Journal of Bio-Molecule Research and Engineering
Vol. 2 | No. 2 |1 – 7 | 2023
e-ISSN: 2962-343X
http://www.e-journal.unair.ac.id/biome

Studies of Yeasts Isolated from Soil as Cellulose Decomposers and Phosphate Solvents

Gading Gunawan Putra[2], Syarifah Salsabila[2], Hanif Nur Rahmawati[2] and Fatimah[1,2*]

[1]University Center of Excellence-Research Center for Biomolecule Engineering, Universitas Airlangga, Surabaya 60115, Indonesia
[2]Department of Biology, Faculty of Science and Technology, Univesrsitas Airlangga, Surabaya 60115, Indonesia

**E-mail address*: fatimah@fst.unair.ac.id

ARTICLE INFO

Article history
Received 10th Sep 2023
Accepted 15th Nov 2023

Keywords:
Candida
cellulose
CMCase
PMEase
phosphate
Rhososporidium

ABSTRACT

The identification of CMCase (Carboxymethylcellulase) and PMEase (Phosphomonoesterase) enzyme activities from yeast genera that produce cellulase and phosphatase is crucial for identifying potential genera that could aid in the development of biofertilizers, serving as an environmentally friendly alternative to chemical fertilizers. This study is based on a review of articles and journals for data collection. The review revealed that the yeast genus *Rhodosporidium* (specifically *Rhodosporidium paludigenum* Y08RA29) is a promising cellulolytic yeast, with CMCase activity approaching 0.500 units. Meanwhile, the potential phosphate-solubilizing yeast genus is *Candida* (*Candida* sp. 3), with PMEase activity ranging from 0.05 to 0.06 units.

1. Introduction

The rhizosphere is the soil layer surrounding the plant root surface that is influenced by root activity [1]. This zone has favorable conditions for the growth of soil microorganisms [2]. The rhizosphere is rich in exudates that contain carbohydrates, amino acids, organic acids, enzymes, and other compounds. These exudates are secreted by plants through root secretion processes [3, 4]. Yeasts are capable of utilizing these exudates for decomposition processes [5]

Yeasts are unicellular, eukaryotic microorganisms classified under fungi, and are found in a wide range of environments such as aquatic, terrestrial, and atmospheric habitats [6]. Yeasts exhibit high resistance to antibiotics, as well as tolerance to salts, acids, and sugars [7, 8]. They also have antimicrobial properties, enabling them to inhibit the growth of other microorganisms such as bacteria and molds [9]. Yeasts are known to produce cellulase enzymes, which break the beta-1,4 glycosidic bonds in cellulose and cellulose derivatives [10].

The production of enzymes by microorganisms in the soil can also enhance the availability of phosphates. Phosphate solubilization can occur through biological or chemical processes, affecting both organic and inorganic phosphates [11]. The conversion of insoluble phosphates into soluble forms in the soil primarily involves the formation of organic and inorganic acids produced by phosphate-solubilizing microorganisms. The chemical dissolution of inorganic phosphate occurs due to the presence of hydroxyl acids that act as chelating agents, forming stable complexes with ions such as Ca^{2+}, Fe^{2+}, Al^{3+}, and Mg^{2+}, thereby converting calcium-bound phosphates into free phosphate forms (monovalent or divalent), which are available to plants. The solubilization of organic phosphate compounds by phosphate-solubilizing

microorganisms involves enzymatic hydrolysis. The enzymes capable of releasing phosphate from organic substrates to a form accessible to plants are collectively known as phosphatases.

Identifying the activities of CMCase (Carboxymethyl Cellulase) and PMEase (Phosphomonoesterase) enzymes from yeast genera that produce cellulase and phosphatase is crucial to determining which genera are most promising for biofertilizer formulation. These biofertilizers could serve as eco-friendly alternatives to chemical fertilizers. The aim of this review is to identify the most promising cellulolytic yeast genera and phosphate-solubilizing yeast genera, as well as to assess the potential of yeasts based on their CMCase and PMEase enzyme activities.

2. Celulolytic Microogranisms

Organic compounds such as carbohydrates, cellulose, pectin, proteins, fats, and organic acids in the soil originate from plants and animals that have been degraded by soil microorganisms [12]. Cellulose is a polymer of glucose that forms a linear chain, with glucose units linked by β-1,4-glycosidic bonds. This linear structure makes cellulose crystalline in nature and insoluble. The chemical and mechanical degradation of cellulose is not an easy process. In nature, cellulose is rarely found in an almost pure state, usually combined with other polysaccharides (called hemicellulose) [13]. Cellulose, hemicellulose and lignin are the main constituents of plant cell waals and important components of natural lignocellulosic materials [14].

Three key enzymes are involved in cellulose degradation: endo-1,4-β-glucanase, exo-1,4-β-glucanase, and β-glucosidase [15]. The internal β-1,4-D-glycosidic bonds of cellulose are randomly cleaved by endo-1,4-β-glucanase in the amorphous regions of the cellulose substrate, producing oligosaccharides and extending the chain ends [16]. The crystalline cellulose chains are then hydrolyzed by exo-1,4-β-glucanase, which cleaves the chain ends, releasing reducing sugars and cellobiose that subsequently hydrolyzed by β-glucosidase to release glucose [17]. Microorganisms capable of hydrolyzing cellulose are referred to as cellulose-degrading microorganisms or cellulolytic microorganisms.

3. Carboxymethil Cellulose (CMCase) Activity

The activity of Carboxymethyl Cellulose (CMCase) enzyme is determined by measuring the amount of glucose released from carboxymethyl cellulose sodium salt (CMC-Na) using the Dinitrosalicylic acid method with glucose as the standard [18]. According to a study conducted in Raja Ampat [19], six strains were identified that were capable of forming a clear zone around their growing colonies (Table 1). The clear zone formation occurs because Congo red cannot bind to reducing sugar forms during CMC hydrolysis, while orange coloration indicates CMC hydrolysis.

The cellulolytic potential of microorganisms in secreting cellulase enzymes can be assessed by testing the cellulolytic index, which is based on the clear zone formed around colonies growing on Carboxymethyl Cellulose (CMC) medium [20]. The cellulolytic index (IS) can be calculated using the formula [21].

$$\text{IS} = \frac{\text{Average of Clear Zone Diameter (mm)}}{\text{Average of Colony Diameter (mm)}}$$

The strains Sporobolomyces poonsookiae (Y08RA07), Rhodosporidium paludigenum (Y08RA29), and Cryptococcus flavescens (Y08RA33) produced the largest clear zones and exhibited the highest cellulolytic index (Table 1) [21]. Among the six yeast strains tested for their ability to hydrolyze CMC, three strains demonstrated the highest CMCase enzyme activity (with enzyme activity values ≥ 0.400 units after 7 days of growth on CMC medium), namely

Sporobolomyces poonsookiae (Y08RA07), Rhodosporidium paludigenum (Y08RA29), and Cryptococcus flavescens (Y08RA33). However, Rhodosporidium paludigenum (Y08RA29) exhibited the highest CMCase enzyme activity, with a value close to 0.500 units, compared to the other strains.

Table 1. Yeast Cellulolytic Index

Species	Average of Clear Zone Diameter (mm)	Average of Colony Diameter (mm)	Cellulolytic Index
Barnettozyma california (Y08RA04)	3.0	2.5	1.20
Sporobolomyces poonsookiae (Y08RA07)	7.0	5.0	1.40
Cryptococcus bestiolae (Y08RA13)	4.0	3.0	1.33
Candida sonorensis (Y08RA23)	6.0	5.0	1.2
Rhodosporidium paludigenum (Y08RA29)	13.0	5.0	2.0
Cryptococcus flavescens (Y08RA33)	8.3	5.0	1.66

4. Phosphate-solubilizing Microorganisms

Phosphorus is an essential element due to its important role in root development and photosynthesis. In Ultisol, Alfisol, Vertisol, and Oxisol soils, phosphorus content is generally less than 0.02% [22]. In general, the availability of phosphorus in the soil rarely exceeds 0.01%, indicating significant variation in phosphorus content across different soil types [23]. According to Isgitani (2005), only about 15-20% of the applied phosphate fertilizer is absorbed by plants, while the remaining 80-85% of phosphorus is adsorbed onto soil colloids.

Phosphorus in the soil exists in both inorganic and organic forms. In its inorganic form, phosphorus is typically found as Al-phosphate, Fe-phosphate, and Ca-phosphate compounds. Organic phosphorus consists of compounds derived from plants and microbes. These organic phosphorus compounds include nucleic acids, phospholipids, and phytic acid [25] . Organic matter from decaying plant debris is rich in organic phosphorus sources. Phosphorus is an integral component in plants for energy storage and transfer [26].

Phosphate solubilization can occur through both biological and chemical processes, affecting both organic and inorganic phosphates [27]. The primary mechanism for converting insoluble phosphate into soluble forms in the soil involves the production of organic and inorganic acids by phosphate-solubilizing microorganisms [28]. Chemical solubilization of inorganic phosphate occurs through the action of hydroxyl acids that chelate metal ions, forming stable complexes with Ca^{2+}, Fe^{2+}, Al^{3+}, and Mg^{2+}. This process releases calcium-bound phosphate, converting it into free phosphate ions (monovalent or divalent) that are available to plants [29] .

Phosphate-solubilizing microbes are those microorganisms capable of converting insoluble phosphate compounds into soluble forms [30]. These microorganisms facilitate the mineralization of organic phosphorus compounds by breaking them down into inorganic phosphate through the decomposition of organic matter from dead plants and animals. Dissolved inorganic phosphate in groundwater or seawater can precipitate as sediments in coral reefs and fossils. Over time, phosphate from these sediments is released and dissolved back into the groundwater and seawater, where it is again absorbed by plant roots, completing a cycle [31]. The solubilization of organic phosphate compounds by phosphate-solubilizing microorganisms is an enzymatic hydrolysis reaction. The enzymes responsible for releasing

phosphate from organic substrates into a form available for plant uptake are collectively known as phosphatases.

5. Phosphomonoesterase (PMEase) Activity

The activity of the enzyme Phosphomonoesterase (PMEase) can be determined through several factors, such as the amount of enzyme produced and its close association with pH regulation. This highlights the role of microorganisms in the phosphorus nutrient cycle within the soil [32]. Soil phosphatases are classified into two main groups based on the optimum pH for phosphate mineralization reactions: acid phosphatases, which are most effective in catalyzing the mineralization of organic phosphates at pH levels below neutral, and alkaline phosphatases, which function optimally at pH levels above neutral [32].

Djuniwati and Prulunggono (2012) reported that acid phosphatases are more dominant compared to alkaline phosphatases in acidic soil environments. A study by Kanti (2006), conducted in the Biological Garden of Papua, identified three isolates out of 20 that were capable of solubilizing calcium phosphate ($Ca_3(PO_4)_2$), forming a clear zone (Table 2). All three isolates were from the same genus, *Candida*. The three *Candida* isolates (*Candida* 1, *Candida* 2, and *Candida* 3) exhibited similar pH-regulating capabilities, as all three lowered the pH to levels between pH 4 and pH 5.

Regarding the activity of the PMEase enzyme during the cultivation period, by day 10, *Candida* isolate (3) showed the highest PMEase activity, with enzyme activity values ranging from 0.05 to 0.06 units. In contrast, *Candida* isolate (1) and *Candida* isolate (2) exhibited lower PMEase activities, with values of 0.01 unit and 0.03 unit, respectively.

Table 2. Calcium Phosphate ($Ca_3(PO_4)_2$)-solubilizing isolate with clear zone

Isolate (Genus)	Clear Zone
Candida (1)	+
Candida (2)	+
Candida (3)	+
Candida (4)	-
Candida (5)	-
Candida (6)	-
Candida (7)	-
Candida (8)	-
Candida (9)	-
Candida (10)	-
Candida (11)	-
Candida (12)	-
Candida (13)	-
Candida (14)	-
Rhodotorula (1)	-
Rhohotorula (2)	-
Rhodotorula (3)	-
Cryptococcus (1)	-
Cryptococcus (2)	-
Debaryomyces (1)	-

6. Conclusion

Based on the journal review, it can be concluded that the yeast genus with the highest cellulolytic potential is *Rhodosporidium* (Rhodosporidium paludigenum (Y08RA29)), while the yeast genus with the highest phosphate-solubilizing potential is *Candida* (*Candida* (3)). The most potent cellulolytic yeast genus, *Rhodosporidium* (*Rhodosporidium paludigenum* (Y08RA29)), exhibited a Carboxymethyl Cellulase (CMCase) enzyme activity value approaching 0.500 units. The most potent phosphate-solubilizing yeast genus, *Candida* (*Candida* (3)), demonstrated Phosphomonoesterase (PMEase) enzyme activity values ranging from 0.05 units to 0.06 units.

References

[1] W. Solomon, T. Janda, and Z. Molnar, "Unveiling the significance of rhizosphere: Implications for plant growth, stress response, and sustainable agriculture," *Plant Physiol Biochem*, vol. 206, p. 108290, Jan 2024, doi: 10.1016/j.plaphy.2023.108290.

[2] S. Hakim *et al.*, "Rhizosphere Engineering With Plant Growth-Promoting Microorganisms for Agriculture and Ecological Sustainability," (in English), *Frontiers in Sustainable Food Systems*, vol. 5, p. 617157, Feb 15 2021, doi: ARTN 61715710.3389/fsufs.2021.617157.

[3] S. K. Upadhyay *et al.*, "Root Exudates: Mechanistic Insight of Plant Growth Promoting Rhizobacteria for Sustainable Crop Production," *Front Microbiol*, vol. 13, p. 916488, 2022, doi: 10.3389/fmicb.2022.916488.

[4] S. S. Zhu, J. M. Vivanco, and D. K. Manter, "Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize," (in English), *Applied Soil Ecology*, vol. 107, pp. 324-333, Nov 2016, doi: 10.1016/j.apsoil.2016.07.009.

[5] K. A. Nimsi, K. Manjusha, A. A. M. Hatha, and K. Kathiresan, "Diversity, distribution, and bioprospecting potentials of manglicolous yeasts: a review," *FEMS Microbiology Ecology*, vol. 99, no. 5, p. fiad044, 2023.

[6] K. Kandasamy, N. M. Alikunhi, and M. Subramanian, "Yeasts in marine and estuarine environments," *J Yeast Fungal Res*, vol. 3, no. 6, pp. 74-82, 2012.

[7] M. Fakruddin, M. N. Hossain, and M. M. Ahmed, "Antimicrobial and antioxidant activities of Saccharomyces cerevisiae IFST062013, a potential probiotic," *BMC Complementary and Alternative Medicine*, vol. 17, pp. 1-11, 2017.

[8] A. Sharma and S. C. Sharma, "Physiological basis for the tolerance of yeast Zygosaccharomyces bisporus to salt stress," *HAYATI Journal of Biosciences*, vol. 24, no. 4, pp. 176-181, 2017.

[9] O. S. Defi and R. Anna, "Potential of yeast at reducing uranium concentration in organic waste TBP-Kerosene contain uranium," 2011.

[10] M. Sohail *et al.*, "Cellulolytic and Xylanolytic Enzymes from Yeasts: Properties and Industrial Applications," *Molecules*, vol. 27, no. 12, p. 3783, Jun 12 2022, doi: 10.3390/molecules27123783.

[11] D. Thakur, R. Kaushal, and V. Shyam, "Phosphate solubilising microorganisms: role in phosphorus nutrition of crop plants-a review," *Agricultural Reviews*, vol. 35, no. 3, pp. 159-171, 2014.

[12] N. S. S. Rao, *Mikroorganisme tanah dan pertumbuhan*. Universitas Indonesia, 2010.

[13] S. Gautam, P. Bundela, A. Pandey, J. Jamaluddin, M. Awasthi, and S. Sarsaiya, "A review on systematic study of cellulose," *Journal of Applied and Natural Science*, vol. 2, no. 2, p. 330, 2010.

[14] H. Chen and H. Chen, "Chemical composition and structure of natural lignocellulose," *Biotechnology of lignocellulose: Theory and practice*, pp. 25-71, 2014.

[15] F. L. Soares Junior *et al.*, "Endo- and exoglucanase activities in bacteria from mangrove sediment," *Braz J Microbiol,* vol. 44, no. 3, pp. 969-76, 2013, doi: 10.1590/s1517-83822013000300048.
[16] T. Perrot, M. Pauly, and V. Ramirez, "Emerging Roles of beta-Glucanases in Plant Development and Adaptative Responses," *Plants (Basel),* vol. 11, no. 9, p. 1119, Apr 20 2022, doi: 10.3390/plants11091119.
[17] R. Datta, "Enzymatic degradation of cellulose in soil: A review," *Heliyon,* vol. 10, no. 1, p. e24022, Jan 15 2024, doi: 10.1016/j.heliyon.2024.e24022.
[18] X. H. Li *et al.*, "Enhanced cellulase production of the Trichoderma viride mutated by microwave and ultraviolet," *Microbiol Res,* vol. 165, no. 3, pp. 190-8, Mar 31 2010, doi: 10.1016/j.micres.2009.04.001.
[19] A. Kanti, N. Sukarno, E. Sukara, and L. K. Darusman, "Cellulolytic Yeast Isolated From Raja Ampat Indonesia," in *Annales Bogorienses*, 2012, vol. 16, no. 1, pp. 27-34.
[20] D. Zahidah and M. Shovitri, "Isolasi, karakterisasi dan potensi bakteri aerob sebagai pendegradasi limbah organik," *Jurnal sains dan seni ITS,* vol. 2, no. 1, pp. E12-E15, 2013.
[21] S. A. Yogyaswari, M. I. Rukmi, and B. Raharjo, "Ekplorasi bakteri selulolitik dari cairan rumen sapi Peranakan Fries Holland (PFH) dan Limousine Peranakan Ongole (LIMPO)," *Jurnal Akademika Biologi,* vol. 5, no. 4, pp. 70-80, 2016.
[22] R. Sánchez-Clemente, M. I. Igeño, A. G. Población, M. I. Guijo, F. Merchán, and R. Blasco, "Study of pH changes in media during bacterial growth of several environmental strains," in *Proceedings*, 2018, vol. 2, no. 20: MDPI, p. 1297.
[23] R. Simanungkalit, D. A. Suriadikarta, R. Saraswati, D. Setyorini, and W. Hartatik, "Pupuk organik dan pupuk hayati," *Balai Besar Penelitian dan Pengembangan Sumberdaya Lahan Pertanian. Bogor,* vol. 312, 2006.
[24] M. Isgitani, S. Kabirun, and S. Siradz, "Pengaruh Inokulasi Bakteri Pelarut Fosfat TerhadapPertumbuhan Sorghum Pada Berbagai Kandungan P Tanah," *Jurnal Ilmu Tanah dan Lingkungan,* vol. 5, no. 2005, 2005.
[25] B. Raharjo, "Pelarutan fosfat anorganik oleh kultur campur jamur pelarut fosfat secara in vitro," *Jurnal Sains dan Matematika,* vol. 15, no. 2, pp. 45-54, 2007.
[26] S. Zhang, N. Merino, A. Okamoto, and P. Gedalanga, "Interkingdom microbial consortia mechanisms to guide biotechnological applications," *Microb Biotechnol,* vol. 11, no. 5, pp. 833-847, Sep 2018, doi: 10.1111/1751-7915.13300.
[27] W. Elhaissoufi, C. Ghoulam, A. Barakat, Y. Zeroual, and A. Bargaz, "Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity," *J Adv Res,* vol. 38, pp. 13-28, May 2022, doi: 10.1016/j.jare.2021.08.014.
[28] N. Prabhu, S. Borkar, and S. Garg, "Phosphate solubilization by microorganisms: overview, mechanisms, applications and advances," *Advances in Biological Science Research: A Practical Approach*, pp. 161-176, 2019, doi: 10.1016/B978-0-12-817497-5.00011-2.
[29] E. T. Alori, B. R. Glick, and O. O. Babalola, "Microbial phosphorus solubilization and its potential for use in sustainable agriculture," *Frontiers in microbiology,* vol. 8, p. 971, 2017.
[30] R. A. Raju and M. N. Reddy, "Effect of rock phosphate amended with phosphate solubilizing bacteria and farmyard manure in wetland rice (*Oryza sativa*)," *Indian Journal of Agricultural Sciences,* vol. 69, no. 6, pp. 451-453, Jun 1999.
[31] S. L. Tisdale, W. L. Nelson, and J. D. Beaton, *Soil fertility and fertilizers*. 1985.
[32] A. Kanti, "Candida sp. yeast solubilizing phosphate isolated from soil in Wamena Biological Garden, Papua," *Biodiversitas Journal of Biological Diversity,* vol. 7, no. 2, 2006.

[33] S. Djuniwati and H. B. Pulunggono, "Pengaruh Pemberian Bahan Organik *(Centrosema pubescens)* dan Fosfat Alam terhadap Aktivitas Fosfatase dan Fraksi P Tanah Latosol di darmaga, Bogor," 2012.