The potency of endophytic bacteria isolated from *Ficus septica* as phytoremediation promoting agent of Cr (VI) contaminated soil

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Abstract. *Rohmah NS, Suharjono, Jatmiko YD, Siswanto D, Mustafa I. 2020. The potency of endophytic bacteria isolated from* Ficus septica *as phytoremediation promoting agent of Cr (VI) contaminated soil. Biodiversitas 21: 1920-1927.* Endophytic bacteria tolerant of chromium have the potential to reduce Cr (VI) to Cr (III) and produce compounds that can improve plant survival in Cr (VI) contaminated soils. This study was performed to isolate and identify Cr (VI)-tolerant bacteria from the endosphere of *Ficus septica* Burm.F growing on the tannery waste contaminated soil, Screening of bacteria was carried out based on the level of Cr (VI) tolerance. High tolerant isolates were tested for their potential of Indole Acetic Acid (IAA) and exopolysaccharide (EPS) production, phosphate solubilizing, and the presence of chromate reductase (*ChR*) genes. The most potential bacterial isolate was identified based on 16S rDNA sequences. The results showed that three isolates (E3, E4, and E8) were tolerant of Cr (VI) up to 900 mg/L. E8 isolates produced the highest IAA production (20.05 μg/mL), phosphate solubilization (127.78 μg/mL), and EPS production (9,70 μg/mL), and all isolates had the *ChR* gene. E8 isolate was found to be 100% identical to *Enterobacter cloacae* ATCC 13047.

Keywords: Endophytic bacteria, *Ficus septica* Burm.F, Phytoremediation, Cr (VI), *Enterobacter cloacae* ATCC 13047

INTRODUCTION

Chromium (Cr) (VI) is one of the toxic heavy metals that contaminate the soil. Chromium (CrVI) is mutagenic, carcinogenic, and teratogenic in humans and other organisms (Schiavon et al. 2008). Cr (VI) mostly produced from leather tanning, welding, and paper production activities in the forms of the anion chromate (CrO_4^{2-}) (Singh et al. 1998). Chromate has structural resembles with tetrahedral sulfate ion (SO_4^{2-}). It can easily pass through cell membranes of microorganisms via SO_4^{2-} transport pathway. The less toxic chromium in nature is Cr (III) (Cervantes et al. 2001; Krishna and Philip 2005).

Heavy metals can be detoxified by adsorption/ accumulation or conventional physicochemical treatments, but they are quite expensive and cumbersome. Therefore, safe and effective technology for detoxification of Cr (VI) contamination is needed, including by using micro-organisms (Malik 2004). Some species of bacteria are tolerant of Cr (VI) and can accumulate and reduce it to Cr (III). Mechanisms of detoxifying Cr (VI) are periplasmic biosorption, intracellular, and extracellular bio-accumulation and biotransformation to less toxic metal oxidation states (Cervantes et al. 1990; Camargo et al. 2003).

Cr (VI) concentrations that are too high in the environment can not be accumulated but will be removed from the cells so as not to interfere with metabolism. The ability to accumulate Cr (VI) by bacteria can be enhanced when bacteria are associated with plants (Hynninen and Virta, 2009; Laghlimi et al. 2015). The genus *Ficus* can be used as phytoremediation agents for heavy metal polluted habitats. Ficus has a wide tolerance for various contaminants, and it has less frequent of biomass removal than herbaceous plants with many adventive roots that allow to penetrate widely into the substrate and increase the efficiency of the phytoremediation process (Yeo and Tan 2011; Luo et al. 2018).

Bacteria as phytoremediation promoting agents produce IAA, solubilize phosphate, and produce EPS. These abilities can improve plant metabolic functions and cell membrane permeability as well as increase plant resistance and its ability to remediate Cr (VI) (Davies et al. 2001; Srivastava et al. 2006; Lugtenberg and Kamilova 2009). Bacteria have the chromate reductase (*ChR*) gene that catalyzes the reduction of Cr(VI) to Cr(III) Patra et al. 2010). *Agrobacterium rhizogenes* can accumulate and reduce heavy metal Cr (VI) and promote plant growth (Rosariastuti et al. 2013). Khan et al. (2015) reported that *Bacillus, Pseudomonas, Enterobacter, Staphylococcus, Microbacterium, Arthrobacter* isolated from *Prosopis juliflora* produce supporting compounds for Cr (VI) phytoremediation.

Many studies on the ability of bacteria to improve phytoremediation have been carried out. However, there are no studies on endophytic bacteria from *Ficus septica* Burm.F that are tolerant of Cr (VI). This study aimed to evaluate the potency of endophytic bacteria from *Ficus septica* Burm.F as phytoremediation promoting agent of Cr (VI) contaminated soil by evaluating IAA production, EPS production, and phosphate solubilization. The association of bacteria and plants has the potential to promote plant growth in Cr (VI) contaminated soil and improve the efficiency of Cr (VI) phytoremediation.

MATERIALS AND METHODS

Description of the sampling area

Leaves of *Ficus septica* Burm.F was collected from the tannery waste disposal area in Malang City, East Java Province, Indonesia. The leaves were collected from three *F. septica* trees that grew in sediments around the tannery wastewater. The leaves were transferred into sterile plastic and stored in a coolbox.

Isolation and screening of Cr (VI) tolerant endophytic bacteria

The leaves of *Ficus septica* Burm.F were washed with sterile aqua dest and surface sterilized using 70% ethanol and 5.25% sodium hypochlorite, then rinsed with sterile aqua dest. Subsequently, 25g of surface-sterilized leaves were homogenized with a pestle and mortar in 225 mL NaCl (0.85%, w/v). Serial dilutions were carried out 10^{-7}. 0.1 ml of each suspension of dilution series was inoculated on Nutrient Agar (NA) medium containing 150 mg/L Cr (VI). The medium was incubated at 28°C for 24 hours. Colonies with different morphologies that grow on the NA medium were picked out and purified by re-streaking onto the NA medium (Harley and Prescot 2002; Khan et al. 2015). Pure bacteria isolates were grown on NA medium containing Cr (VI) with multilevel concentrations from 150 mg/L to 1000 mg/L by streaking plate method. All the plates were incubated at 28°C for two days, and the appearance of bacterial growth was observed (Khan et al. 2015).

Determination of IAA production by endophytic bacteria
Qualitative IAA production

Qualitative and quantitative methods used to determine IAA production. Qualitative methods are carried out based on the modified method of Brick et al. (1991) using Salkowski's reagent. Salkowski's reagent is a mixture of 1.05 g ferric chloride ($FeCl_3$) and 60 mL sulfuric acid (H_2SO_4). The reagent was cooled down and stored in a brown bottle with proper labelling (Gordon and Weber 1951). One ose of bacterial culture was inoculated on 25 mL Tryptic Soy Broth (TSB) medium containing 2% L-tryptophan and Cr (VI) 150 mg/L. The culture was incubated at 28°C for 24 hours. Bacterial cultures with a density of 10^7-10^8 or optical density (OD) 0.5 were inoculated by spreading it on Tryptic Soy Agar (TSA) medium containing 2% L-tryptophan and Cr (VI) 150 mg/L. After inoculation, 0.45 μm nitrocellulose membranes placed onto agar plates and incubated at 28°C for 48 hours. The membrane was then flooded with Salkowski's reagent and incubated at room temperature in the dark for 30 min and observed for pink color development as an indication of IAA production.

Quantitative IAA production

The bacteria isolates that able to produce IAA in the qualitative assay were quantitatively analyzed with three replicates. One ose of bacterial culture was inoculated on 50 mL Tryptic Soy Broth (TSB) medium containing 2% L-tryptophan and Cr (VI) 150 mg/L. The culture was incubated at 28°C for 24 hours. After incubation, five

milliliters of cultures (from the previous culture) were inoculated on 50 mL Tryptic Soy Broth (TSB) medium containing 2% L-tryptophan and Cr (VI) 150 mg/L and incubated at 28°C for 96 hours. Three milliliters of bacterial suspension was taken out every 24 hours and centrifuged at 10000 rpm for 10 minutes. Two milliliters of Salkowski's reagent was added into 1 mL of the supernatant and incubated for 30 minutes in a dark room. Pink color development indicated IAA production. The absorbance of the sample is measured at 535 nm using a UV-VIS spectrophotometer (UV Mini 1240). IAA concentrations were calculated based on IAA standard curves.

Determination of phosphate solubilizing by endophytic bacteria
Qualitative phosphate solubilization

One ose of bacterial culture was inoculated on Pikovskaya Broth media added 0.5% Tricalcium phosphate (TCP) and Cr (VI) 150 mg/L. The culture was incubated at 28° C for 24 hours. Sterile paper disks were inoculated with a 20 μL bacterial culture at a density of 10^7-10^8 or OD 0.5 and then placed on the Pikovskaya agar medium. The medium was incubated at 30°C for 72 hours. Comparative solubilization index measurement was carried out on day three of incubation by measuring halo zone and colony diameters. Phosphate solubilization index was determined by using the following formula: the ratio of the total diameter (colony + halo zone) and the diameter of the colony (Premono et al. 1996).

Quantitative phosphate solubilization

One ose of bacterial culture was inoculated on Pikovskaya Broth media added 0.5% Tricalcium phosphate (TCP) and Cr (VI) 150 mg/L. Each isolate was determined with three replicates. The culture was incubated at 28°C for 24 hours. After incubation, five milliliters of bacterial culture with a density of 10^7-10^8 or OD 0.5 were grown on Pikovskaya Broth media added with 0.5% Tricalcium phosphate (TCP) and Cr (VI) 150 mg/L and incubated at 28°C for 120 hours. Two milliliters of bacterial suspension were harvested every 24 hours and centrifuged at 10000 rpm for 10 minutes. And then, ten milliliters of chloromolybdate reagent and 0.1 mL of chlorostannous acid was added to 1 mL of the supernatant. The suspension was diluted to 25 mL using sterile aqua dest, homogenized, and incubated for 10 minutes. The absorbance of the sample was measured at 690 nm using a UV-VIS spectrophotometer (UV Mini 1240). Solubilized phosphate concentration is calculated based on a standard phosphate curve (Tirry et al. 2018).

Determination of EPS production by endophytic bacteria
Qualitative EPS production

Bacteria were inoculated in two types of Nutrient Agar (NA) medium containing: (1) 2% (w/v) sucrose and Cr (VI) 150 mg/L and (2) 2% (w/v) glucose and Cr (VI) 150 mg/L. The culture was incubated at 30°C for 48 hours. Colonies of bacteria that form thick mucous and slimy indicate that bacteria capable of producing EPS (Tallgren et al. 1999). Pawar et al. (2013) reported that NB medium contains

sucrose and glucose as carbon sources at the same concentration indicated that NB media with sucrose as carbon source produced more EPS. It shows that sucrose metabolism is easier than glucose in bacteria isolates, therefore EPS quantitative tests used sucrose as the carbon source

Quantitative EPS Production

Exopolysaccharide (EPS) production quantified according to the modified method of Rida et al. (2015). One ose of the bacterial isolate was inoculated into Nutrient Broth (NB) medium containing 2% (w/v) sucrose and Cr (VI) 150 mg/L, then incubated at 30°C for 24 hours. Each isolate was determined in three replicates. Then, five milliliters of bacterial isolate with a density 10^7-10^8 or OD 0.5 was taken out and grown on NB medium containing 150 mg /L Cr (VI) and enriched with sucrose 2% (w/v), then incubated at 30°C for 48 hours. A total of 10 mL of samples were centrifuged at 10000 rpm at 4°C for 30 minutes. The supernatants were boiled at 100°C for 15 minutes. After cooling down, the supernatant was added with trichloroacetic acid (4% w/v) to precipitate protein and centrifuged at 10000 rpm at 4°C for 30 minutes. The supernatants were precipitated overnight at 4°C in 20 mL ethanol for EPS precipitation. The suspension was centrifuged at 10000 rpm at 4°C for 30 minutes, the pellets obtained were dried at 100°C for 15 minutes and the EPS weight was calculated (Cerantola et al. 2000).

Data analysis

Qualitative data were analyzed by descriptive method, while quantitative data were analyzed by two-way ANOVA using the univariate model to evaluate interactions between treatments at a 95% confidence level and followed by Tukey test to assess differences in the ability of each endophytic bacterial isolate using SPSS 16.0 software program for Windows.

Detection of chromate reductase (*ChR*) gene

Bacterial DNA chromosomes were extracted using the Zymo DNA Extraction Kit. Detection of the chromate reductase gene in the bacterial genome DNA was carried out by PCR amplification method using a specific primer chromate reductase Gene (*ChR*) Forward 5'TCACGCCGGAATATAACTAC- 'and Reverse 5'CGTACCCTGATCAATCACTT3'. The PCR program used for *ChR* gene amplification was: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min and a final extension at 72°C for 10 min. The PCR product was qualitatively confirmed using DNA electrophoresis in 1.5% agarose gel and visualized using a UV transilluminator (EBOX VX2) (Patra et al. 2010).

Identification of selected endophytic bacteria based on 16S rDNA

DNA chromosome of the most potential endophytic bacteria was extracted using the Zymo DNA Extraction Kit. The sequence of 16S rDNA was amplified using universal primers 27f (5 '-GAG AGT TTG CTG GCT ATC CAG- 3') and 1492r (5 '-CTA CGG CTA TGT CCT TAC

GA- 3 '). PCR amplification of 16S rDNA gene was carried out in PCR Mastercycler Personal (EPPENDORF) subjected to denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1,5 min and a final extension of 72°C for 10 min. Amplicons of 16S rDNA were purified and sequenced in First BASE, Malaysia. The 16S rDNA sequences of isolates were aligned with reference strains from BLAST and analyzed using the MEGA V.6 program. Phylogeny tree was constructed and inferred with the Neighbor-joining algorithm with the Tamura-Nei model using 1000 replicates bootstraps (Patra et al. 2010).

RESULTS AND DISCUSSION

Isolation and screening of Cr (VI) tolerant endophytic bacteria from *Ficus septica* Burm.F

The population density of endophytic bacteria on NA medium containing 150 mg/L Cr (VI) was 2,23 x10^5 CFU/g with population density in the control media (NA without Cr (VI)) was 9,68x10^5 CFU/g. A total of eight bacterial strains with different morphological colonies were isolated from endosphere of *Ficus septica* Burm.F All isolates were tolerant to Cr (VI) 500 mg/L. In contrast, those tolerant to Cr (VI) 900 mg/L were only three isolates (E3, E4, and E8). Three other isolates could not grow at the highest concentration of 1000 mg/L (Table 1). Each bacterial isolate has a different tolerance level to Cr (VI).

The previous study by Khan et al. (2015) reported that endophytic bacteria isolated from *Prosopis juliflora* were tolerant of Cr (VI) up to 3000 mg/L. Another study by Ramirez et al. (2019) showed that endophytic bacteria from *Prosopis laevigata* were tolerant of Cr (VI) 1474 mg/L. Dimitroula et al. (2014) showed that endophytic bacteria from *Juncus acutus* were tolerant of Cr (VI) 100 mg/L. Some mechanisms for Cr (VI) tolerance by bacteria were chromate regulator protein synthesis (*ChrA* and *ChrB*) by the chrBCAF operon which acts as a sensitive chromate regulator; balancing oxidative stress-induced Cr (VI) by activating ROS scavenging enzymes (e.g. catalase and superoxide dismutase), DNA repair with SOS response enzymes (RecA, RecA, RecG, RuvAB) to fight DNA damage and regulation of iron absorption to prevent the production of hydroxyl radicals through the Fenton reaction (Morais et al. 2011; Ahemad, 2014).

Table 1. Bacteria isolated from endosphere of *Ficus septica* Burm F that are tolerant of Cr (VI)

Isolate	Cr (VI) concentration (mg/L)									
	150	200	300	400	500	600	700	800	900	1000
E1	+	+	+	+	+	-	-	-	-	-
E2	+	+	+	+	+	+	-	-	-	-
E3	+	+	+	+	+	+	+	+	+	-
E4	+	+	+	+	+	+	+	+	+	-
E5	+	+	+	+	-	-	-	-	-	-
E6	+	+	+	+	+	-	-	-	-	-
E7	+	+	+	+	+	+	+	-	-	-
E8	+	+	+	+	+	+	+	+	+	-

Note: + (growth), -(no growth)

The potency of endophytic bacteria to produce IAA

Three endophytic bacteria (E3, E4, and E8) produced a pink color on the nitrocellulose membrane indicated IAA production. The high concentrations of IAA produce a deep pink color, and the lower concentrations yield a light pink color after exposure to the Salkowski reagent (Figure 1a).

Pink color intensity indicates indole formation from the IAA complex and the reduction of $Fe^{3+.}$ The difference in the intensity of the pink color is caused by the presence of various indole compounds as products of tryptophan metabolism, such as indolpirufic acid and indoleacetamide (Bric et al., 1991). Singh et al. (2014) reported that red or pink color was an indication of indole detection in IAA. Quantitative data on IAA production corresponding with qualitative determination showing that all three isolates (E3, E4, and E8) have varying abilities in producing IAA (Figure 1b). The three isolates produce an optimal IAA hormone at 48 hours of incubation. E8 isolate produced the highest IAA (20.05 μg/mL) (p <0.05). All isolates showed decreased IAA concentrations at 72 hours of incubation. The optimum IAA production was in the final logarithmic growth phase goes to the stationary phase. A decrease in IAA production in E8 isolates was after 72 and 48 hours of incubation indicates a phase of death.

According to Lugtenberg and Kamilova (2009), at the beginning of the stationary phase, microbial growth is constant. The number of nutrients in the medium decreases and the product of secondary metabolites were generally increased. Nutrients in the medium are used to maintain the number of living cells available. In the microbial death phase, the microbes die due to the reduced amounts of nutrients and increased toxic metabolites in the medium so that the number of biomass decreases. A decrease in IAA concentration after the initial stationary phase indicates a reduction of nutrients in the medium, so bacteria utilize IAA as a source of nutrition. Donoso et al. (2017) reported that bacteria used IAA as a carbon and energy source employing the iac gene under controlled of tautomerase superfamilies. Iac gene regulation is also regulated by the response of the MarR-type transcriptional repressor.

Javaid and Sultan (2012) reported that *Streptomyces matansis* was able to produce 16 μg/mL IAA in media containing 50 mg/L Cr (VI). Oves et al. (2013) showed that *Pseudomonas aeruginosa* OSG41 isolate was able to produce 24.2 μg/mL IAA in the media containing Cr (VI) 200 mg/L and L-Tryptophan 1%. The ability of endophytic bacteria *Pseudomonas aeruginosa* OSG41 to produce IAA is almost the same as E8 isolates. In this study, IAA was detected early because this hormone is the main parameter that increases the adaptation and tolerance of plants which are gripped by heavy metals by inducing physiological changes (Liphadzi et al., 2006). Tandon et al. (2015) show that exogenous IAA increases phytoremediation efficiency in wastewater treatment. The difference in IAA production potential is due to differences in isolates, L-Tryptophan and Cr (VI) concentrations contained in the medium.
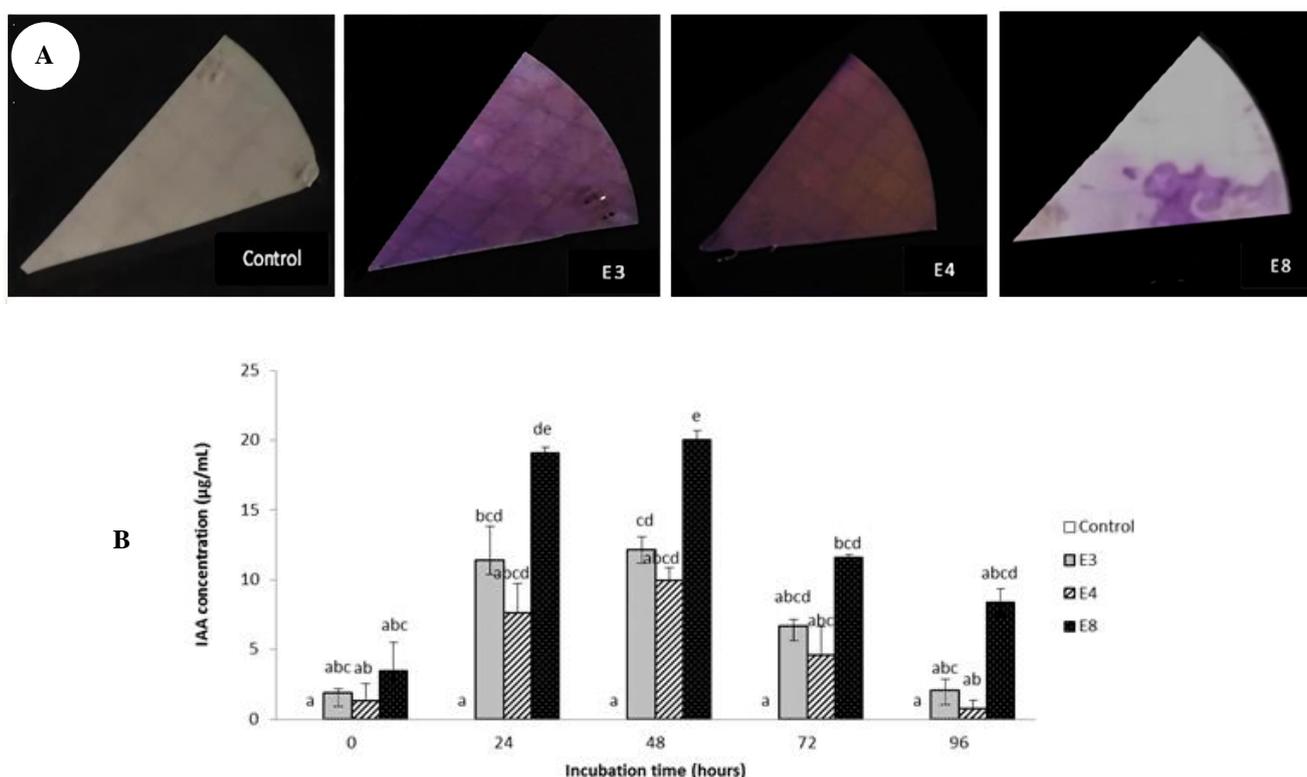

Figure 1.A. Pink color formation by bacteria after reacting with Salkowski"s reagents on the nitrocellulose membrane indicated the production of IAA. B. The concentration of IAA production by endophytic bacteria. Data were expressed as the mean ± standard deviation of three replicates using Two-way ANOVA test at a = 0.05. Different notations indicate significant difference among treatments (p < 0.05).

Table 2. Solubilization index (SI) and EPS production of three endophytic bacterial isolates at two different carbon sources

Isolate	SI*	EPS		
		Glucose	Sucrose	Total EPS (μg/mL)*
Control	1 ± 0^a	-	-	0^a
E3	1.54 ± 0.05^c	-	+	7.66 ± 0.09^c
E4	1.32 ± 0.05^b	+	+	5.70 ± 0.09^b
E8	1.52 ± 0.07^c	+	+	9.70 ± 0.01^d

Note: *Data were expressed as mean ± standard deviation, notation differences show significant differences in the Anova test ($\alpha = 0.05$); + (slime formed), - (not slime formed)

The potency of endophytic bacteria in phosphate solubilizing and EPS production

Qualitative results showed that the three isolates were able to dissolve phosphate that indicated by the formation of halo zones around the disk paper and the Solubilization index (SI). The highest SI was performed by isolate E3 (1.54) which was not significantly different (p> 0.05) from isolate E8 (Table 2).

Formation of halo zones indicates the capability of bacteria to dissolve tricalcium phosphate (Ca3 (PO4) 3OH) in the media as a source of phosphate in the presence of phosphatase enzyme (acid phosphatase (AcPase)) (Thakuria et al., 2004) The results of the qualitative determination are corresponding with the quantitative determination. E8 isolate was able to dissolve the highest phosphate (129.33 μg/mL) and the fastest at 48 hours of incubation time. The concentration of dissolved phosphate decreased significantly at 72 hours of incubation time. E3 isolate was able to dissolve phosphate of 127.78 μg/mL at 72 hours of incubation that was not significantly different (p> 0.05) from E8 isolate at 48 hours of incubation (Figure 2). The optimum condition for phosphate solubilization indicates that the bacterium is in the final logarithmic growth phase towards stationary. The decrease in dissolved phosphate concentration indicates bacterial isolates in the stationary phase leading to death. Bacteria *P. aeruginosa* OSG41 can dissolve phosphate to a concentration of 185.6 μg/mL on media containing 200 mg/L Cr (VI) (Oves et al. 2013).

The qualitative determination of EPS production showed variations of EPS production due to differences in carbon sources in NA medium. EPS production in bacterial isolates is characterized by thick slime and the formation of mucus in the colony. E3 isolate showed the accumulation of mucus in glucose-containing medium, while isolates E4 and E8 both using glucose and sucrose as carbon sources that indicated by the mucous formation in NA media containing glucose and sucrose (Table 2). Pawar et al. (2013) reported that in the media containing sucrose as carbon source produces higher total EPS than that of glucose as a carbon source at the same concentration. It shows that sucrose can be more metabolized than glucose, so in quantitative determination, the carbon source used is sucrose.

Quantitative determination showed that endophytic bacteria; E8 produced the highest total of EPS (9.70 μg/mL) (p <0.05) whereas E3 and E4 produce EPS 7.66 μg/mL and 5.70 μg/mL, respectively (Figure 3). Rida et al. (2015) reported that *Pseudomonas aeruginosa* Rb-1 and *Ochrobactrum intermediate* Rb-2 grown on medium containing Cr (VI) 1000 mg/L produce total EPS 4.15 μg/mL and 13.63 μg/mL, respectively. Harish et al. (2012) showed that *Enterobacter cloacae* produced EPS 78 μg/mL in a medium containing Cr (VI) 100 mg/L.

Differences in the ability to dissolve phosphate and EPS production is due to differences in the species of bacteria and the concentration of Cr (VI) in the medium. IAA production and phosphate solubility decrease while EPS production increases with increasing concentration of Cr (VI) at a particular concentration (Dogan et al. 201; Oves et al. 2013).
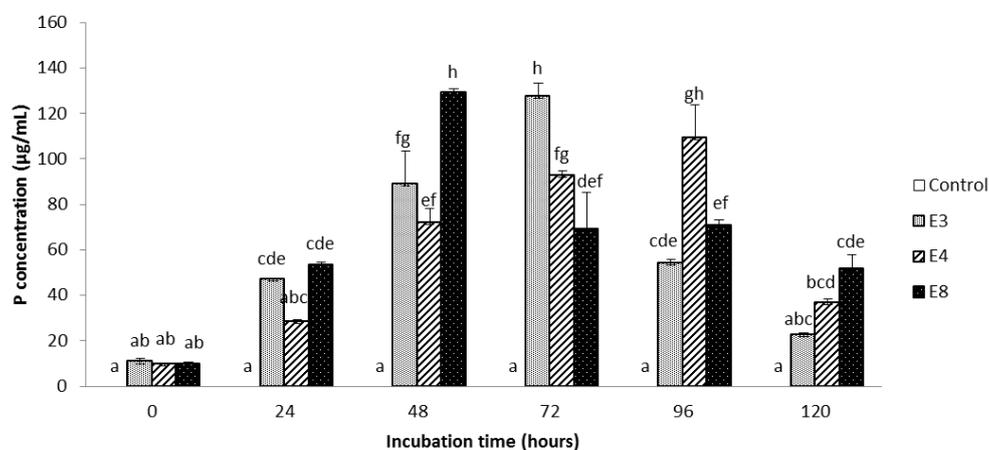

Figure 2. Phosphate solubilizing ability of endophytic bacteria. Data were expressed as the mean ± standard deviation of three replicates using Two-way ANOVA test at a = 0.05. Different notations indicate significant difference among treatments (p < 0.05).
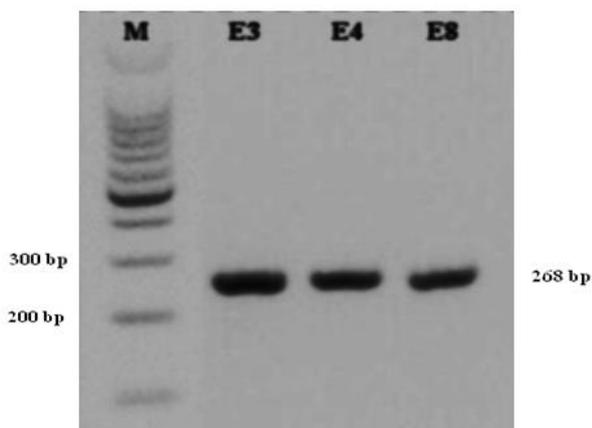
Figure 4. Results of *ChR* gene fragment amplification

The chromate reductase (*ChR*) gene in Endophytic bacteria

Three endophytic bacteria (E3, E4, and E8) were tolerant of Cr (VI) were analyzed for their *ChR* gene. The results showed that these isolates had a *ChR* gene of 268 bp when confirmed using DNA ladder (Figure 4). Similar results were reported by Patra et al. (2010) that the *ChR* gene in *Arthrobacter aurescens*, *Bacillus atrophaeus*, and *Rhodococcus erythropolis* was 268 bp. Research by Sanjay et al. (2018) showed that *Klebsiella pneumoniae* and *Mangrovibacter yixingensis* also had a *ChR* gene of 268 bp. The *ChR* gene encodes an enzyme that catalyzes the reduction reaction of Cr (VI) to Cr (III) (Wani and Adeosun 2017).

Wang et al. (1990) reported that *Escherichia coli* ATCC 33456, *Orchobactrum anthtopi*, and *Enterobacter cloacae* HO1 possess the *ChR* gene. These bacteria can reduce Cr (VI) aerobically by involving NAD(P)H extracellular reductase. These bacteria are also able to reduce Cr (VI) to Cr (III) anaerobically by involving amino acids, nucleotides, carbohydrates, vitamins and organic acids as electron donors and Cr (VI) as the terminal electron. The reduction mechanism of Cr (VI) associated with cell membranes requires H_2 or glucose as electron donors (Cheung and Gu 2007).

Potential endophytic bacterial species as Cr (VI) phytoremediation promoting agent

Phylogeny tree of the most potential endophytic bacteria was constructed based on the similarity of 16S rDNA sequences The result of Figure 5 showed that E8 isolate was found to be 100% identical to *Enterobacter cloacae*. Chitraprabha and Sathyavathi (2018) reported that *Enterobacter cloacae* associated with *Tagetes erecta* could accumulate and reduce Cr (VI). Kumar et al. (2014) identified *Enterobacter aerogenes* that resistant to Cr (VI) up to 600 mg/L and was able to produce IAA and solubilize phosphate. Khan et al. (2015) identified *Enterobacter* sp. That is tolerant to Cr (VI) and capable of producing phytoremediation supporting compounds.

Three endophytic bacteria isolates (E3, E4, and E8) were tolerant of Cr(VI) up to 900 mg/L and able to produce IAA, dissolve phosphate, and produce EPS. The three isolates have the potential to support *Ficus septica* Burm.F in remediating the polluted ecosystem of Cr (VI), with E8 as the highest potential isolate. IAA produced by bacteria plays an important role in increasing plant IAA and metal detoxification. IAA stimulates the production of root exudates, the proliferation of lateral and adventitious roots, and induces the synthesis of ACC synthase. It supports the absorption of nutrients and metals and increases plant adaptation and tolerance to heavy metals (Liphadzi et al. 2006).
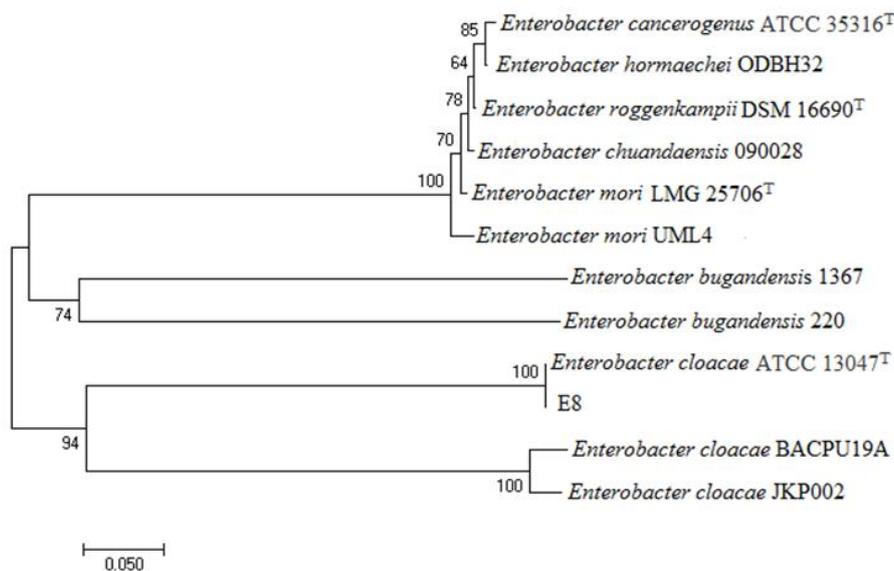

Figure 5. Phylogenic trees of E8 isolates and reference isolates based on the similarity of 16S rDNA sequences with Neighbor-Joining algorithm

The ability of bacteria to dissolve phosphate can increase bioavailability and absorption of metals and nutrients in the soil by plants. Phosphate solubilizing bacteria can also release bonds of precipitated metal sulfides so that they accumulate for plant growth (Sharma et al. 2013). Exopolysaccharides act as barriers layer to heavy metals through biosorption which includes the exchange of metal ions, complexation with negative charges of functional groups, adsorption, and precipitation. Besides, EPS also acts as a protection against pathogens, phagocytosis, and infections by bacteriophages. Endophytic bacteria can support the phytoremediation of Cr (VI) due to the presence of a *ChR* gene that can reduce the toxic Cr (VI) to the less toxic Cr (III) (Oves et al. 2013). This result was confirmed by Rosariastuti et al. (2013) which shows that the *Agrobacterium rhizogenes* bacterium can accumulate and reduce the heavy metal Cr (VI) in the shoots and stimulate plant growth.

The Cr (VI) tolerant endophytic bacteria associated with *Ficus septica* Burm.F were potential to protects the plant against the inhibitory effects of Cr (VI), reduced heavy metals toxicity, and leading to enhanced remediation of contaminated soil and water and environmentally and economically sustainable plant biomass production.

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