

PAPER • OPEN ACCESS

A simple method for analysis of *Saccharomyces cerevisiae* morphology by applying a high vacuum mode of the scanning electron microscopy and without chemical fixatives

To cite this article: M F Karimy *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **462** 012048

View the [article online](#) for updates and enhancements.

You may also like

- [Modeling the minimum enzymatic requirements for optimal cellulose conversion](#)
R den Haan, J M van Zyl, T M Harms et al.
- [Towards instantaneous cellular level bio diagnosis: laser extraction and imaging of biological entities with conserved integrity and activity](#)
L Ren (), W D Robertson, R Reimer et al.
- [Ni²⁺ adsorption performance of immobilized *Saccharomyces cerevisiae* in fractionated clay from Southeast Sulawesi](#)
N Sasria, Fahmiati and Mashuni



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

A simple method for analysis of *Saccharomyces cerevisiae* morphology by applying a high vacuum mode of the scanning electron microscopy and without chemical fixatives

M F Karimy*, E Damayanti, A E Suryani, E Prasetyo, R Nurhayati, M Anwar and A S Anggraeni

Research Division for Natural Product Technology, Indonesian Institute of Sciences (LIPI) Gunungkidul, D I Yogyakarta, Indonesia, 55861

*Corresponding author's email: mfaizkarimy@gmail.com;
emadamayanti80@gmail.com

Abstract. A simple method for the preparation of a specimen for observing *Saccharomyces cerevisiae* B-18 by using a high vacuum mode setting of scanning electron microscopy (SEM). The method without chemical fixatives (F0) was very simple, the culture was directly taken from Chloramphenicol Yeast Glucose Agar (CYGA) by using a loop needle and coated with a film of gold (Au) without using any chemical fixative and only air dehydrating procedures. The method with chemical fixatives (F1) like osmium tetroxide (OsO₄), is highly toxic and corrosive. The methods also take much time for the preparation of samples before observation. The morphology of *S. cerevisiae* B-18 was observed of two magnifications, 5K and 10 K and over a range of time setting of ion sputter and one current that is ten mA. The treatments of ion sputter current exposure in this study were ten mA for 10 (T10), 20 (T20), 30 (T30), 40 (T40), 50 (T50), and 60 (T60) seconds. This preparation procedure could be most useful for *S. cerevisiae* morphological screening of rapidly and safely than using chemical fixatives before using adequate preparation procedure with chemical fixatives and could substitute light microscopy method with better magnification. The best result is the ion sputter setting ten mA for 60 seconds (T60) without any charging phenomenon disturbances. We have suggestions for F0; the first is the maximum duration of the sample that had air-dried is about three hours because more than its period, the resolution would be not proper. The optimum period for observation in the chamber in the high vacuum condition is about two hours and the second to developing methods so that representation for cell diameter measurements can be even better than this study.

1. Introduction

Scanning electron microscopy (SEM) was not a new technology in the field of microscopy, but the technology embedded in SEM itself continues to develop. Commercial products available in the electron microscope market have existed around 1965 and are the first technology that can show the three-dimensional structure of an object [1]. The SEM magnification is around 10 – 300.000 times and the resolution reaches below 1 nm even smaller depending on the type of SEM. The primary purpose of SEM can be used to find out morphology, topography, even the composition of a material by different imaging colorations [2].



The achievement to get high-quality SEM images, the samples have to be good electrical conductivity, thermal stability, and a high secondary electrical yield to acquire the high quality of SEM image [3]. The accumulation of electrons excess on a low conductive sample surface can result in a charge-discharge phenomenon (charging effect) and sensitive to radiation damage [4-6].

Poor conductivity in organic samples can be improved by making sample preparations first until the sample was ready for observed using SEM. According to [1], the requirement for organic/biological samples before conducting the observation using SEM, the sample must be free from impurities, stable in vacuum condition, must remain stable after being exposed to electrons, and must be able to emit enough secondary electrons to produce images the good one. Imaging using SEM with a sample that has low conductivity can be used vacuum in low mode setting because if SEM is operated in high vacuum mode, it could cause image distortion.

The sodium cacodylate $[(CH_3)_2AsO_2Na \cdot 3H_2O]$ is used as a buffer for glutaraldehyde fixative since 1963 by Sabatini *et al.* and widely used in electron microscopy because of the effectiveness as a buffer and its resistance to bacterial contamination during storage [7]. The fixative, like osmium tetroxide (OsO_4), is highly toxic and corrosive. The methods also take much time for the preparation of samples before observation. Improvement specimen preparation has resulted in better resolution at higher magnification [8]. The study of budding of *Saccharomyces* with the electron microscope had been done since 1953, and the preparation method of the yeast using OsO_4 was carried out by the method of Small and Marszalek since 1969 [9]. Therefore in this study, we have reported a quick and straightforward method of specimen preparation, which can be used to observe the morphology of *Saccharomyces cerevisiae* (B-18). The culture was directly sampled from CYGA media and coated with a film of gold (Au) without using any chemical fixatives.

2. Materials and methods

2.1. Object

The cultures of *S. cerevisiae* was obtained from the Research Division for Natural Product Technology (BPTBA), Indonesian Institute of Sciences (LIPI). Yeast strain isolated from the colon of Javanese duck (*Anas Javanicus*) and depend on a phylogenetic tree showed that *S. cerevisiae* B-18 was closely related to *S. cerevisiae* mt 21s (accession number X00149.1). *S. cerevisiae* B-18 may serve a potential isolate for assimilating cholesterol. The culture isolation and the method of preparation of the *S. cerevisiae* B-18 cultures were sited from [10]. *S. cerevisiae* had registered at Indonesian Culture Collection (INACC) and had not been registered at genbank for the accession number.

2.2. Method of preparation

In this study, we used two different preparation samples of *S. cerevisiae* B-18, without chemical fixatives preparations (F0) and chemical fixatives preparations (F1).

2.2.1. Method without chemical fixatives preparations.

Directly sampling using loop needle from the Casein Yeast Glucose Agar (CYGA) medium (oxoid) of *S. cerevisiae* B-18 and swab on carbon tape and then air-dried for a few minutes until dry out. The sample coating used Au by ion sputter MC1000 (Hitachi Corp.).

The fresh sample of *S. cerevisiae* B-18 for SEM evaluation, which cannot be stored even though the sample has been coated with ion sputter. This method, especially for isolate screening before used adequate methodology for SEM preparation. The maximum duration of the sample that had air-dried for about three hours; more than that duration, the resolution is not proper. The optimum duration for observation in the chamber in the high vacuum condition is about two hours.

2.2.2. Method chemical fixative preparations.

Biological sample preparation following [11-12] method was started by growing *S. cerevisiae* on CYG (casein yeast glucose) broth medium for 30 °C, 48 hours, and then the biomass of isolate was taken by

centrifuging 3000 rpm, 20 minutes. Glutaraldehyde 2.5% solution (in phosphate buffer or cacodylate buffer) was added to the isolates biomass. The isolates suspension allowed to stand for 2 hours. Each suspension was centrifuged again (3000 rpm; 20 minutes), the biomass was taken and added with a fixation solution of 2% tannic acid, allowed to stand for 2 hours. The suspension was centrifuged again, and the bacterial cell biomass is taken. Phosphate buffer or cacodylate buffer was added in isolates biomass, then allowed to stand for 10 minutes. This stage was repeated 2 times. The suspension was re-centrifuged, and bacterial cell biomass is taken. Osmium tetroxide 1% was added, allowed to stand for 1 hour. The suspension was centrifuged, cell biomass was taken and then the dehydration process was carried out with 50% alcohol, 70% alcohol, 80% alcohol, 95% alcohol and absolute alcohol. After dehydration, cell biomass was suspended in t-butanol and then dried with the freeze-drying method.

All of *S. cerevisiae* B-18 samples in this research (F0 and F1) only used one level of ion sputter i.e 10 mA with six different duration of time current exposures i.e 10 seconds (T10), 20 seconds (T20), 30 seconds (T30), 40 seconds (T40), 50 seconds (T50), and 60 seconds (T60).

2.3. Scanning Electron Microscopy (SEM) settings

The SEM instrument Hitachi SU3500 settings were accelerating voltage (Vacc) 3kV and 5kV, spot intensity 30%, working distance 6 mm, and magnification 5K and 10K.

2.4. Analysis

The analysis was carried out qualitatively based on the level of clarity for analyzing the morphological changes (to identify cell with bud and without bud) cells and resolution for quality of images which have different time of ion sputter setting condition. Quantitatively analysis was conducted for measuring the *S. cerevisiae* B-18 diameter using ImageJ software as the combined effect of sample preparation treatment (2 levels) and the coating duration time (6 levels). The effect was statistically analyzed by Analysis of variance with completely randomized factorial design and the calculation was carried out by Microsoft Excel 2010 using data analysis two factors with replication module.

3. Results and discussions

One of the biggest challenges, if we have to use SEM analysis for a biological sample, was being able to display the appropriate structural image without changing the structure during the process of analysis. If it failed, it would not be used for analysis in a biological sample. Water is an essential component of living biological samples, together with complex organic macromolecules such as proteins, lipids, and carbohydrates. The various elements that make up the cell are 59% Hydrogen (H), 24% Oxygen (O), 11% Carbon (C), 4% Nitrogen (N), 2% Others – Phosphorus (P), Sulfur (S), etc. [13-15]. A biological sample consisted mostly of water when it inserted into the SEM chamber; the water will evaporate rapidly due to the low air pressure in the SEM chamber. Morphological changes occur when observed in biological samples with cells consisting of fluid. Therefore the biological sample was prepared by freeze-drying to remove water in frozen conditions (sublimation) so as not to change the shape and morphology of the sample.

Accelerating voltage (Vacc) is essential to interpret the image of such material properly [16]. The VAcc Increased, cause more penetration into the sample. Using a high VAcc, more internal structure information is obtained, therefore fine detail on the surface structure will be lost, otherwise using low VAcc, the penetration of beam is low, but more information regarding the surface structure is obtained, so increasing the VAcc cause more penetration into the sample will occur [17] and if we want to find the surface much more detail using lower voltage [16]. The VAcc often used in SEM image analysis of biological samples varies from 5 kV until 20 kV. However, most samples can achieve various benefits from the combination of low voltage and low vacuum [18]. In this study, we try to imagine the *S. cerevisiae* B-18 with low voltage setting and high vacuum mode without any

chemical fixatives, and neither drying by freeze dryer. Treatment with chemical fixatives as positive controls.

Figure 1 showed that the results of *S. cerevisiae* B-18 observations with the setting of Au coating at the current ten mA for 10 seconds. SEM results showed that the image of the *S. cerevisiae* B-18 sample was observed from one and another. The cell wall thicknesses of “mother” cells are known to be thicker than those of the smaller “daughter” cells; because of that, it may have a different response to osmotic pressure [19]. Changes in the growth medium, external pH, growth temperature, and oxygen level will significantly affect the composition, structure, and thickness of the cell wall [20]. In figure 1, the results of the *S. cerevisiae* image show luminescence around the yeast (charging phenomenon) from both treatment (F0 and F1), the obscurity of yeast surface morphology (low resolution), and images that could not focus. The resolutions are bad for SEM images.

Another thing to note was that damage caused by electron radiation doesn't occur in yeast samples with the smallest current coating of 10 mA. Damage also doesn't happen during observation at VAcc 3 kV and 5 kV. Thermal change or chemical change happening on a sample due to electron beam irradiation was referred to as beam damage. The temperature rise of the sample due to the electron beam was dependent on number factors, including accelerating voltage, the intensity of the beam, observation area, observation time, specific heat, and heat conductivity of the sample. Biological samples were generally susceptible to heat and readily damaged thermally by the electron beam [21]. In these results, it was known that *S. cerevisiae* B-18 does not damage, this was due to the strong yeast cell wall so that it could withstand the heat generated by radiation of electrons at 3 kV and 5 kV acceleration voltages.

Biological tissues are generally good insulators (nonconductive) and result in imaging caused by charging in SEM. This charging phenomenon shows that there is a build-up of electron charges on the surface of this yeast sample. Au coating for 10 seconds (T10) was not optimal enough to reduce the charging effect. Then the coating was increased for 20 seconds (T20) and 30 seconds (T30) in figure 2 and figure 3. In figure 2 and figure 3, image clarity was improved, but charging still occurs even though there was a decrease in charging rates. However, the surface morphology of *S. cerevisiae* has not been fully visible.

The comparison between the acceleration voltage (VAcc) used was between 3kV and 5kV in figure 1, figure 2 and figure 3 is not very significant when viewed from the image quality. However, testing with a higher acceleration voltage of 5kV results in an image with a higher charging rate compared to the 3kV acceleration shown in figure 1, figure 2 and figure 3. The VAcc is needed to increase the depth penetration of electrons into the sample to get a proper resolution, but not good for observe surface structure [16-17]. On the other hand, if a nonconductive sample such as a yeast sample uses a high acceleration voltage it can cause charging to be higher so that will not a good image is obtained but will make the image even worse.

In figure 4 (T40) with coating for 40 seconds, the charging effect still occurs, but in figure 5 (T50) with a coating for 50 seconds, charging has been wholly reduced at 3kV and 5kV. The level of clarity of the image also increases.

The best result for F0 is shown in figure 6 (T60). The thick coating will increase the conductivity so that it will reduce charging at higher acceleration voltages. Because charging has been completely reduced, then the level of clarity, resolution, cleanliness of the image will increase. Figure 6 (T60) showed an obvious resolution of the *S. cerevisiae* B-18 cell morphology. When compared with the results of the acceleration voltage between 3kV and 5kV in Figure 6, an image with 5kV acceleration is of higher quality due to deeper electron penetration depth without any charging phenomenon disturbances. This setting is appropriate with [18] that for biological samples VAcc varies from 5 kV until 20 kV.

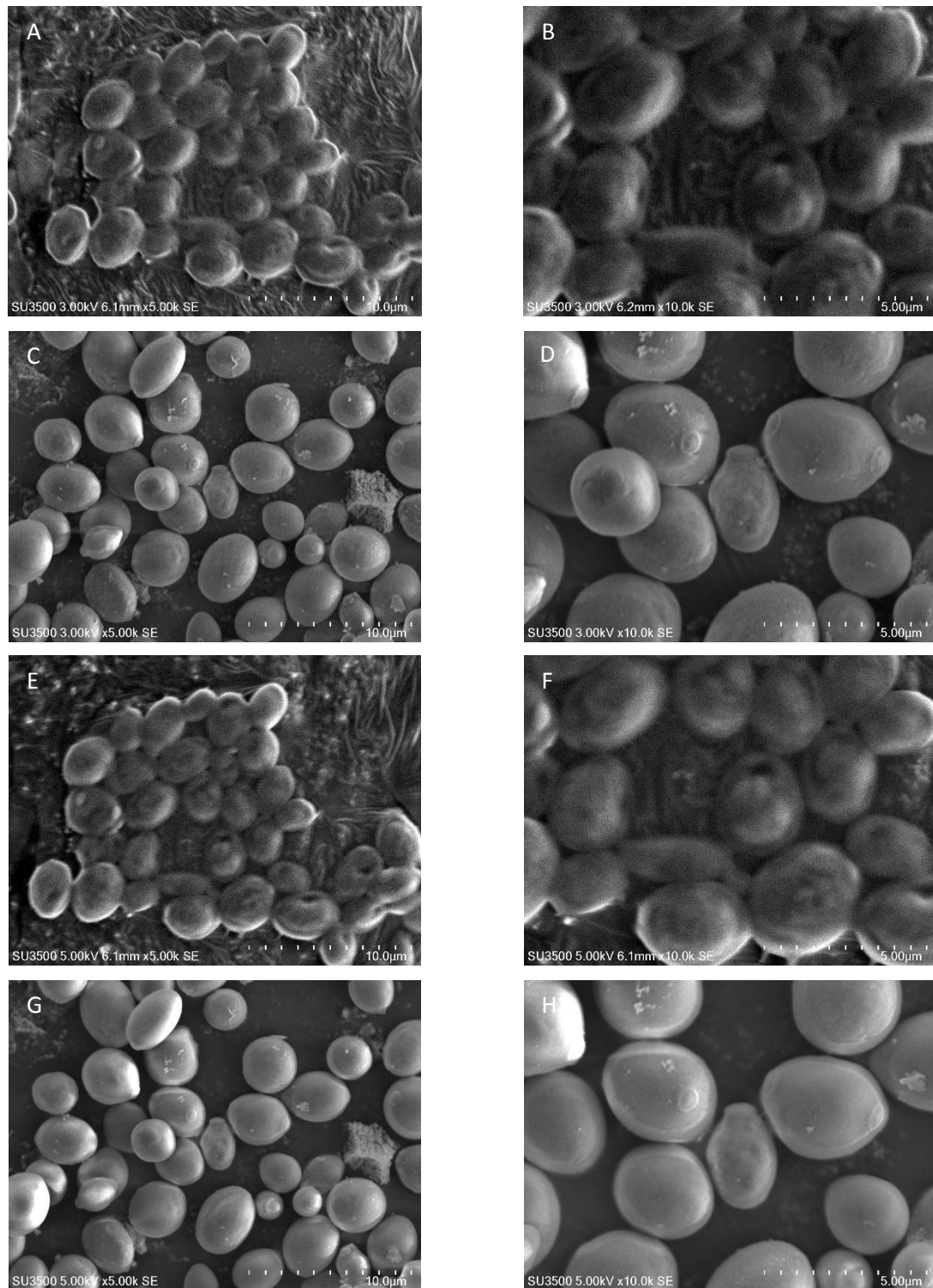


Figure 1. T10: F0 SEM image of *Saccharomyces cerevisiae* B-18 at the different accelerating voltage 3 kV (A and B) and 5 kV (E and F) and magnification, 5 K (left figure) and 10K (right figure). F1 As positive controls to compare the figures could be seen in figure C and D for accelerating voltage 3 kV and figure G and H for accelerating voltage 5 kV, 5 K (left figure) and 10K (right figure). All of the figures were shown the charging phenomenon has still occurred and the resolutions were poor.

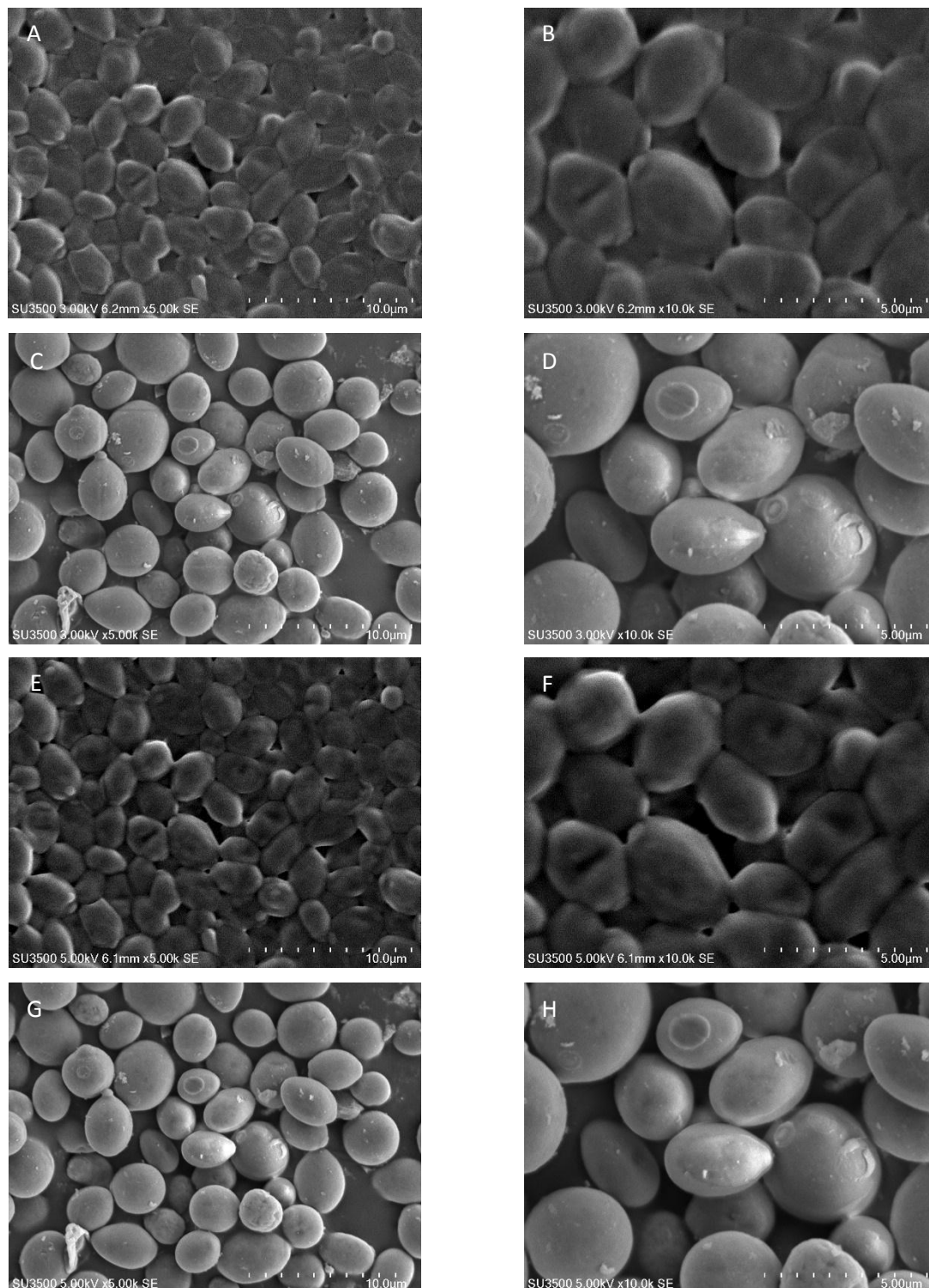


Figure 2. T20: F0 SEM image of *Saccharomyces cerevisiae* B-18 at the different accelerating voltage 3 kV (A and B) and 5 kV (E and F) and magnification, 5 K (left figure) and 10K (right figure). F1 As positive controls to compare the figures could be seen in figure C and D for accelerating voltage 3 kV and figure G and H for accelerating voltage 5 kV, 5 K (left figure) and 10K (right figure). All of the figures were shown the charging phenomenon has still occurred and the resolutions were poor.

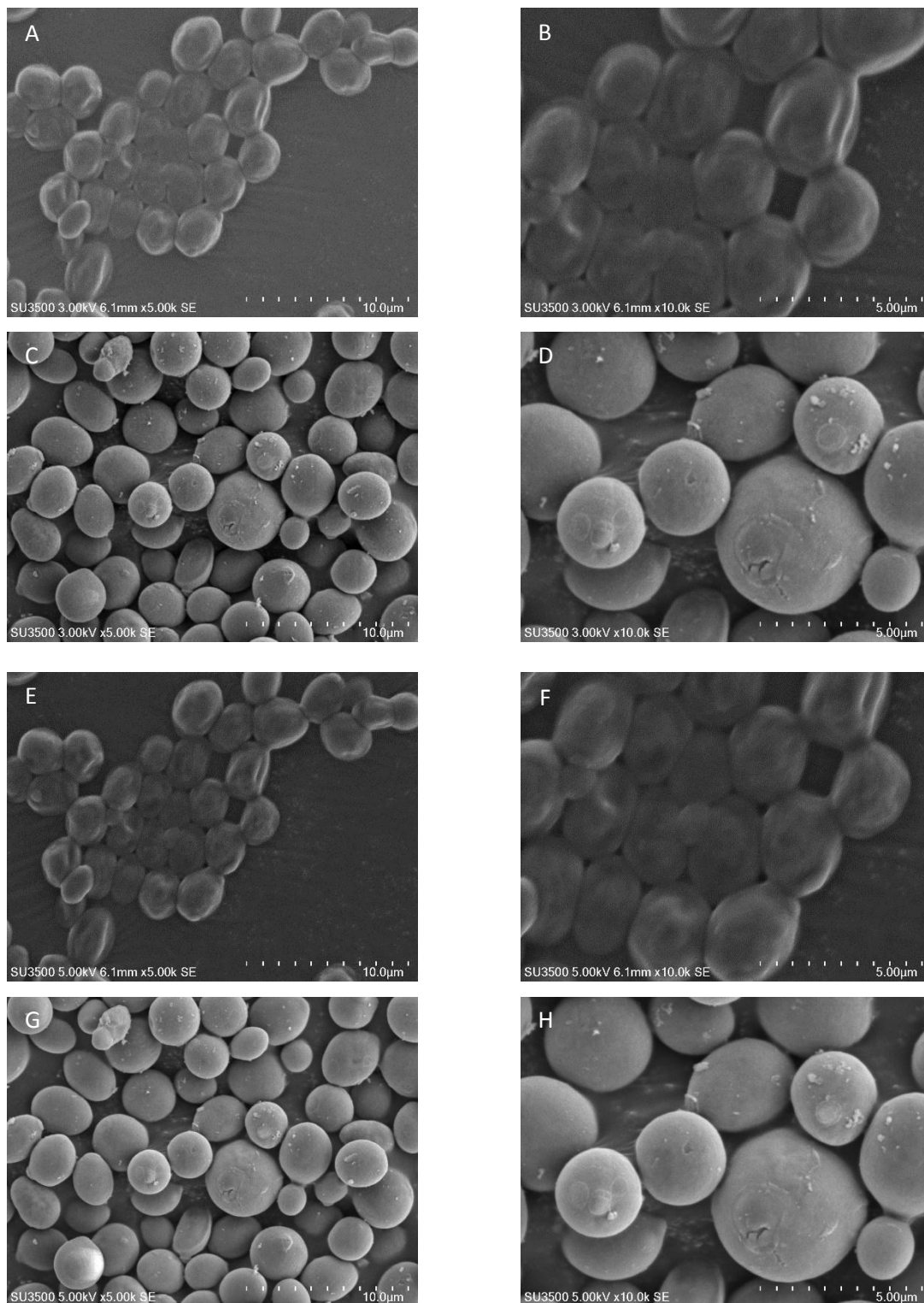


Figure 3. T30: F0 SEM image of *Saccharomyces cerevisiae* B-18 at the different accelerating voltage 3 kV (A and B) and 5 kV (E and F) and magnification, 5 K (left figure) and 10K (right figure). F1 As positive controls to compare the figures could be seen in figure C and D for accelerating voltage 3 kV and figure G and H for accelerating voltage 5 kV, 5 K (left figure) and 10K (right figure). Figure G was shown the slight charging effect, but the resolution is better.

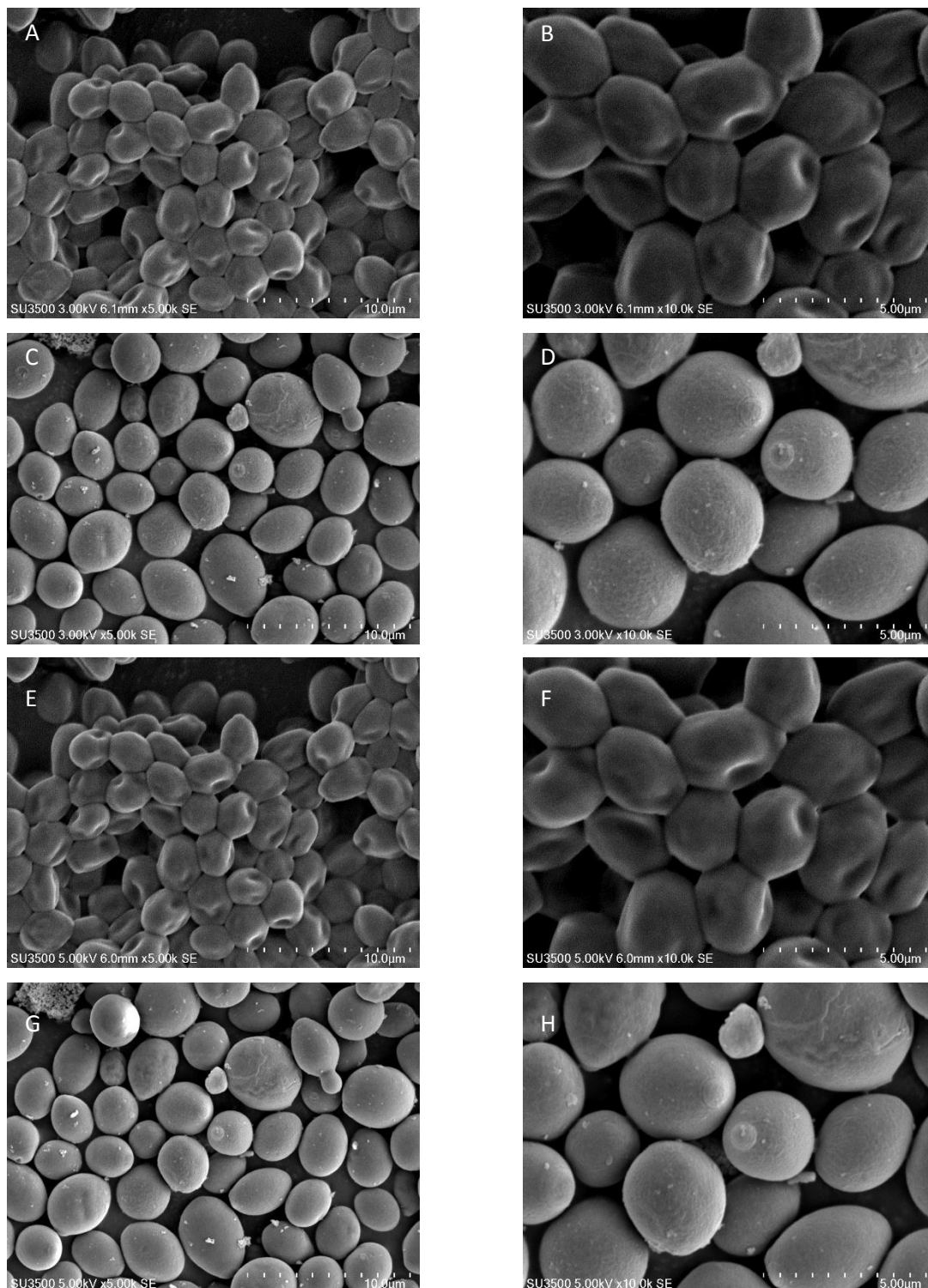


Figure 4. T40: F0 SEM image of *Saccharomyces cerevisiae* B-18 at the different accelerating voltage 3 kV (A and B) and 5 kV (E and F) and magnification, 5 K (left figure) and 10K (right figure). F1 As positive controls to compare the figures could be seen in figure C and D for accelerating voltage 3 kV and figure G and H for accelerating voltage 5 kV, 5 K (left figure) and 10K (right figure). Figure G was shown the slight charging effect, but the resolution is better. Figures A, B, E, and F were shown; the cell surface is good but not detail yet.

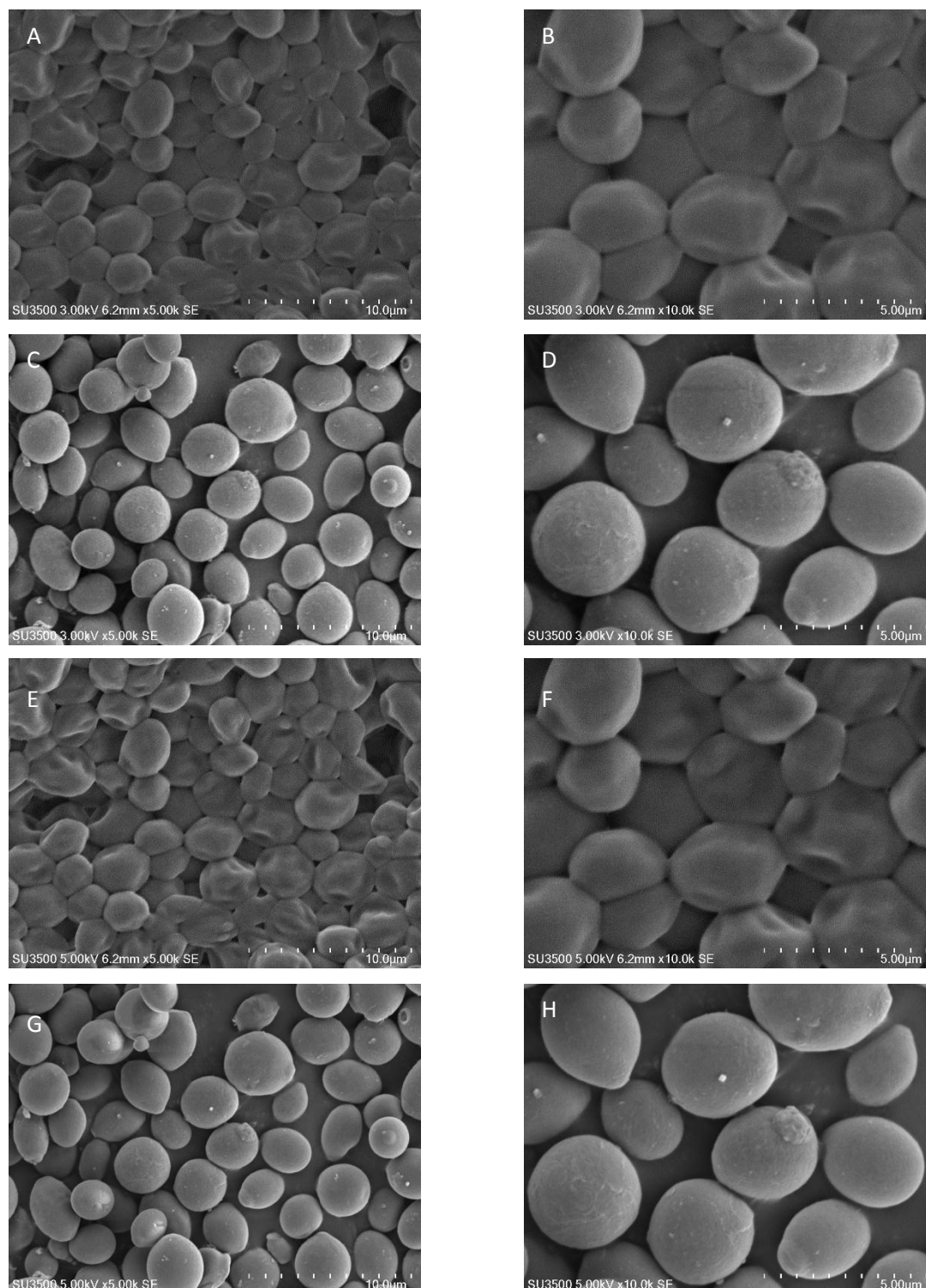


Figure 5. T50: F0 SEM image of *Saccharomyces cerevisiae* B-18 at the different accelerating voltage 3 kV (A and B) and 5 kV (E and F) and magnification, 5 K (left figure) and 10K (right figure). F1 As positive controls to compare the figures could be seen in figure C and D for accelerating voltage 3 kV and figure G and H for accelerating voltage 5 kV, 5 K (left figure) and 10K (right figure). All of the figures were shown no charging effect, and the resolutions were good. The detail of Figures A, B, E, and F starting was shown.

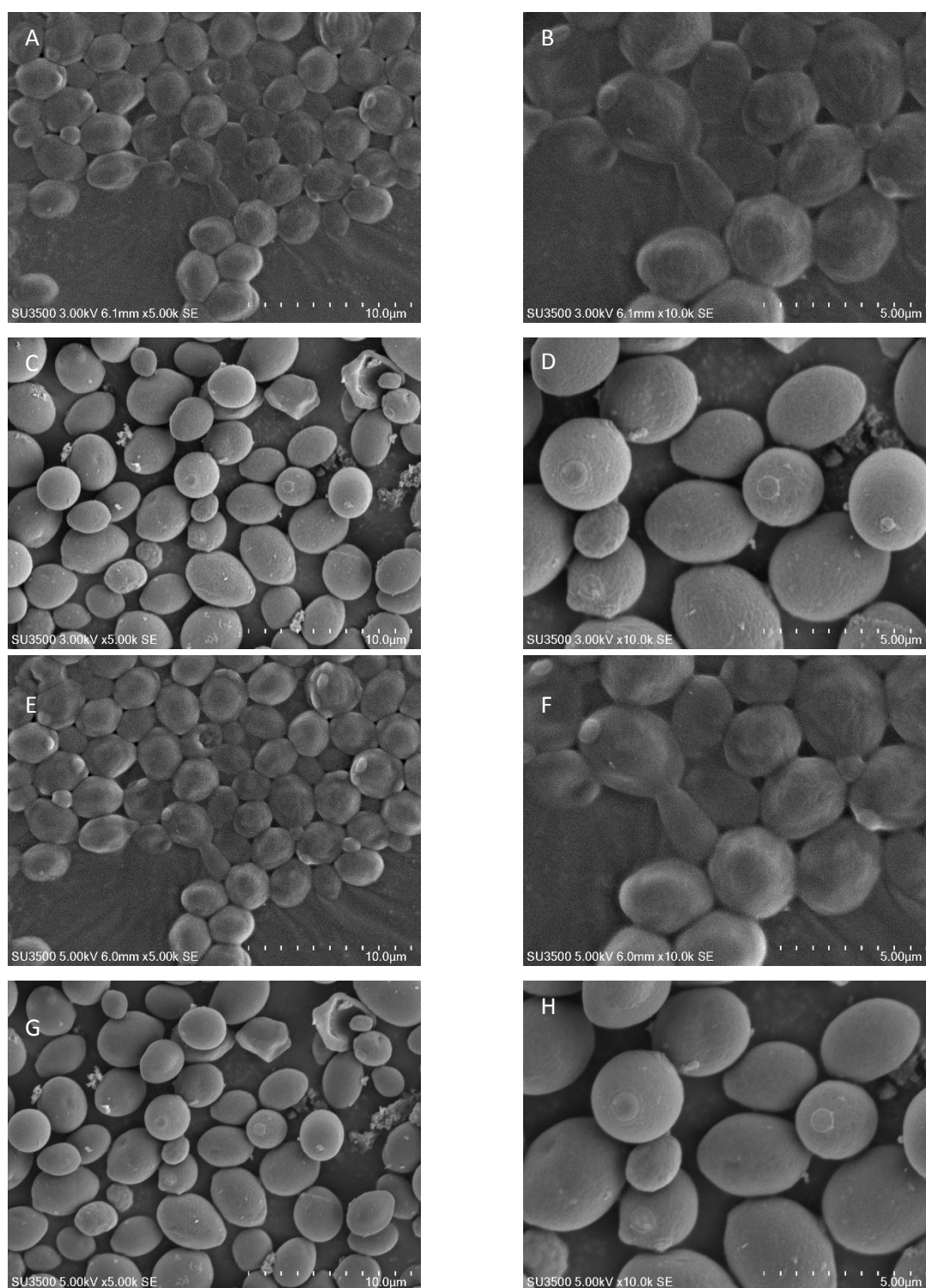


Figure 6. T60: F0 SEM image of *Saccharomyces cerevisiae* B-18 at the different accelerating voltage 3 kV (A and B) and 5 kV (E and F) and magnification, 5 K (left figure) and 10K (right figure). F1 As positive controls to compare the figures could be seen in figure C and D for accelerating voltage 3 kV and figure G and H for accelerating voltage 5 kV, 5 K (left figure) and 10K (right figure). All of the figures were shown no charging effect, and the resolutions were good. The detail of Figures A, B, E, and F was good shown.

Table 1 is shown the statistical analysis of the diameter measurement of *S. cerevisiae* B-18 was not significantly different ($P>0.05$) for variation of time ion sputter exposures (T10-T60); however, there is significantly different ($P<0.05$) between preparation variation treatment F0 and F1. F0 diameters were around 3.162-3.440 μm , whereas the F1 diameters were around 3.565-4.038 μm , the shortest different between F0 and F1 is 0.125 μm , and the longest different between F0 and F1 is 0.803. The diameters of F1 significantly different from F0; these can be due to F1 have cells outline better than F0 for deciding the point of measurement using ImageJ software.

In this study, *S. cerevisiae* B-18 has microscopic characteristics of small budding, according to [22], the type of germination consists of no, small, medium, and large type of budding. The microscopic morphology of *S. cerevisiae* in the form of blastopores that are oval, cylindrical, or ovoid short and long affected by its strain. The SEM images qualitatively could be seen that T40, T50, and T60 have better resolution than T10, T20, and T30 (for F0). For F1 the resolution of T50 and T60 was better than T10, T20, T30, and T40 with no charging phenomenon effect. The morphology of *S. cerevisiae* B-18 and the formation of “daughter” cells that called bud could be seen very good in T60 either using magnification 5K or 10 K.

Yeast cell typically has around five until ten micrometers in diameter, and the cells reproduce through a process called budding. Budding has resulted from asymmetric asexual cell division. If once a “mother” cell reaches a maximum size, it gives rise to a “daughter” cell called the bud. It showed an entirely new surface [23]. The other study stated that the critical diameter of single cells was 7,94 μm if it grows on temperature above 18,5 °C. The growth will exponentially increase up to 10,2 μm if the temperature reaches below 18,5 °C. The size of the bud linearly depends on μ_{max} , and it is between 50% at 5°C, and 90% at 31°C of an averaged single cell [24].

In this research, the average cell size was about 3.162 - 4.038 μm in diameter; the diameter is smaller than [23] and [24]. It is could be the *S. cerevisiae* B-18 cells because of blocking in aerobic respiratory chain pathway, which generates ATP, thus unable to grow on nonfermentable carbon source (such as glycerol or ethanol), and form small anaerobic-sized colonies when grown in the presence of fermentable carbon source (such as glucose), its called the yeast petite mutant, and they all produce wild type progeny when crossed with a wild-type strain [25]. Petit cell synthesis could contribute to maintain optimum structure and functions of the cell membranes and improve cell resistance to inactivation [26].

From the results of the experiments obtained that if the coating is thicker, the image quality is better. However, the negative effect of the thickening coating causes a change in the microstructure of the yeast. So that when carried out observations with high magnification, the image doesn't match the natural because the surface is stacked with thick Au coating.

If compared with another SEM results by [27] on normal cell morphology of *S. cerevisiae*, and [28] that given lyticase treatment, so that invagination of *S. cerevisiae* cells walls caused a damaged cell morphology, can be seen that this simple method (F0). This simple method can be used as a screening process to observe cell morphology with good image quality in high vacuum conditions and high magnification by using SEM analysis. The best result of without chemical preparation (F0) and with chemical fixatives (F1) were T60.

This simple preparation method proved that the *S. cerevisiae* B-18 cells were strong enough in high pressure and high magnification in the SEM chamber only through a coating process without any chemical fixatives preparation process, which is marked by no invagination of the cells. This preparation could also help observe the morphology of *S. cerevisiae* B-18 even the budding process can also be seen well, by using this method we can replace observations under a light microscope that has limitation of magnification (1000x), by using SEM with better results without going through a long preparation process by staining cells with Giemsa as was done by [29] to observe the budding period between the “mother” cell and its “daughter”.

Table 1. Diameter measurement of *S. cerevisiae* B-18 by SEM using different treatment preparation method and different current ion sputtering duration of exposure of the gold (Au) coating

Ion Sputter Setting	Treatments		Mean different (μm)	P		
	F0 (μm) ^b	F1 (μm) ^b		Ion Sputter Setting	Treatment	Ion Sputter Setting x Treatment
T10 ^a	3.319±0.380	3.890±0.312	0.571	NS ^c	* ^d	NS ^c
T20 ^a	3.351±0.379	4.038±0.516	0.687			
T30 ^a	3.162±0.184	3.682±0.248	0.520			
T40 ^a	3.175±0.388	3.978±0.624	0.803			
T50 ^a	3.393±0.408	3.763±0.348	0.370			
T60 ^a	3.440±0.047	3.565±0.404	0.125			

^a T10, 20, 30, 40, 50, and 60 = Time duration of ion sputter current exposure for 10, 20, 30, 40, 50, and 60 seconds.

^b Preparation *S. cerevisiae* B-18 for SEM analysis i.e without (F0), and with chemical preparation (F1)

^c NS P> 0.05

^d * P<0.05

4. Conclusion

The method described in this paper is so simple, fast without any expertise, the minimum skill only how to take the sample from the CYGA media. This method can be used for screening, routine identification, and for known the budding cells of *Saccharomyces cerevisiae* B-18 using a scanning electron microscope with high-pressure vacuum mode without disturbing their cell morphology. We have suggestions for F0; the first is the maximum duration of the sample that had air-dried is about three hours because more than its period, the resolution would be not proper. The optimum period for observation in the chamber in the high vacuum condition is about two hours; the second to developing methods so that representation for cell diameter measurements can be even better than this study.

Acknowledgment

Authors would like to thanks Hendra Herdian for statistical support, Madina Nurohmah, for technical support in this study and Research Division for Natural Product Technology (BPTBA), Indonesian Institute of Sciences (LIPI) for supportive SEM instrumentation.

References

- [1] Hariono B 2009 *Mikroskopi Elektron* (Indonesia, Yogyakarta: Kanisius Press)
- [2] Prasetyo E, Jatmiko T H, Karimy M F 2018 *IOP Conf Ser: Earth Environ.Sci.* **251** 012010
- [3] Liu J 2000 *Mater. Charact* **44** 353–363
- [4] Endo A, Yamada M, Kataoka S, Sano T, Inagi Y, Miyaki A 2010 *Colloid Surf. A* **357** 11-16
- [5] Yamada M, Yoshihara T, Arima H, Kobayashi T 1997 *J. Electron Microsc* **46** 311-314
- [6] Stevenskalceff M 2003 *Plant Breeding* **130** 569-573
- [7] Weakley B S 1977 *J Microscopy* **109** 249-251
- [8] Kumar V, Bharti A, Gusain O, Bisht G S 2011 *Scanning* **33** 446-449
- [9] Belin J M 1972 *J. Antonie van Leeuwenhoek* **38** 341-349
- [10] Istiqomah L, Anwar M, Anggraeni A S, Damayanti, E 2018 *J. Indon. Trop. Anim. Agric.* **43** 149-158
- [11] Goldstein J I, Newbury D E, Echlin P, Joy D C, Romic Jr A D, Lyman C E, Fiori C, and Lifshin E 1992 *Scanning Electron Microscopy and X-ray Microanalysis: A Text for Biologist, Materials Scientist, and Geologist* 2nd ed (USA, New York: Plenum) 820 p
- [12] Nurhayati R dan Dianing E 2018 *konferensi nasional hasil penelitian pangan dan pertanian* 369-375

- [13] Kashi A M, Tahermanesh K, Chaichian S, Joghataei M T, Moradi F, Tavangar S M, Najafabadi A S M, Lotfibakhshaiesh N, Pour S, Beyranvand, Yazdi A F A, Abed S M 2014 *Galen Medical Journal*. **3** 63-80
- [14] Echlin P. 2009 *Handbook of Sample Preparation for Scanning Electron Microscopy and X-Ray Microanalysis* (UK, Cambridge: Springer)
- [15] Alberts A, Johnson J, Lewis J, Raff M, Roberts k, Walter P 2007 *Molecular Biology of the Cell*. 5th Ed (USA, New York: Garland Science)
- [16] Stowe S, Parirokh M, Asgary S, Eghbal M J 2004 *Aust Endod J* **30** 5-10
- [17] Pretorius E 2010 *Microscopy Research and Technique* **73** 225-228
- [18] Oho E, Asai N, Itoh S 2000 *J Electron Microsc* **49** 761-763
- [19] Stenson J D 2008 A thesis The University of Birmingham. pp 192-199
- [20] Uscanga A and Francois B J M 2003. *Letter in Applied Microb*. **37** 268-274
- [21] Holmes J L, Bachus K N and Bloebaum R D 2000 *SCANNING*. **22** 243–248
- [22] Saito T L, Ohtani M, Sawai H, Sano F, Saka A, Watanabe D, Yukawa M, Ohya Y, and Morishita S 2004 SCMD: *Saccharomyces cerevisiae* Morphological Database. *Nucleic Acids Research* **32** 319-322.
- [23] Nguyen K, Murray S, Lewis J A, Kumar P 2017 Morphology, cell division, and viability of *Saccharomyces cerevisiae* at high hydrostatic pressure. arXiv preprint arXiv:1703.00547. 1-19
- [24] Zakhartsev M and Reuss M 2018 *Yeast Ressearch* **18** 1–16
- [25] Day M 2013 *Advance in Applied Microbiology Chapter one* **85** 1-41 ISSN 0065-2164 <http://dx.doi.org/10.1016/B978-0-12-407672-3.00001-0>
- [26] Niu L, Nomura K, Iwahashi H, Matsuoka H, Kawachi S, Suzuki Y, Tamura K 2017 *Biophysical Chemistry* 231 79-86
- [27] Coluccio A and Neiman A M 2004 *Microbiol*. **150** 3189-3196
- [28] Tang S-Y, Zhang W, Soffe R, Nahavandi S, Shukla R, Khoshmanesh K 2014 *PLoS ONE* **9** 1-9 e104109 doi: 10.1371/journal.pone.0104109
- [29] Hashimoto T Conti S F and Naylor H B 1959 *J. Bacteriol*. **77** 344–354