with search and analysis tools to explore these data, enabling the discovery of functional relationships between sequence and gene products in fungi and higher organisms.

The Human Aging Genomic Resources (HAGR) (http://genomics.senescence.info/genes/human.html) is the publicly benchmark databases and tools designed to help researchers study the

genetics of human aging, divided into two main sections: human potential aging-associated genes and longevity-associated genes in model organisms.

Gene expression using Real Time – Polymerase Chain Reaction

Presently (27.04.2021), GenAge database (https://genomics.senescence.info/genes/search.php?organism=Saccharomyces+cerevisiae&show=5) contains 911 aging-associated genes in *Saccharomyces cerevisiae* as model organism, based on current evidence. The most studied aging-associated genes in *S. cerevisiae* are presented in Table 2. *SIR*2 from *S. cerevisiae* plays a significantly role in modulation of cellular senescence and increasing ribosomal DNA (rDNA) longevity and stability under the *TOR* inhibition (Ha and Huh, 2011). The involvement of the *TOR* (target of rapamycin) pathway in aging and lifespan regulation, by gene deletion of *Sch9* (functional ortholog of mammalian *S6K*) has been studied extensively (see review Papadopoli et al., 2019). *GLR1* has a significant role to play in protecting and avoiding cellular oxidative stress and *GPX1* is recognized as one of the antioxidant enzymes that removes the free radicals and lipid peroxide (Grant et al., 1996; Harrison et al., 2009; Shirazi et al., 2013).

The RT-PCR assay was carried out to investigate the effects of plant extracts on several aging-associated genes expression level.

Table 2. List of some aging-associated genes in *S. cerevisiae* from GenAge database, mentioned in this paper

Gene	Known functions and activities	Lifespan Effect
TOR1	PIK-related protein kinase and rapamycin target	Deletion increased mean and maximum lifespan by 20%
SCH9	Controls cAPK activity, required for nitrogen activation of the FGM pathway	Mutations increase resistance to oxidants and extend lifespan by up to threefold. Stress-resistance transcription factors Msn2/Msn4 and protein kinase Rim15 are required for this life-extension.
SIR2	Conserved NAD+ dependent histone deacetylase of the Sirtuin family	Involve in the regulation of the aging process
URH1	URidine Hydrolase	Replicative lifespan increased
UTH1	Uth1p	Mutation increases lifespan and stress resistance.
SOD1/SOD2	Cytosolic/mitochondrial superoxide dismutases. Protects cells against oxygen toxicity	Overexpression of *Sod1* and *Sod2* extends survival

Natural overexpression of *SIR2* gene could be correlated with the replicative life spans of yeast cells (Guo et al., 2011). Hesperidin derived from *Citrus* genus has been reported to produce anti-aging effects in yeast via the *UTH1* gene expression, and increase in *SIR*2 activity (Sun et al., 2012). In another study was evidenced the anti-aging effects of the polyphenolic compound in apple extract by upregulation gene expression of aging pathway (*SIR2*) and antioxidant (*SOD1 and SOD2*) in yeast via RT-PCR (Xiang et al., 2011). Parishin from *Gastropodia elata* extends the lifespan of yeast by increasing of the *SIR2* gene expression, and inhibition of the *UTH1/TOR* signalling pathway (Lin et al., 2016). By using RT-PCR have been demonstrated that the potential of roselle petal extract at 300 ppm concentration was able to upregulate of genes involved in tolerance mechanism opposed to oxidative stress (*GLR1* and *GPX1*) and key genes in aging pathway like *SIR2* (Sarima et al., 2019).

Quantifying telomere length

Examination of the end fragment of the Terminal Restriction (TRF) is the initial technique established for telomere duration and is thus often referred to as a form of "gold standard."

This technique uses a cocktail of regular cutting restriction enzymes to exhaustively digest genomic DNA that do not have recognition positions in the telomeric and subtelomeric region (and thus do not "recut" telomerical DNA).

The preserved telomeres are then resolved by size of all chromosomes. The various lengths of telomeres, the size and strength of the strain are compared to a DNA ladder consisting of defined fragment dimensions (Allshire et al., 1989; Harley et al., 1990; Kimura et al., 2010).

For this technique and for all other approaches for quantifying telomere length, the integrity of derived genomic DNA is important. Clearly, the degradation of DNA - a mechanism by which DNA is fragmented into smaller fragments - can contribute to inaccuracies in telomere length evaluation and to a tendency towards shorter lengths.

DNA degradation may be caused by many different factors, including the frequent thawing and freezing of the DNA, long time-out of DNA at room temperature and incorrect purification of residual nuclears. Cautionary steps must also be taken to avoid deterioration in the handling and extraction of genomic DNA (Aubert et al., 2012).

This provides the ability to compare findings from other investigators and to include an estimation of the kilobase size for the telomere period. Furthermore, since this approach does not entail the use of expensive advanced equipment, proof-of-concept experiments can be an appropriate technique. A drawback is to use subtelomeric DNA contiguous to the telomere, thus over-estimating the true telomere length, by using the restriction enzymes. Subtelomeric and telomeric areas can also contain polymorphisms that may confuse data understanding. Even if various restriction enzymes are used, the findings can vary from laboratory to laboratory. Other shortcomings of the TRF test include a large quantity of DNA (micrograms) needed for which this procedure is more commonly used than other tissue samples for the analysis of telomere length in blood samples. This technique is intensive and cannot detect short telomeres on a limited number of chromosomes, which represent hybridizing limitations because very short telomeres cannot connect the sample efficiently (Martin-Ruiz et al., 2015). These short coming are significant limits for using the TRF method for evaluating telomeres for studies involving large numbers of people using epidemiological study design approaches, and for the fact that the TRF value is expressed as a mean of the smear dimension not providing information on single telomeres (non-clear recognition of the range or values at the extremities of the smears spectrum) (Aubert et al., 2012). Zubko et al. (2016) developed an advanced PCR-method refined as ATLAS (A-dvanced T-elomere L-ength A-nalysis in *S. cerevisiae*) for direct visualization of telomere length differences in routine experiments with *Saccharomyces cerevisiae,* showing strong correlation of results with data obtained by Southern blot hybridization.

CONCLUSIONS

The results which we reviewed prove that *S. cerevisiae* is the ideal candidate to demonstrate the anti-aging effects of various plant extracts or active substances isolated from plant sources. The small and relatively well-defined genome makes yeast a prime model in a basic setting for researching complex cellular processes. Initial drug development using yeast was initiated by several pharmacological scans; often with other laboratory models the screening technique is unworkable. As a screening medium for anti-aging compounds, yeast has been long time underestimated. The increasing number of instruments and methods available to measure lifetime in a high-performance manner further opens up the opportunity for anti-aging screens dependent on the yeast. Therefore, it is recommended using that more than one method for the study of the influence of anti-aging effect of the plant extracts on yeast cells. In this way, it will obtain full and complexes results that can be helpful to understand the mechanisms of the aging process. Yeast viability is a powerful readout which makes translative use in research fields of bioactive compounds apart from aging based on the hypothesis that compounds that reduce viability might be good and applicable against development of anarchic cells.

Although the categorical anti-aging effects of certain plants have been proven on *S. cerevisiae* and rarely on small animals, we must not interpret this fact as being categorical for the human body as well.

Yeast has a wide capacity to unveil innovative pharmacological approaches against aging and we are confident that they will remain substantially involved in drug development in the field.

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