Chapter

The Budding Yeast *Saccharomyces cerevisiae* as a Valuable Model Organism for Investigating Anti-Aging Compounds

Yanni Sudiyani, Muhammad Eka Prastya, Roni Maryana, Eka Triwahyuni and Muryanto

Abstract

Saccharomyces cerevisiae, the budding yeast was long history as industrial baker's yeast due to its ability to produce numerous product such as ethanol, acetate, industrial bakers etc. Interestingly, this yeast was also important tools for studying biological mechanism in eukaryotic cells including aging, autophagy, mitochondrial response etc. *S. cerevisiae* has arisen as a powerful chemical and genetic screening platform, due to a rapid workflow with experimental amenability and the availability of a wide range of genetic mutant libraries. Calorie restriction (CR) as the reduction of nutrients intake could promote yeast longevity through some pathways such as inhibition of nutrient sensing target of rapamycin (TOR), serine–threonine kinase (SCH9), protein adenylate cyclase (AC), protein kinase A (PKA) and ras, reduced ethanol, acetic acid and apoptotic process. In addition, CR also induces the expression of antioxidative proteins, sirtuin2 (Sir2), autophagy and induction of mitochondrial yeast adaptive response. Three methods, spotting test; chronological life span (CLS) and replicative life span (RLS) assays, have been developed to study aging in *S. cerevisiae*. Here, we present strategies for pharmacological anti-aging screens in yeast, discuss common pitfalls and summarize studies that have used yeast for drug discovery.

Keywords: *Saccharomyces cerevisiae*, anti-aging, calorie restriction, spotting test, chronological life span, replicative life span

1. Introduction

The budding yeast *Saccharomyces cerevisiae* is unicellular eukariotic fungi that divide asexually by budding. This particular yeast cells has an individual cell size of 5–10 μm and are pigmented, which cream color emerged in surface-grown colonies (**Figure 1**). Taxonomically, *S. cerevisiae* belongs to division of Ascomycota, class of Saccharomycetes, family of Saccharomycetaceae and genus of Saccharomyces. Yeast *S. cerevisiae* breaks down glucose in the medium through aerobic respiration in presence of oxygen. If oxygen is absent, the yeast will then undergo through anaerobic fermentation. This yeasts is widely distributed in the natural environment,

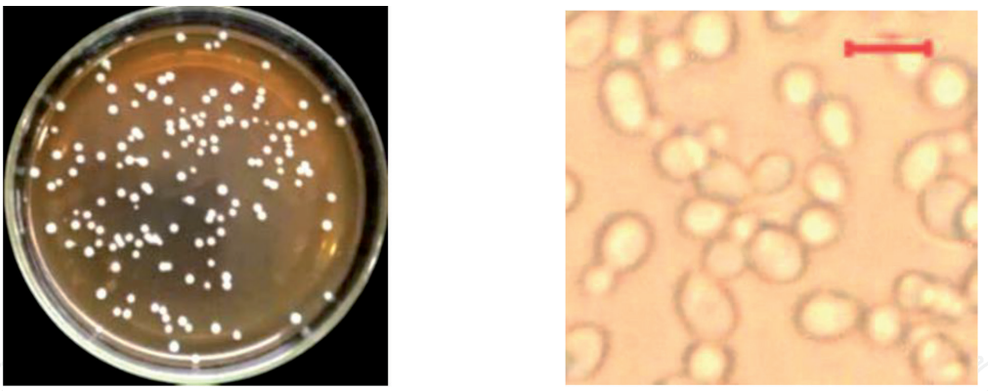

Figure 1.
The budding yeast S. cerevisiae*, (A) cells colony in agar plate medium; (B) cells morphology observed under microscope observation with 1000x magnification. Bars represent 5 μm. (picture permitted from [1], originally published in IOP Conf. Series: Earth and Environmental Science. doi: 10.1088/1755-1315/299/1/012059).*

including soil, water, plants, animals, and insects. Notably, *S. cerevisiae* are eukaryotic cells that contain all major organelles that are also common to animal cells like nucleus, mitochondria, endoplasmic reticulum, vacuole, golgi apparatus, cytoskeleton and many others organelles [2].

Scientist stated that *S. cerevisiae* was the first eukaryotic genome that was completely sequenced in 1996. In fact, the chromosomes of *S. cerevisiae* is compactly organized on 16 chromosomes and the genome has about 12.156.677 base pairs with 6.275 genes along with about 5.800 are believed to be functionally genes. Interestingly, it is estimated that this particular yeast have at least 31% of its genes homologous with that of humans belonging [3, 4]. As for, mating between haploid cells must occur to return to the diploid state. Both of haploid and diploid phases are morphologically similar, but with larger cells for diploid. In the asexual reproduction, bud grows to reach the size of the mother cell while nuclear division happens [5]. The separation would process after a nucleus is passed to the daughter cells with the smaller size particularly than mother cells. *S. cerevisiae* has a long history of uses in the area of food processing as leavening for bread and as a fermenter of wine production and alcoholic beverages, along with as a vitamin supplement due to it contains 50% protein and is a rich source of niacin, folic acid and B vitamins. In addition, *S. cerevisiae* is also clearly stated as the most ideal eukaryotic cells for biological studies. The incredible power of yeast genetics has made a legendary tools and is the envy of those who work with higher eukaryotic organisms [4, 6].

On the other hand, aging is a multifaceted process of accumulation of cellular, molecular and organ damage, leading to loss of function and increased vulnerability to disease following with the death. Indeed, there is a profound overlap between cellular and molecular pathways that influence aging and those linked to neurodegeneration, cancer, metabolic syndrome, and cardiovascular disorders. Therefore, recent efforts have emerged at the identification of compounds that decelerate the aging process and thus may act as a preventive measure that collectively ameliorates age-related diseases [7]. In fact, studies of aging in mammalian cells are limited by the long lifespan of common model organisms. Rats and mice live 3–5 years and primates up to 40. Nevertheless, aging studies, particularly in rodents, have been highly informative, of the prospective understanding of the genetic factors for modulating longevity [6]. Alternatively, a second approach that has dramatically accelerated aging research is the use of invertebrate model organisms, which age more rapidly and are readily amenable to environmental and genetic manipulation. Even though a variety of organisms have been investigated, a majority of

studies have employed worms (*Caenorhabditis elegans*) [8], fruit flies (*Drosophila melanogaster*) [9], or yeast (*S. cerevisiae*) [10]. The use of model organisms at least in part due to its conserve some pathways that exist in all of these creatures, besides similarly in cellular or molecular events. One of the most popular conserve pathway is the nutrient-sensing pathway namely target of rapamycin (TOR) that exist in all of model organism starting from yeast to mammals [11].

The budding yeast *S. cerevisiae* has served as a model of organism and cellular aging for more than 50 years. In this particular yeast, many pathways that are relevant for aging and disease in humans are well conserved, including nutrient signaling, DNA repair mechanisms, cell cycle regulation, protein folding, lipostasis, mitochondrial homeostasis, stress response, secretion, proteostasis, and regulated cell death [6, 7]. In addition, the molecular mechanisms for cellular aging are also conserved from budding yeast to humans, primarily in particular condition with the nutrient depletion in growth medium, which retards aging and prolongs the lifespan. The conserved protein kinase, TORC1 (target of rapamycin complex 1), that is activated by nutrients and insulin, is a key regulator of the lifespan from eukaryotes organisms. The TORC1 inhibitor, namely rapamycin, retards aging and extends the lifespan of various species from yeast to mammals. In addition, this yeast has the advantages of speediness, simplicity, low cost, and good reproducibility. Furthermore, the genome DNA is not complex and has high homology with mammals. Indeed, all of DNA sequences are known and molecular mechanism research handling is relatively easy to perform, as well [6, 12, 13]. Some recent studies reported the utilization of *S.* cerevisase as a promising model organism to unravel anti-aging mechanism of compounds derived from various sources. Carmona-Gutierrez et al. [14] has shown anti-aging activity of flavonoid 4,4-dimethoxychalcone in *S. cerevisiae* by promoting authopagy mechanism. Lin et al., [15] reported anti-aging activity of Cucurbitacin B through regulating autophagy and oxidative stress in *S. cerevisiae*. In addition, Sudharsan et al., [16] also succeded to uncover the anti-aging mechanism of Astaxanthin in this yeast by decreasing oxidative stress and apoptosis mechanisms.

In this brief chapter, we discussed the utilization of *S. cerevisiae* as a model organism in drug discovery research, and how this simple eukaryote has been employed not only as a production vehicle due to its capability to produce some products i.e. wine, alcoholic beverage, and proteins but also as a valuable tools in understanding biological aging. In fact, *S. cerevisiae* has a long history as the workhorse of pharmaceutical discovery research [17]. The uses of yeast explained in this chapter are including primary pathways involved in the mechanism of yeast aging belong to pro- or anti-aging mechanisms, and sections on the use of yeast for elucidating anti-aging compounds through the popular methods that was already utilized by previuos researcher's. To this end, we would state that there are still limitation in regard with anti-aging study or review of using yeast as a model organism for elucidating anti-aging compounds in our country, Indonesia. At the same time, this country has an abundant sources of medicinal compounds derived from terestrial to aquatic regions. Therefore, we hope that this book chapter could provide new alternatively insight for pharamaceutical study focusing to discover active compounds with anti-aging properties.

2. Aging interventing mechanisms in *S. cerevisiae*

Biological aging in *S. cerevisiae* involved complicated mechanisms including, cellular, physiological and molecular as well as intervented by environmental growth conditions. Interestingly, almost all of yeast model, particularly *S. cerevisiae* has

sophisticated response while growth in Calorie Restriction (CR) condition, which scientist's oftenly used those phenomenon to learn aging pathway in yeast model. CR, a reduction in nutrient availability without malnutrition, is known to expand lifespan in a wide range of organisms from yeast to primates [11, 18]. Of note, deeply effort has been devoted to understanding the pathways that mediate the benefits of dietary restriction, since interventions that target these pathways may be effective in humans against the diseases of aging. In the yeast anti-aging assay, dietary restriction is usually generated by reducing glucose concentration from 2% to 0.5 or 0.05% in the growth culture [6], as well as restriction for amino acids has also been reported to exert lifespan [19].

Ultimately, CR reported could affect in some distinctive pathways in yeast cells. The first pathway is nutrient-sensing which reduced activity of two major nutrient sensing pathways, due to CR condition, could extend yeast life span. Both nutrient sensing pathways are focused on an amino acid-sensing pathway, including the serine–threonine kinase SCH9 and the target of rapamycin (TOR). Notably, deletion or inhibition of SCH9 and TOR causes an increase of up to several fold in yeast life span. Alterations to reduce nutrient and protein synthesis in CR condition are strongly implicated in extension of yeast lifespan by reduced TOR/SCH9. Extension of yeast lifespan by reduced activity of the TOR pathway depends on the transcription factor Gis1, which activates many protective systems including Mn-SOD [6, 20].

Further, the second pathway includes three proteins including adenylate cyclase (AC), protein kinase A (PKA), and Ras which will inhibit by CR conditions. The activation of two transcription factors (Msn2 and Msn4) that control cellular protection systems is required to mediate the effect of reduced Ras-AC-PKA signaling on yeast lifespan extension. Extension of yeast lifespan by these pathways needs the antioxidant enzyme Mn-SOD (superoxide dismutase), which scavenges the superoxide free radical [11, 21]. Intriguingly, superoxide level increases during yeast aging and is reduced in yeast mutants deficient in Tor-SCH9 or Ras-AC-PKA signaling. As for the yeast cells grow in the high glucose medium, could produce ethanol or acetic acid, which also contribute to chronological aging. Interestingly, deletion of SCH9 or TOR1 promotes removal of ethanol and acetic acid and accumulation of glycerol in the medium and further extend chronological life span by mechanisms similar to those of dietary restriction [22]. More importantly, decreased signaling by the Tor-SCH9 and Ras-AC-PKA pathways is important in response to glucose restriction as well as increased transcriptional activity of Msn2 and Msn4, and the consequent affecting the expression of Pnc1 [nicotinamide deaminase that promotes the activity of the nicotinamide adenine dinucleotide (NAD)- dependent deacetylase Sirtuin 2/Sir2]. The Sir2 have been extensively studied for their potential role as conserved modulators of anti-aging in a various of organisms, including mammals [23]. One mechanism by which Sir2 activity promotes yeast longevity is by suppressing homologous recombination in the rDNA that can promote the formation of extrachromosomal rDNA circles (ERCs). In fact, rDNA instability in general suggested the primary defect causing senescence and cell death [24].

Notably, another crucial mechanism that closely related with yeast aging is autophagy. This cellular process is reported as a highly conserved in organisms from yeast to human, which involves degradation of damaged organelles, circulation of amino acids, proteins, and other metabolites. It also regulates the genomic integrity via suppression of cell division in yeast under CR condition. Notably, decreased or dysfunction expression of autophagy genes leads to shorter lifespan in yeast and fruit fly. Conversely, enhanced autophagy promotes the longevity in aging models and suggested could protect against aging and age-related disorders [25, 26]. Another mechanisms which closely related with yeast aging is mitochondrial

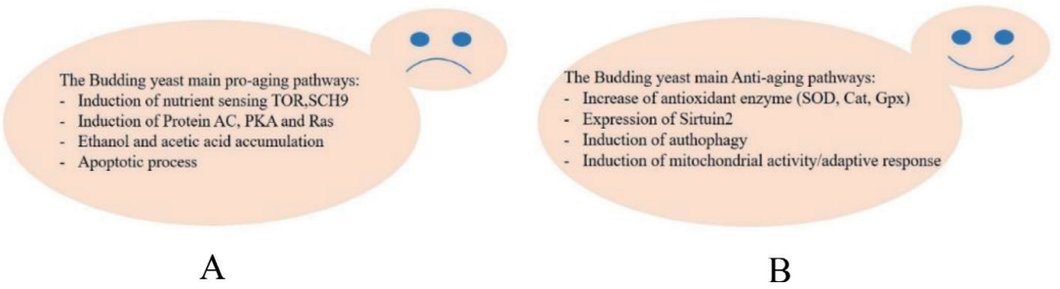

Figure 2.
(A) Some main pro-aging and (B) anti-aging pathways in S. cerevisiae*. (TOR: target of rapamycin, SCH9: serine–threonine kinase, AC: Adenylate cyclase, PKA: Protein kinase A, SOD: Superoxide dismutase, Cat: Catalase, Gpx: Glutathione peroxidase).*

adaptive response signaling. Mitochondrial organelle is known to have a basic role in aging and age-related diseases. This organelle contributes to the ATP production, cell homeostasis, and imbalanced reactive oxygen species (ROS) creating a basic role of the cells regulation [27]. In common condition, mitochondrial produces toxic ROS as by product of respiration process. However, on the CR condition, mitochondrial would be more active due to the shift metabolic process occurs from fermentation to respiration (CR) resulting ROS at the initial growth phases, as well. Consequently, yeast cells will adapt (pre-adaptation) with the ROS molecules strating from early growth stage and thus might activate defence mechanisms in the late of growth phase ensuring protection against higher doses of ROS. Those defence mechanisms likely activated antioxidative enzyme i.e. superoxide dismutase (SOD), catalase or glutathione peroxidase and therefore increase yeast lifespan (**Figure 2**) [28, 29].

Other than the above mechanisms in relation with CR condition resulting yeast lifespan extension. CR was also reported as having other substantial effects in yeast cells i.e. apoptotic process that accelerate aging process, repairing protein damage, NAD+ homeostasis, vacuolar function, genome stability, ribosom biogenesis, proteolysis regulation, and cell hyperthrophy [30]. These valuable insights reflect that CR condition in yeast cells could modulate numerous pathways affected in biological aging mechanism. As for forefront anti-aging methods strategy, yeast cells growth on CR oftenly use as for positive control, whereas growing yeast cells on the normal growth medium with 2% glucose utilize as treatment for anti-aging compounds screening. If the yeast cells viability derived from compounds treatment has similar or higher than positive control, it suggested that corresponding compounds have anti-aging activity in yeast cells. Further research usually applied to investigate the precise mechanisms which modulated by those promising anti-aging compounds inside yeast cells.

3. Methods for investigating anti-aging activity in *S. cerevisiae*

On the basis of the current literatures, there were established various potential methods for investigating anti-aging activity derived from chemical compound by using *S. cerevisiae* as a model organism. It is including spotting test, Chronological Life Span (CLS) using Total Plate Count (TPC) analysis, Replicative Life Span (RLS) assay using microscopic observation, Propidium Iodida – Flow Cytometry Analysis (PI-FCA) analysis (see detail in Ocampo and Barrientos, 2011), Bac-light method (see detail in [31] Zhang and Fang, 2004), staining with methylen blue or phloxin B, physiological assay using luceferin reaction or rhodamine B (see detail in [32], and High-Throughput Rapid Chronological Lifespan (HTRCL) based on MTT

assay (See detail in [33]). However, in this section we would highlight only the most 3 popular methods including spotting test, CLS, and RLS analysis.

3.1 Spotting test

This particular method is commonly used in order to observe the viability of *S. cerevisiae* cells through the phenotype spotting shape on the surface of solid growth medium. In fact, this method is also applied not only for *S. cerevisiae*, but also for other yeast to assay anti-aging activity of compounds, including *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Candida albicans*, and *Cryptococcus neoformans* [7]. In addition, microbiologist oftenly utilize this method to search the specific microbial mutant that sensitive to particular conditions existing in the solid medium i.e. oxidative stress, antibiotic resistance, heat, osmotic, nutrient limitation, etc. Briefly, spot test was conducted by inoculating the fresh logarithmic *S. cerevisae* cells to the yeast liquid medium in the test tube with an appropriate initial OD600 of 0.05–0.1. Subsequently, the corresponding compound supplemented to the same yeast liquid medium with a certain concentration, and culture were incubated until reached the stationary phase (20–25 days) at the optimal growth temperature. Periodically, yeast treatment culture (usually at day 5, 10, 15, and 20) spotted to the yeast solid culture. As for the spotting test was initially performs by serially dilution of the treatment culture at the selected incubation time until 10^{-4} dilution value, then spotting applied from each dilution to the yeast solid medium. Further, the results are incubated for 3 days at the optimal growth temperature prior observed. The density of the grown cells from each spot is considered as the viability of *S. cerevisiae* cells (**Figure 3**) (see detail in [1, 34]).

Spotting test was reported as having some advantages in relation to assay anti-aging compounds including simple, fast handling and relatively low cost compare than CLS or other methods. However, this method could only represent a qualitative result of yeast cells viability through the yeast cells density, and thus it should be supported by other methods. As long as for the preliminary screening of numerous compounds acting as anti-aging, spotting method will be recommended. Some previous studies reported the usefulness of spot test to examine the particular compound for delaying aging in *S. cerevisiae*. Xiang et al., [35] was succeded to promote anti-aging mechanism in this yeast using phloridzin (an apple polyphenol) treatment. Apple extract, artemisinin, and roselle petal extract were also reported could increase *S. cerevisiae* lifespan using spotting test method [36–38]. Along with that, *S. cerevisiae* was succesfully used for obtaining the anti-aging activity of clove bud extract and some medicinal plant extract [1, 39].

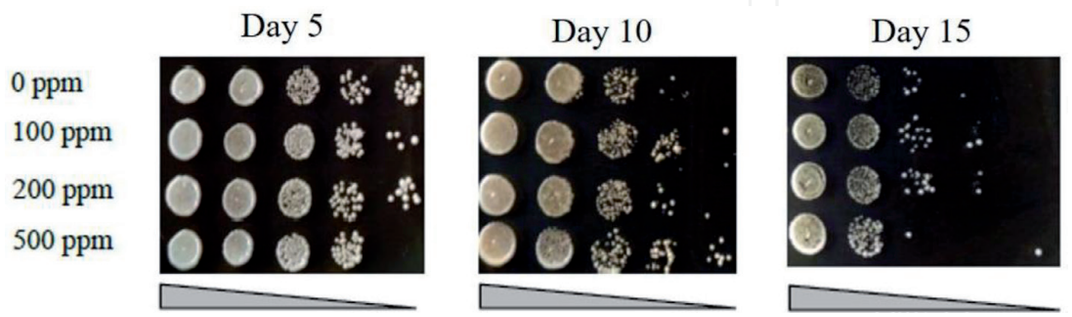

Figure 3.
Result of spotting test from clove bud extract using S. cerevisiae *as model organism. Cells grown in yeast culture without extract addition (0 ppm) is designed as a control. The density of the grown cells from each spot considered as the viability of* S. cerevisiae *cells. Spotted at day 15 after treatment with 100 and 200 ppm compound showed higher density than 0 ppm (control treatment), indicating anti-aging activity of corresponding compound to exert the yeast lifespan (picture permitted from [1], originally published in IOP Conf. Series: Earth and Environmental Science. doi: 10.1088/1755-1315/299/1/012059).*

3.2 CLS method using TPC analysis

CLS defines to the length of time a non-dividing cell can maintain viability, as refers to the its ability to re-enter the cell cycle process after a prolonged period of quiescence. Thus, CLS has been exhibited as a model of the viability of post-mitotic yeast cells [12]. Traditionally, CLS has been examined by culturing fresh logarithmic yeast cells on a particular flask until reached the stationary phase (20–25 days) in liquid culture with an appropriate initial OD600 of 0.05–0.1. As for compound treatment is applied to the liquid culture medium soon thereafter yeast innoculated. Further, the yeast cell survival is measured as a function of time by dilution and plating onto a nutrient-rich agar medium at the periodic time (i.e each 3 days). Subsequently, viability is then calculated on the basis of the number of colonies (colony forming units: CFUs) on the nutrient plate agar arising [40, 41]. The budding yeast *S. cerevisiae* reported entering a stationary/death phase after 20–25 days incubation [6]. Therefore, if compound treatment could prolong the yeast lifespan beyond 25 days, it suggested as the promising anti-aging materials. Of note, CLS assay is widely applied not only for *S. cerevisiae* but also for other yeast i.e. *S. pombe* or *K. lactis* for observing the bioactivity of prospective anti-aging compounds (i.e **Figure 4**, CLS applying in *S. pombe*).

Ultimately, CLS method requires a relatively large investment of materials, investigator time and belong to laborious, therefore is not suited for high-throughput screening anti-aging compounds. However, through this particular method, it was obtained the quantitatively results and thus could provide deeply insight for representing the cell viability of the yeast cells. Numerous studies has been applied CLS method for assaying anti-aging compounds derived from multi-resources i.e. Nakaya et al., [42] assayed Beauveriolide I isolated from mushroom or Sunthonkun *et al*., [40] examined anti-aging of pigmented rice. In addition, other compounds including Dimethylchalcone, Cucurbitacin B, and Astaxanthine was exhibited anti-aging activity in *S. cerevisiae* through CLS analysis [14–16].

3.3 RLS method

RLS assay is simple conceptually and shows an advantage of the fact that *S. cerevisiae* cells divide by asymmetric budding, with the daughter cells that is produced

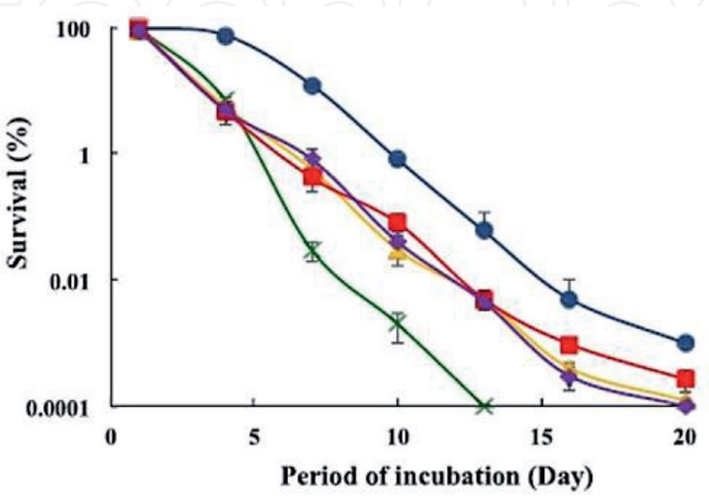

Figure 4.
CLS result using S. pombe *(green line as a control treatment) after treatment with some compounds (red, orange, purple and yellow lines showed higher viability day than control treatment). In* S.pombe*, the stationary phase will reach after 13–15 days incubation at the optimal temperature (Prastya et al., 2020).*

being smaller than the mother cells of which it is derived. Daughter cells were isolated on a solid growth media and, once they started dividing, all daughter cells were removed. Longo (2012) founded that an individual cells do not divide forever, instead they would stop after a limited number of cells divisions (around of 20–25) and going to a short post-replicative state followed by death cells. The RLS method determines the number of daughters of a single mother cell, which can asexually produce prior to senescence. The mother-daughter cell asymmetry in *S. cerevisiae* cells can be easily observed under the light microscope, allowing the development of the RLS assay [6, 43].

As for RLS assay, the *S. cerevisiae* cells inoculated onto 5 mL galactose or glucose liquid medium and further incubated in a shaking incubator for 48 h at the optimal temperature. Subsequently, 1 mL of yeast cells culture was centrifuged and pellet washed with distilled water or phosphate buffer solution (PBS). After counting using a hemocytometer, 4000–5000 cells are plated on agar plates medium containing chemical compounds need to be assay for antiaging activity, and plates were further incubated for 2 days at the optimal temperature. The 40 microcolonies that formed on the agar plates were randomly observed under a microscope, and daughter cells were counted. Recently, *S. cerevisase* yeast cells reported as the most powerfull model organism for RLS analysis [44, 45]. The results of RLS assay usually show in the particular graphic that indicating the viability number from each generations of the yeast cells. If the viability of the yeast cells after compound treatment is higher than control treatment, it is indicating the potential anti-aging properties.

RLS method is reported as having some major weakness which it makes less effective for high-throughput approaches. It is including time-consuming, laborious and relatively intricate in technique due to applying microscopic cells observation prior for plating in the plate medium during assays. Nevertheless, this particular methods will devote precisely quntitatively results and thus could represent the number of yeast cells generation between control and anti-aging compound treatments. To date, some previous studies were reported for using *S. cerevisiae* RLS method to examine anti-aging compound derived from various natural products, including Ganodermasides isolated from mushroom, or Hesperidin from citrus [46, 47]. Current studies were also informed anti-aging assay of some compounds i. e Parishin and Cucurbitacin B using *S. cerevisiae* RLS method, as well [15, 45].

4. Conclusions

Antiaging study in yeast was popular using CR condition which has numerous response to prolong yeast lifespan. Aging pathway in CR belong to pro-and anti-aging pathways. As for pro-aging are including TOR, SCH9, Ras protein, AC, PKA, ethanol accumulation, and apoptotic process. On the other hand, anti-aging pathways are including induction of antioxidative enzymes, sirtuin2, autophagy and adaptive response thorough mitochondrial adaptive ROS signaling. CR condition usually use for positive control, while treatment conducted in non-CR/high 2% glucose medium. There are numerous methods for anti-aging study, which the most popular is spotting test, CLS and RLS assays.

Recently, anti-aging in *S. cerevisiae* research was developed sophisticated, derived from previous sources including natural compound derived from terestrial or aquatic organism. In fact, other sources also potentially developed i.e. semisynthetic or synthetic compound as the preliminary screening for anti-aging compounds.

Even the data from studies could be revisited and mined for potential bioactive substances, data obtained in yeast should not be over-interpreted unduly, and when aiming for applications in humans, validation of compounds in multicellular organisms should be done. So far, the potential of yeast to unravel novel pharmacological interventions against aging is far-reaching, however that it will continue to contribute substantially not only to drug discovery but also in other field such as fermented food, biochemical and bioenergy production.

Acknowledgements

The authors would like to thank Dr. Rika Indri Astuti Department of Biology, IPB University, Indonesia for permitting the pictures.

Conflict of interest

On behalf of all authors, we declare no conflict of interest.

Author details

Yanni Sudiyani*, Muhammad Eka Prastya, Roni Maryana, Eka Triwahyuni and Muryanto
Research Center for Chemistry, Indonesian Institute of Sciences, Serpong Tangerang Selatan, Banten, Indonesia

*Address all correspondence to: sudiyani@gmail.com

References

[1] R. I. Astuti, S. Listyowati, and W. T. Wahyuni, "Life span extension of model yeast *Saccharomyces cerevisiae* upon ethanol derived-clover bud extract treatment," in *IOP Conference Series: Earth and Environmental Science*, 2019, vol. 299, no. 1, p. 12059.

[2] G. M. Walker and N. A. White, "Introduction to Fungal Physiology," *Fungi*. pp. 1-35, Nov. 22, 2017, doi: doi:10.1002/9781119374312.ch1.

[3] A. Goffeau *et al.*, "Life with 6000 genes," Science (80-.)., vol. 274, no. 5287, pp. 546-567, 1996.

[4] R. Montes de Oca *et al.*, "Yeast: description and structure," *Yeast Addit. Anim. Prod. Tamilnadu, PubBioMed Cent. Res. Publ. Serv.*, pp. 4-13, 2016.

[5] M. H. Barros, F. M. da Cunha, G. A. Oliveira, E. B. Tahara, and A. J. Kowaltowski, "Yeast as a model to study mitochondrial mechanisms in ageing," Mech. Ageing Dev., vol. 131, no. 7-8, pp. 494-502, 2010.

[6] V. D. Longo, G. S. Shadel, M. Kaeberlein, and B. Kennedy, "Replicative and chronological aging in *Saccharomyces cerevisiae*," Cell Metab., vol. 16, no. 1, pp. 18-31, 2012.

[7] A. Zimmermann, S. Hofer, T. Pendl, K. Kainz, F. Madeo, and D. Carmona-Gutierrez, "Yeast as a tool to identify anti-aging compounds," *FEMS Yeast Res.*, vol. 18, no. 6, Sep. 2018, doi: 10.1093/femsyr/foy020.

[8] A. Olsen, M. C. Vantipalli, and G. J. Lithgow, "Using Caenorhabditis elegans as a model for aging and age-related diseases," Ann. N. Y. Acad. Sci., vol. 1067, no. 1, pp. 120-128, 2006.

[9] S. L. Helfand and B. Rogina, "Genetics of aging in the fruit fly, Drosophila melanogaster," Annu. Rev. Genet., vol. 37, no. 1, pp. 329-348, 2003.

[10] M. Kaeberlein, C. R. Burtner, and B. K. Kennedy, "Recent developments in yeast aging," *PLoS Genet*, vol. 3, no. 5, p. e84, 2007.

[11] L. Fontana, L. Partridge, and V. D. Longo, "Extending Healthy Life Span—From Yeast to Humans," *Science (80-.).*, vol. 328, no. 5976, pp. 321 LP – 326, Apr. 2010, doi: 10.1126/science.1172539.

[12] C. J. Murakami, C. R. Burtner, B. K. Kennedy, and M. Kaeberlein, "A method for high-throughput quantitative analysis of yeast chronological life span," Journals Gerontol. Ser. A Biol. Sci. Med. Sci., vol. 63, no. 2, pp. 113-121, 2008.

[13] M. Kaeberlein, "Lessons on longevity from budding yeast," Nature, vol. 464, no. 7288, pp. 513-519, 2010.

[14] D. Carmona-Gutierrez *et al.*, "The flavonoid 4, 4′-dimethoxychalcone promotes autophagy-dependent longevity across species," Nat. Commun., vol. 10, no. 1, pp. 1-17, 2019.

[15] Y. Lin *et al.*, "Cucurbitacin B Exerts Antiaging Effects in Yeast by Regulating Autophagy and Oxidative Stress," *Oxid. Med. Cell. Longev.*, vol. 2019, 2019.

[16] S. SJ, B. Veerabhadrappa, S. Subramaniyan, and M. Dyavaiah, "Astaxanthin enhances the longevity of *Saccharomyces cerevisiae* by decreasing oxidative stress and apoptosis," *FEMS Yeast Res.*, vol. 19, no. 1, Jan. 2019, doi: 10.1093/femsyr/foy113.

[17] P. Ross-Macdonald, "Growing Yeast for Fun and Profit: Use of *Saccharomyces cerevisiae* as a Model System in Drug Discovery," Model Org. Drug Discov., pp. 9-39, 2003.

[18] R. M. Anderson and R. Weindruch, "The caloric restriction paradigm: implications for healthy human aging," Am. J. Hum. Biol., vol. 24, no. 2, pp. 101-106, 2012.

[19] J. C. Jiang, E. Jaruga, M. V Repnevskaya, and S. M. Jazwinski, "An intervention resembling caloric restriction prolongs life span and retards aging in yeast," FASEB J., vol. 14, no. 14, pp. 2135-2137, 2000.

[20] M. Kaeberlein *et al.*, "Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients," Science (80-.)., vol. 310, no. 5751, pp. 1193-1196, 2005.

[21] P. Fabrizio, F. Pozza, S. D. Pletcher, C. M. Gendron, and V. D. Longo, "Regulation of longevity and stress resistance by Sch9 in yeast," Science (80-.)., vol. 292, no. 5515, pp. 288-290, 2001.

[22] P. Fabrizio *et al.*, "Sir2 blocks extreme life-span extension," Cell, vol. 123, no. 4, pp. 655-667, 2005.

[23] T. Finkel, C.-X. Deng, and R. Mostoslavsky, "Recent progress in the biology and physiology of sirtuins," Nature, vol. 460, no. 7255, pp. 587-591, 2009.

[24] D. L. Lindstrom, C. K. Leverich, K. A. Henderson, and D. E. Gottschling, "Replicative age induces mitotic recombination in the ribosomal RNA gene cluster of *Saccharomyces cerevisiae*," *PLoS Genet*, vol. 7, no. 3, p. e1002015, 2011.

[25] D. C. Rubinsztein, G. Mariño, and G. Kroemer, "Autophagy and aging," Cell, vol. 146, no. 5, pp. 682-695, 2011.

[26] J. K. Tyler and J. E. Johnson, "The role of autophagy in the regulation of yeast life span," Ann. N. Y. Acad. Sci., vol. 1418, no. 1, pp. 31-43, Apr. 2018, doi: 10.1111/nyas.13549.

[27] M. T. Lin and M. F. Beal, "Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases," Nature, vol. 443, no. 7113, pp. 787-795, 2006.

[28] Y. Pan, E. A. Schroeder, A. Ocampo, A. Barrientos, and G. S. Shadel, "Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling," Cell Metab., vol. 13, no. 6, pp. 668-678, 2011.

[29] G. S. Shadel, "Live longer on MARS: a yeast paradigm of mitochondrial adaptive ROS signaling in aging," *Microb. cell (Graz, Austria)*, vol. 1, no. 5, pp. 140-144, Apr. 2014, doi: 10.15698/mic2014.05.143.

[30] P. Fabrizio and V. D. Longo, "The chronological life span of *Saccharomyces cerevisiae*," Aging Cell, vol. 2, no. 2, pp. 73-81, 2003.

[31] T. Zhang and H. H. P. Fang, "Quantification of *Saccharomyces cerevisiae* viability using BacLight," Biotechnol. Lett., vol. 26, no. 12, pp. 989-992, 2004, doi: 10.1023/B:BILE.0000030045.16713.19.

[32] M. Kwolek-Mirek and R. Zadrag-Tecza, "Comparison of methods used for assessing the viability and vitality of yeast cells," FEMS Yeast Res., vol. 14, no. 7, pp. 1068-1079, 2014.

[33] Z. R. Belak, T. Harkness, and C. H. Eskiw, "A rapid, high-throughput method for determining chronological lifespan in budding yeast," *J. Biol. Methods*, vol. 5, no. 4, 2018.

[34] M. E. Prastya, R. I. Astuti, I. Batubara, and A. T. Wahyudi, "Bacillus sp. SAB E-41-derived extract shows antiaging properties via ctt1-mediated oxidative stress tolerance response in yeast Schizosaccharomyces pombe," *Asian Pac. J. Trop. Biomed.*, vol. 8, no. 11, p. 533, 2018.

[35] L. XIANG *et al.*, "Anti-Aging Effects of Phloridzin, an Apple Polyphenol, on Yeast via the SOD and Sir2 Genes," Biosci. Biotechnol. Biochem., vol. 75, no. 5, pp. 854-858, 2011, doi: 10.1271/bbb.100774.

[36] V. Palermo, F. Mattivi, R. Silvestri, G. La Regina, C. Falcone, and C. Mazzoni, "Apple can act as anti-aging on yeast cells," *Oxid. Med. Cell. Longev.*, vol. 2012, 2012.

[37] D. Wang, M. Wu, S. Li, Q. Gao, and Q. Zeng, "Artemisinin mimics calorie restriction to extend yeast lifespan via a dual-phase mode: a conclusion drawn from global transcriptome profiling," Sci. China Life Sci., vol. 58, no. 5, pp. 451-465, 2015, doi: 10.1007/s11427-014-4736-9.

[38] A. R. I. Sarima and A. Meryandini, "Modulation of aging in yeast *Saccharomyces cerevisiae* by roselle petal extract (Hibiscus sabdariffa L.)," Am. J. Biochem. Biotechnol., vol. 15, no. 1, pp. 23-32, 2019.

[39] P. Dakik *et al.*, "Discovery of fifteen new geroprotective plant extracts and identification of cellular processes they affect to prolong the chronological lifespan of budding yeast," *Oncotarget*, vol. 11, no. 23, p. 2182, 2020.

[40] P. Sunthonkun, R. Palajai, P. Somboon, C. L. Suan, M. Ungsurangsri, and N. Soontorngun, "Life-span extension by pigmented rice bran in the model yeast *Saccharomyces cerevisiae*," *Sci. Rep.*, vol. 9, no. 1, p. 18061, 2019, doi: 10.1038/s41598-019-54448-9.

[41] M. E. Prastya, R. I. Astuti, I. Batubara, H. Takagi, and A. T. Wahyudi, "Natural extract and its fractions isolated from the marine bacterium Pseudoalteromonas flavipulchra STILL-33 have antioxidant and antiaging activities in Schizosaccharomyces pombe," *FEMS Yeast Res.*, vol. 20, no. 3, p. foaa014, 2020.

[42] S. Nakaya, S. Mizuno, H. Ishigami, Y. Yamakawa, H. Kawagishi, and T. Ushimaru, "New rapid screening method for anti-aging compounds using budding yeast and identification of beauveriolide I as a potent active compound," Biosci. Biotechnol. Biochem., vol. 76, no. 6, pp. 1226-1228, 2012.

[43] R. K. Mortimer and J. R. Johnston, "Life span of individual yeast cells," Nature, vol. 183, no. 4677, pp. 1751-1752, 1959.

[44] S.-J. Lin and N. Austriaco, "Aging and cell death in the other yeasts, Schizosaccharomyces pombe and Candida albicans," FEMS Yeast Res., vol. 14, no. 1, pp. 119-135, 2014.

[45] Y. Lin, Y. Sun, Y. Weng, A. Matsuura, L. Xiang, and J. Qi, "Parishin from Gastrodia elata extends the lifespan of yeast via regulation of Sir2/Uth1/TOR signaling pathway," *Oxid. Med. Cell. Longev.*, vol. 2016, 2016.

[46] Y. WENG *et al.*, "Ganodermasides C and D, Two New Anti-Aging Ergosterols from Spores of the Medicinal Mushroom Ganoderma lucidum," Biosci. Biotechnol. Biochem., vol. 75, no. 4, pp. 800-803, 2011, doi: 10.1271/bbb.100918.

[47] K. SUN, L. XIANG, S. ISHIHARA, A. MATSUURA, Y. SAKAGAMI, and J. QI, "Anti-Aging Effects of Hesperidin on *Saccharomyces cerevisiae* via Inhibition of Reactive Oxygen Species and UTH1 Gene Expression," *Biosci. Biotechnol. Biochem.*, vol. advpub, p. 1202232809, 2012, doi: 10.1271/bbb.110535.