Eco. Env. & Cons. 26 (June Suppl. Issue) : 2020; pp. (S51-S56)
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ISSN 0971–765X

The potential of yeast isolated from agricultural soil in atrazine degradation

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(Received 27 September, 2019; Accepted 10 March, 2020)

ABSTRACT

Atrazine herbicides are the most widely used pesticides in the world and are persistent on the ground. This makes atrazine an environmental concern because it can pollute the soil and water. Yeast as a degrading agent has many advantages because of its tolerance to extreme conditions and has a good response to toxic compounds. The purpose of this study was to determine the ability of yeast isolates to degrade atrazine. The isolates used were TB1, TB2, TB3, TB4, TB5, TB6, TB7, TB8, TB9, TB10, TB11, TB12, TB13,TB14, TB15, TB16, JA1, JA2, JA3, JA4, JA5, UF1, UF3, UF5, UF7, UF8, SS4, and SSU6. Isolates were screened through clear zone measurements until the best three isolates were obtained, that are TB1, UF5, and UF7. The three isolates and control were cultured in liquid Mineral Salts Medium (MSM) with 120 µg/L atrazine addition. The percentage of atrazine degradation was tested using spectrophotometric methods and yeast cell viability using the TPC method. The results showed that all isolates were able to degrade atrazine. The type of treatment and incubation time affected the ability of atrazine degradation, with the most effective isolate was TB1 which had a degradation percentage value of 94.26% and TPC of 9.27×10^4 CFU/mL on the 10th day.

Key words: *Agricultural soil, Atrazine degradation, Yeast*

Introduction

Pesticides have an important role to increase agricultural production and productivity. This is because pesticides function in pest control, thus bringing huge economic benefits for farmers. Until now there is still a perception that the more pesticides are used, the higher the agricultural production. By 2050 it is assumed that the use of pesticides will be 2.7 times greater than in 2000. This is associated with an increase in agricultural production due to the more intensive use of pesticides (Joko *et al.*, 2017).

The persistence of pesticides makes it easy to exist to the soil, causing a decrease in soil function. The use of pesticides in agriculture can also damage public health. Humans as living things that are at the very end of the food chain can get the greatest biomagnification effect due to bioaccumulation of pesticide residues by the levels of organisms below it (Jayaraj *et al.*, 2016).

The s-triazine herbicide is the most widely used pesticide in agriculture and forestry throughout the world (Abigail *et al.*, 2013). Atrazine, 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine is one of the s-triazine herbicides which is widely used to control grass and broadleaf weeds in corn, sorghum, sugarcane, pineapple, conifers, macadamia nuts, and others (Abigail *et al.*, 2013; Abigail and Das, 2012).

Atrazine in nature is a compound that is persistent in soil and has a half-life ranging from 21 days to 1 year. This makes atrazine an environmental threat because the residue of the parent compound and its derivatives can pollute the soil and water. Atrazine is also an endocrine disruptor and inducer of liver cancer in humans and other non-target organisms (Abigail *et al.,* 2013).

Biodegradation by microorganisms is one of the solution to solve atrazine residues, one of which uses yeast. Yeast as a degrading agent has many advantages because it is more effective in breaking down chemical components and has environmental stress-resistant properties (Bahafid *et al.,* 2017; Sui *et al.*, 2015).

The mechanism of atrazine degradation carried out by yeast begins with initial dechlorination to produce hydroxyatrazine, then hydrolyzed to form N-isopropilammelide and cyanuric acid. At the end of biodegradation, no persistent and accumulative products are found, which means that the metabolites have been completely degraded. The enzymes that work in the atrazine degradation activity are atrazine chlorohydrolase, hydroxyatrazine ethylaminohydrolase, and N-isopropylammelide isopropylaminohydrolase (Abigail *et al.,* 2013).

Research on atrazine degradation using yeast is still limited. Some yeasts, such as *Saccharomyces cerevisiae* (Wu *et al.,* 2018), *Pichia kudriavzevii* (Abigail *et al.,* 2013), and *Cryptococcus laurentii* (Abigail *et al.,* 2012) are reported to be able to use atrazine as a carbon and nitrogen source. Atrazine contamination in nature is detected at concentrations of 1-108 μg/L even after 22 years (Lu *et al.,* 2016). However, laboratory testing showed that *S. cerevisiae* was able to degrade atrazine <2 mg/L without any metabolites remaining for 7 days (Wu *et al.,* 2018), *P. kudriavzevii* degraded atrazine to 500 mg/L for 7 days (Abigail *et al.,* 2013), whereas *C. laurentii* can degrade atrazine up to 150 mg/L for 9 days (Abigail *et al.,* 2012).

In a previous study, yeast was isolated from agricultural land around the ITS area (Institut Teknologi Sepuluh November) with the code UF1, UF3, UF5, UF7, UF8, SS3, SS4, and SSU6, besides that, yeast was also isolated from sugarcane and corn agricultural land contaminated with herbicide in Mojokerto with the code TB1 to TB16, and JA1 to JA5. The ability of these yeast isolates to degrade atrazine herbicides has never been tested. Based on this background, we tested the potential of yeast isolated from agricultural land to degrade atrazine.

Materials and Methods

Yeast isolates

Yeast were isolated from agricultural soil around the ITS area with the code UF1, UF3, UF5, UF7, UF8, SS4, and SSU6.

Yeast isolates from soil contaminated with herbicides with the code TB1, TB2, TB3, TB4, TB5, TB6, TB7, TB8, TB9, TB10, TB11, TB12, TB13, TB14, TB15, TB15, TB16, JA1, JA2, JA3, JA4, and JA5 obtained from the isolation of sugarcane and corn plantation land in the Mojokerto area which has been exposed to herbicides for ± 3 months.

Mediums

YMEA (Yeast Malt Extract Agar) media used for isolates maintenance and cell viability tests were made by dissolving 3 grams of yeast extract, 3 grams of malt extract, 5 grams of peptone, 10 grams of glucose, and 2% (w/v) of agar in 1 L of aquadest (Kurtzman and Fell, 1998). Atrazine Mineral Salts (AMS) media for screening test, dose-response, percentage of atrazine degradation, and cell viability were prepared by dissolving 0.5 g of $MgSO_4.7H_2O$, 0.2 g of KH_2PO_4, 0.4 g of K_2HPO_4, 0.1 g of NaCl, 0.01 g of $FeSO_4.7H_2O$, 0.01 g of $MnSO_4.H_2O$, and 0.01 g of Na_2MoO_4 in 1 L of aquadest (Abigail *et al.,* 2013). In the dose-response test 2% (w/v) of agar, atrazine 30, 60, 90, 120, 150 mg/L, and 1 mL methanol (as a carrier solvent) were added. Atrazine with a concentration of 30 mg/L, 1 mL methanol, and 2% (w/v) of agar were also added to the screening test. In the percentage degradation and cell viability test, 120 mg/L of atrazine and 1 mL of methanol were added without agar (Abigail *et al.,* 2012; Topp *et al.,* 2000; Putra *et al.,* 2015).

Subculture and yeast screening for atrazine degradation

Yeast isolates were subcultured using the continuous streak method on the YMEA slant media then incubated at 30 ºC for 24-48 hours (Chen *et al.,* 2012). Subculture of yeast isolates were then inoculated on a Petri dish containing AMS with atrazine 30 μg/L using the line streak method (Topp *et al.,* 2000), then the Petri dish was wrapped in aluminum foil (Baghapour *et al.,* 2013) to be incubated for 7 days at 30 °C (Chen *et al.,* 2012). Screening of three atrazine degrading isolates was determined from the formation of the largest clear zone around the colony us-

ing the formula below:

$$\text{Clear Zone Index} = \frac{\text{Total diameter} - \text{Diameter of the colony}}{\text{Diameter of the colony}}$$

(Alfisyahri *et al.*, 2018).

Atrazine dose-response test

Add 1 loop of Each selected yeast isolate into 10 mL of 0.85% physiological saline solution, then vortexed for 15 seconds (Pfaller *et al.*, 1988). The turbidity of the cell suspension was measured using a spectrophotometer at λ 600 nm until reach absorbance value of 0.1 (Abigail *et al.*, 2013). 200 µL isolates were subcultured in sterile Petri dishes, then each AMS solid media containing atrazine with concentrations starting from 0, 30, 60, 90, 120, and 150 µg/L was added aseptically (pour plate method). The culture was incubated for 48 hours at room temperature and made two replications.

Identification of yeast isolates to the genus level

Yeast identification to genus level based on Kurtzman *et al.* (2011).

Inoculum preparation

The isolate was suspended in 50 mL of 0.85% physiological saline solution, then vortexed for 15 seconds (Pfaller *et al.*, 1988). Suspension turbidity was measured at λ 600 nm and absorbance value of 0.1 was determined (Abigail *et al.*, 2013).

Percentage of atrazine degradation

The isolate suspension was taken as 10% (v/v) to be inoculated into a bottle containing 100 mL AMS 120 µg/L. In the control medium, no suspension of isolates was added. The bottle is wrapped in Aluminium foil (Baghapour *et al.*, 2013). The culture was incubated on a rotary shaker at a speed of 120 rpm with a temperature of 30ºC for 10 days (Abigail *et al.*, 2013; Chen *et al.*, 2012). Percentage of degradation measurements were done every 2 days (0, 2, 4, 6, 8, and 10) (Chen *et al.*, 2012; Pawar *et al.*, 2016), by centrifuging the suspension at a speed of 9000 x g for 20 minutes (Abigail *et al.*, 2013). The supernatant was filtered through a 0.2 µm membrane filter (Pawar *et al.*, 2016), then the filtrate was absorbed using λ 222 nm (Sigmon, 2016). The percentage of atrazine degradation is determined using the formula:

$$\text{Percent degradation} = \frac{Ab - Aa}{Ab} \times 100$$

(Rokade and Mali, 2013).

Ab : Absorbance of the compound before degradation at λ 222 nm

Aa: Absorbance of compounds at the same wavelength (222 nm) after degradation.

Yeast cell viability

The number of yeast cells was measured by the method of Total Plate Count (TPC) on the YMEA medium. The culture was incubated for 48 hours at 30 ºC.

Results and Discussion

Screening and dose-response test

Based on the screening test of yeast isolates, all isolates are able to degrade atrazine by forming clear zones. TB1, UF5, and UF7 isolates showed the highest ratio of clear zones compared to other isolates, which were 0.259, 0.195, and 0.194 cm. The clear zone in Figure 1 shows that atrazine degradation has occurred in the medium. Figure 1 shows the same characteristics of the three best isolates screening results, which is the presence of pseudohyphae.

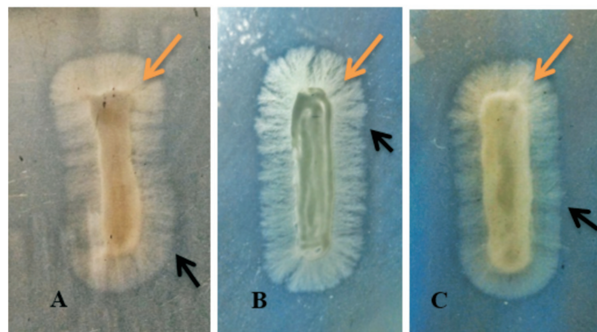

Fig. 1. Three isolates which have the largest clear zone ratio. Description: (A) Isolate TB1; (B) Isolate UF5; (C) Isolate UF7 (the clear zone is indicated by a black arrow, whereas pseudohyphae are indicated by an orange arrow).

The larger the clear zone area, the better the isolate degrades atrazine. This is due to the low solubility of atrazine in the water, which is 33 mg/L (Adams, 2014) so that if atrazine is added in the media which has the largest content of water, then the media will form a suspension and cause opaque properties. If atrazine is degraded, it will produce a compound which is simpler and polar in nature and has higher water solubility. Higher solubility in wa-

ter will cause loss of opaque, so the media will be clear.

Pseudohyphae, which results from imperfect budding formation, in which cells lengthen, but remain attached after division, can help yeast cells to absorb atrazine more effectively (Moore *et al.*, 2011).

Overall, the three isolates were tolerant of atrazine because they were able to survive with the emergence of many colonies in the AMS test medium despite being given atrazine at doses up to 150 µg/L, but at the highest concentrations, it also began to notice a decrease in the number of colonies. The average total number of colonies of the three isolates was also higher than the other concentrations, which was 10.04×10^2 CFU/mL, so the concentration of 120 µg/L was chosen as the dose of atrazine used in the percentage degradation and cell viability test.

Identification of the best atrazine degrading yeast

Based on morphological and physiological-biochemical observations of TB1, UF5, and UF7 isolates showed characteristics similar to the *Candida* genus according to the book "The Yeast A Taxonomic Study". *Candida* characteristics include cell shape varying from round, oval, cylindrical to elongated, rarely apiculate, ogival, triangular or bottle shape with or without pseudohyphae. Asexual reproduction with multilateral budding. Does not have carotenoid pigments so they are white to creamy. Some species have ascospores that belong to the *Ascomycetes* group and can form pseudohyphae, reproduce by budding, have capsules, and are able to ferment glucose (Kurtzman and Fell, 1998).

Percentage of atrazine degradation

Figure 2 shows the percentage value of atrazine degradation (%). An increase in the percentage of degradation proves that yeast isolates can use atrazine as a source of carbon and nitrogen.

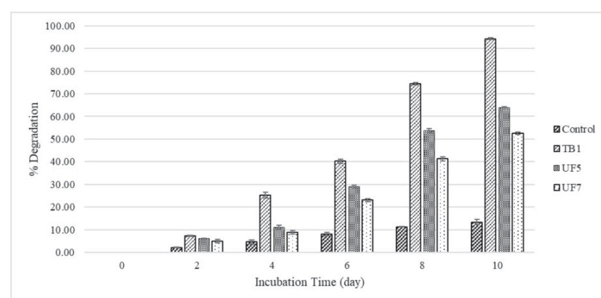

Fig. 2. Percentage of atrazine degradation

Based on Figure 2, the highest percentage of degradation was 94.26% which was owned by TB1 isolates on the 10th day. This shows that TB1 isolates were better at degrading atrazine compared to other isolates at 10 days incubation time. The next best value of degradation percentage on the 10th day was shown by UF5 isolate at 64.01% and UF7 isolate at 52.51%.

The control medium that did not do the addition of isolates, on the 10th day showed a percentage value of atrazine degradation of 13.41%. This is likely to occur due to the addition of methanol as a carrier solvent for atrazine, so that the solubility of atrazine increases from 33 mg/L in water to 18,000 mg/L in methanol (CH_3OH) which is an organic solvent, so that makes the structure of atrazine unstable and easy hydrolyzed. The composition of the test medium containing Manganese (Mn) and Iron (Fe) can also degrade organic pollutants such as atrazine, where Mn has a relatively high redox potential and is often an important component in the oxidation (and degradation) of organic compounds after Fe. Changes in temperature contribute to the degradation of atrazine, where the solubility of atrazine increases when the temperature is above 27 ºC so that atrazine will be more easily hydrolyzed by water (Adams, 2014).

All treatments showed that the longer the incubation time, the higher the percentage value of atrazine degradation, which meant that the levels of atrazine in the test medium were also reduced (Abigail *et al.*, 2013).

Effects of atrazine on yeast cell viability

Based on Figure 3, On the 4th, 6th, and 8th day, all isolates showed a constant increase in the number of colonies, but the average number of the largest colonies still to TB1 isolates, which obtained an average

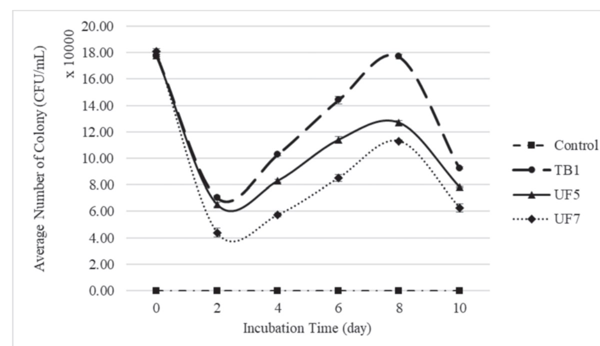

Fig. 3. The average value of the yeast colony (CFU/mL)

number of 1.03×10^5, 1.44×10^5, and 1.77×10^5 CFU/mL, respectively.

On the 10th day, all isolates showed a decrease in the number of colonies, where the highest average number of colonies was shown by TB1 isolates at 9.27×10^4 CFU/mL, then UF5 isolates at 7.83×10^4 CFU/mL, and finally the UF7 isolates with an average number of 6.27×10^4 CFU/mL.

The constant increase in the number of colonies is likely because yeast is in the logarithmic growth phase, where the speed of growth is strongly influenced by environmental conditions, besides this increase can also be interpreted that yeast is able to utilize atrazine contained in the test medium as an energy source for its growth, resulting in an increase in the population and activity of yeast (Romero *et al.*, 2004). The greater the number of yeast colonies, the more enzymes released by yeast to degrade atrazine (Abigail *et al.*, 2012). The decrease in the number of colonies can be caused by the yeast being in a phase of death, where most of the population begins to die because the nutrients in the medium are depleted, the presence of toxic substances, and depletion of energy reserves in cells. In the control treatment, there was no yeast growth, because there was no addition of yeast isolates into the control medium.

The *Candida* genus is a dominant isolate found in untreated soils and treated soils, such as fertilizer or pesticides (Romero *et al.*, 2004), so that these microbes have adapted to their environment and are able to release specific enzymes that can detoxify compounds to be degraded, for later use as a source of carbon and nitrogen. The *Candida* genus has been widely reported as a yeast group that is good at degrading pollutants, such as polycyclic aromatic hydrocarbons and biarilyc compounds (Romero *et al.*, 2004).

Conclusion

Three isolates coded TB1, UF5, and UF7 belonging to the genus Candida have a good ability to degrade atrazine because it produces the largest clear zone ratio. TB1, UF5, and UF7 isolates can degrade atrazine up to 94.26%, 64.01%, and 52.51% and also have an average colony of 9.27×10^4, 7.83×10^4, and 6.27×10^4 CFU/mL, respectively on the 10th day of incubation.

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