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Selective abiotic and biotic stressors influence the *acd*S gene abundance in the rhizospheric soil of pineapple plants in Lampung, Indonesia

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ABSTRACT

Indonesia has faced extensive poor soil quality and health for years.Anthropogenic, climatic, and plant pathogenic activities are the major contributing factors that degrade the soil's essential life-supporting system, such as the functional gene pool. One such gene is the rhizobacterial *acd*S, which codes for synthesizing the ACC (1-aminocyclopropane-1-carboxylate) deaminase that cleaves ACC as an ethylene hormone precursor in the stressed plant growing under stressful conditions. This study aims to investigate the abundance of the *acd*S gene in different abiotic and biotic stressors of the rhizospheric soil of pineapple plantation area that has been exposed incidentally to herbicides, flooding, and pathogenic *Phytophthora* spp infections. Soil samples were collected by using simple randomized sampling. A quantitative real-time polymerase chain reaction (qPCR)-based method was applied to assess the gene abundance. This study reveals that the abundance of *acd*S in the rhizospheric soil of herbicide-treated and *Phytophthora*-infected symptomatic plants is relatively lower than that of non-treated ones. The highest abundance was found in the *Phytophthora*-infected soil of asymptomatic plants. However, there was no significant difference in abundance between the flooded rhizospheric soil and the non-exposed ones. Hopefully, this data will help us better understand proper land-use management practices for pineapple plants.

Keywords: ACC deaminase, *acd*S gene abundance, *Phytophthora*-infected root, pineapple, qPCR

INTRODUCTION

Poor soil quality and soil health have been an alarming attention issue in Indonesian agricultural lands nowadays. Good quality of soil maintains and sustains biodiversity, productivity, and nutrient cycling and supports plant growth. However, the area of quality soil for food production has been shrinking rapidly (Hartemink, 2003). Anthropogenic, climatic, and pathogenic stressors have become the primary causes of soil degradation, which leads to a low yield of many food crops in Indonesia. Soil degradation, a decline in soil quality caused by anthropogenic activities, has also been a major global issue during the twentieth century and will remain an international project of great concern in the twenty-first century (MoEF and UNCCD, 2015). Such concerns about poor quality soil also happen in some areas of a pineapple plantation in Lampung Province. Herbicide treatments and flooding have intensified, causing the pineapple leaves to turn reddish to yellowish. *Phytophthora* spp also rot the pineapple roots. It has been hypothesized that all the stressors must contribute substantially to the soil microbiome structure and function (Rocca et al., 2019), especially the abundance of beneficial genes of plant growth-promoting rhizobacteria (PGPR).

Recent studies have focused on attenuating the abiotic stressor effects (Meena et al., 2017; Nephali et al., 2020) and phytopathogenic effects (Zhang et al., 2013) through an integrated land-use management approach. The trend of using this approach will continue in order to achieve sustainable agriculture. Plant-microbial interaction plays an essential, mutually beneficial role in attenuating the effects of environmental stressors on the plant. This interaction shows a complex ecosystem hosting microbes in the rhizosphere (Müller et al., 2016). It eventually implicates the presence and expression

of plant-beneficial functional genes in PGPR that contribute to plant growth and health effects. One of these genes whose expression is affiliated with environmental stressors is the *acd*S gene (Timmusk et al., 2011; Liu et al., 2021). This gene is a structural gene that codes ACC deaminase, which plays a role in suppressing the excessive synthesis of ethylene produced by stressed plants through the degradation of its immediate precursor, the ACC, into ammonia and á-ketobutyrate (Honma and Shimomura, 1978). At the end, Rhizobacteria consume the ACCs to maintain the equilibrium between the plant tissue's internal and external ACC levels. As feedback, Rhizobacteria produce indole acetic acid (IAA) and promote plant growth (Glick, 2014). Bruto *et al.* (2014) found that the *acd*S gene is one of the 23 PGPR genes contributing to plant-beneficial functions.

Soil quality can be evaluated using a variety of indicators. However, fewer indicators may be more practical in providing the necessary information for specific purposes (Kostov, 2017; Lima et al., 2013). Revealing the abundance of the *acd*S gene in the total rhizobacteria can be one critical indicator of the molecular biological health of the soil. Consequently, identifying and practicing farming management that sustains soil health is essential in maintaining an equilibrium between high productivity and soil fertility. However, molecular studies investigating the correlation between environmental stressors and the abundance of the *acd*S gene in agricultural land have rarely been conducted, especially in Indonesia. Most previous studies have focused on the biochemical level of ACC deaminase encoded by the *acd*S gene rather than the molecular level. Monitoring the microbial community through the abundance of the *acd*S gene will more accurately reflect the microbiological conditions, such as those of the pineapple plant rhizosphere. Therefore, land-use management practices should place greater emphasis on controlling environmental stressors, such as well-managed herbicides, pesticides, chemical fertilizers, irrigation, and specifically by applying ACC deaminase-producing rhizobacteria as a bio-inoculant to anticipate stressors interference. This study intends to investigate the abundance of the *acd*S gene in different abiotic and biotic stressors of the rhizospheric soil of pineapple plants.

MATERIALS AND METHODS

As displayed in Figure 1, this study was carried out in several steps. The first step was rhizobacterial DNA extraction from soil samples. As the first step was carried out, previous studies analyzed the soil's chemical properties. Isolated DNAs were then amplified using real-time PCR reaction to detect their *acd*S gene. The amplified genes were analyzed based on the Cq value of the qPCR run and interpreted in the end.

Research Site and Soil Sample Condition

This study was conducted at the Agricultural Microbiology Laboratory, Research Center for Applied Microbiology, Indonesian Institute of Sciences (National Research and Innovation Agency – BRIN), in Cibinong between October 2017 and May 2018. Soil samples originated from a sample collection of the laboratory, which was carefully sampled from a pineapple plantation belonging to PT Great Giant Foods (GGF) in Central Lampung, Indonesia, in October 2017.

The pineapple plants were incidentally exposed to the three types of environmental stressors; two of them were abiotic stressors (herbicide and flooding), and another was a biotic stressor (*Phytophthora* spp). Seven types of soil samples were collected, namely non-stressed soil from

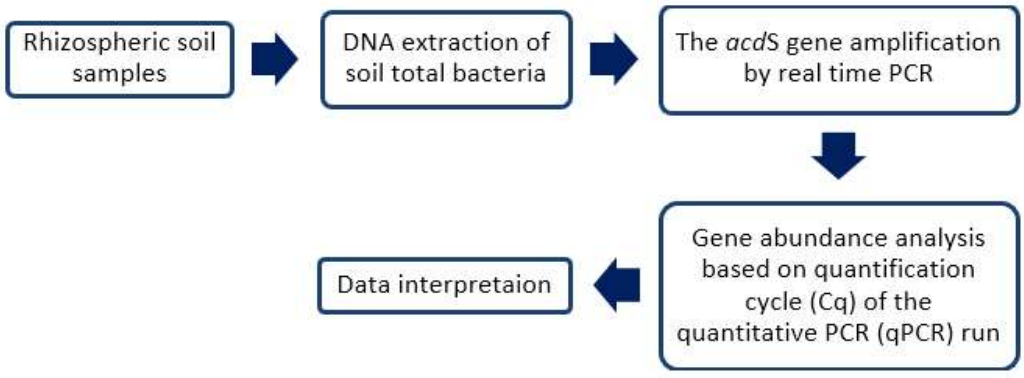

Figure 1. Experimental steps of this study, from DNA extraction to gene abundance interpretation

asymptomatic plants (NSA), herbicide-stressed soil from asymptomatic plants (HSA) and symptomatic plants (HSS), flooding-stressed soil from asymptomatic plants (FSA) and symptomatic plants (FSS), and biotic stressors, *Phytophthora* spp-infected root from asymptomatic plants (PSA) and symptomatic plants (PSS). A 100-gram sample of rhizospheric soil was taken from 6- to 12-month-old plants. Each soil sample was sampled from five plants and combined into one sample using simple randomized sampling. The soil samples were carefully collected from a 20 cm depth. Each soil sample was placed into a 3.5 × 5 inches polyethylene plastic bag with a zip and stored in a cool condition. Lastly, soil samples were stored at 4°C in the laboratory before downstream application.

DNA Extraction of Total Rhizobacteria

Bacterial DNA from soil samples was extracted using Power Soil® DNA Isolation Kit (Mo Bio Inc. USA) following the manufacturer's protocol. Firstly, 0.25-gram soil was put into a Bead Tube and vortexed briefly. A 60 µl of dissolved Solution C1 was added into the Bead Tube and inverted several times. The Bead Tube was secured horizontally using a Vortex Adapter tube holder and vortexed at maximum speed for 10 min. The Bead Tube was rotated freely in the centrifuge (Effendorf 5415 R, Germany) without rubbing at 10,000 × g (times gravity) for 30 sec at room temperature. The sample supernatant was then transferred into a clean 2 mL Collection Tube. Secondly, 250 µl of Solution C2 was added into the Collection Tube containing supernatant, vortexed for 5 sec and incubated at 4°C for 5 minute. The sample was centrifuged at room temperature for 1 minute at 10,000 x g. The pellet was avoided, and 600 µL of supernatant was transferred into a clean 2 ml Collection Tube. Thirdly, 200 µL of Solution C3 was added, vortexed briefly, and incubated at 4°C for 5 min. The tube was centrifuged at room temperature for 1 minute at 10,000 × g. The pellet was avoided, and 750 µL of supernatant was transferred into a clean 2 mL Collection Tube. Fourthly, 1200 µL of Solution C4 was added into the supernatant, vortexed for 5 min. Approximately 675 µL of the sample was loaded onto a Spin Filter and centrifuged at 10,000 × g for 1 minute at room temperature. Three loads for each sample processed were required. Fifthly, 500 µL of Solution C5 was added into the sample, centrifuged at room temperature for 30 sec at 10,000 × g, and the flow-through was discarded. The sample was centrifuged at room temperature for 1 minute at 10,000 x g. The Spin Filter was carefully placed in a clean 2 mL Collection Tube. Sixthly, 100 µL of Solution C6 was added to the center of the white filter membrane. The sample was centrifuged at room temperature for 30 sec at 10,000 × g. The Spin Filter was discarded, and the DNA sample in the tube is now ready for any downstream application. Finally, DNA concentration was measured by NanoPhotometer® (IMPLEN 7122, Germany) at A260/280 ratio, and quality was confirmed at 1.8-2.0.

Amplification of *acd*S Gene by Real-Time PCR

Bacterial DNA was extracted from 0.25-gram rhizospheric soil of each sample and stored at -20°C before use. The soil DNA's partial *acd*S gene copy number was assessed using a quantitative real-time PCR (qPCR) assay. A couple of primers, 105F-*acd*S 5'-TGCCAAGCGTGAAGACTGC-3' and 244R-*acd*S 5'-GGGTCTGGTTCGACTGGAT-3', constructed by Jaya et al. (2019), were used to amplify a 140 bp of partial *acd*S sequence. The qPCR reaction was performed in a 20 µL reaction volume consisting of 10 µL KAPA SYBR® Fast qPCR Master Mix (2X) (Promega, United States), 1 µL (0.2 µM) of each primer, 6 µL nuclease-free water, and 2 µL DNA template. A non-template control (NTC) was used as a control reaction. The qPCR protocol was set at 95 °C for 3 minute, followed by 40 cycles of 95 °C for 30 sec and 58 °C for 30 sec. After the qPCR process, the amplification cycle chart was analyzed. The qPCR reaction was conducted for triplicates using a real-time PCR thermal cycler (Bio-Rad, USA). The data were then presented in CFX Manager software for analysis purposes.

Statistical Analysis

The experimental design was a complete randomized design with three replicates per treatment. The effect of abiotic and biotic stressors was analyzed using a one-way ANOVA. Post-hoc mean separation was then performed using Duncan's Multiple Range Test at $P \leq 0.05$ using the SAS 9.3 software for Windows.

RESULTS AND DISCUSSION

Samples Origin and Soil Analysis

The rhizospheric soil samples originated from the laboratory collection, which had previously been carefully collected by previous researchers from the pineapple plantation. Seven types of soil samples, each derived from five plants, have been published.

Table 1. The chemical properties of soil samples

Variable	Data interpretation
Total nitrogen (%)	Similar among the seven soil samples
Total phosphorus (ppm)	Higher in stressed soil samples than that of control (NSA) and the highest in herbicide-stressed soil from symptomatic plant (HSS)
Total Potassium (ppm)	The lowest in herbicide-stressed soil from asymptomatic plants (HSA) and the highest in herbicide-stressed soil from symptomatic plants (HSS)
Organic-C (%)	Similar among the seven soil samples but the highest in non-stressed soil from asymptomatic plant (NSA)
C:N ratio	The highest in non-stressed soil from asymptomatic plants (NSA), while o ther samples were somewhat lower

Reference: Ratnaningsih et al., 2023

The soil chemical properties of the samples consist of total N, total P, total K, organic-C, and C:N ratio (Ratnaningsih et al., 2023) (Table 1).

The soil samples were categorized based on stressor type. Two types of abiotic stressors and one biotic stressor, namely herbicide, flooding, and fungal pathogen *Phytophthora* spp, respectively, were exposed to pineapple plants. Overtreated herbicide during pineapple cultivation causes the total phosphorus and potassium, lowercase to remain the highest in the rhizosphere of the symptomatic plant (HSS), the unhealthy plant. It leads to the lowest phosphatase activity of the rhizobacteria, while waterlogging happens in particular areas of the plantation during cultivation and causes poor drainage and irrigation when rain comes. The fungal pathogens cause root rot in the symptomatic pineapple plants, the unhealthy plants (Ratnaningsih et al., 2023). The soil samples were non-stressed soil from asymptomatic plants (NSA), herbicide-stressed soil from asymptomatic plants (HSA) and symptomatic plants (HSS), flooding-stressed soil from asymptomatic plants (FSA) and symptomatic plants (FSS), and *Phytophthora* spp-infected soil from asymptomatic plants (PSA) and symptomatic plants (PSS). The herbicide treatment was overtreated to soil during pineapple cultivation; certain areas were flooding, and pineapple root was infected incidentally by *Phytophthora* spp with well-known specific symptom, root rot. Generally, asymptomatic plants are characterized by green and fresh leaves, while symptomatic plants have reddish to yellow leaves. In addition, leaf tips and margins eventually become necrotic, and the root system is dead caused by the biotic stressor.

The Abundance of *acd*S Gene

Table 2 describes the purity and concentration of DNA. All the samples have a relatively small amount of DNA, ranging from the lowest (7.97 ng

Table 2. DNA sample and copy number of acdS gene from total rhizobacteria of soil sample.

Soil samples	A 260/280	DNA concentration (ng μL^{-1} 0.25-g^{-1} soil)	Relative abundance of *acd*S gene (RFU±StDv)
NTC	-	Primer (1 µL (200 nM))	(5.84±2.20) x 10^3
NSA	1.867	13.90 (2 µL)	(7.76±0.82) x 10^3
HSA	1.600	7.97 (2 µL)	(8.51±0.69) x 10^3
FSA	1.889	8.47 (2 µL)	(8.28±1.19) x 10^3
PSA	1.833	11.00 (2 µL)	(8.69±1.39) x 10^3
HSS	1.692	11.00 (2 µL)	(8.32±0.72) x 10^3
FSS	2.000	8.96 (2 µL)	(8.10±1.53) x 10^3
PSS	1.917	10.50 (2 µL)	(6.80±2.37) x 10^3

Note: NTC (non-template control), NSA (non-stressed soil from the asymptomatic plant), HSA (herbicide-stressed soil from the asymptomatic plant) and HSS (symptomatic plant), FSA (flooding-stressed soil from the asymptomatic plant) and FSS (symptomatic plant), PSA (*Phytophthora* spp-infected soil from the asymptomatic plant) and PSS (symptomatic plant). Means followed by the same letter are not significantly different using Duncan's Multiple Range Test at $P \leq 0.05$.

μL^{-1} 0.25-g^{-1} soil) to the highest (13.90 ng μL^{-1} 0.25-g^{-1} soil). However, these were good enough for qPCR analysis. The soil samples of HSA and HSS have the lowest purity ratio below 1.8, at 1.600 and 1.692, respectively. This low purity may indicate contamination of protein, phenol, or humic substances from soil origin that may remain in the samples.

The rhizobacterial *acd*S were quantified to describe soil quality and health. Generally, the *acd*S abundance in the non-stressed soil (NSA) was higher than that of other stressed soils but not from those of flooding-stressed soil (FSA and FSS). Interestingly, the *acd*S abundance in PSA soil was the highest among the samples with a relative abundance of $8.69 \pm 1.39 \times 10^3$ 0.25-g^{-1} soil and the lowest in PSS soil with a relative abundance of 6.80 $\pm 2.37 \times 10^3$ 0.25-g^{-1} soil (Table 2). Furthermore, the *acd*S abundance was not significantly different between stressed soil from asymptomatic and symptomatic plants, except between *Phytophthora* spp-infected soil from asymptomatic (PSA) and symptomatic plants (PSS). However, the trend of lower Cq values of the soil in the asymptomatic plants than those in the symptomatic plants of the same category makes this result unique, indicating the different amounts of *acd*S-containing PGPR (Figure 2).

The abundance of plant-beneficial functional genes in rhizobacteria is an accurate, sensitive, and widely used indicator to study real soil quality and health. Measurements of soil health include gene abundance and function, which has been possible with recent advancements in DNA analysis. The highly conserved 16S rRNA gene is most sequenced, although gene regions encoding specific functional enzymes have also been sequenced, such as *cbb*L (carbon fixation), *nif*H (nitrogen fixation), *amo*A (ammonium oxidizing) (Keshri et al., 2015; Aparna et al., 2016), *pqq*B (phosphate solubilizing), *ipd*C (auxin synthesis), *acd*S (ACC deamination) (Bruto et al., 2014; Akinola et al., 2020), *thi*C (thiamine biosynthesis) (Idris et al., 2018), and others. Nevertheless, one of the specific concerns of many today's studies is the *acd*S gene since its role in expressing ACC deaminase ameliorates plant growth under stressful conditions. Many studies focusing on the *acd*S gene abundance were conducted by Bouffaud et al. (2018), Tyler (2021), and Manter et al. (2023). However, the study of *the acd*S gene in Indonesia at the molecular level, especially on the *acd*S gene abundance and gene expression, needs to be revised. Therefore, the study of *the acd*S gene in Indonesia through this study has just begun to intensify.

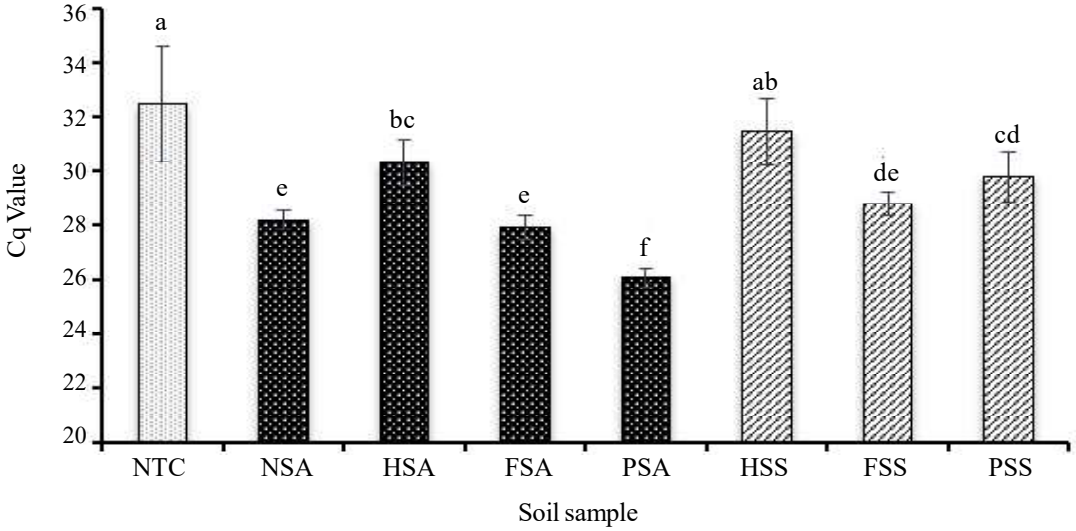

Figure 2. Cq value of rhizobacterial DNA from soil samples. Non-template control (NTC), non-stressed soil from the asymptomatic plant (NSA), herbicide-stressed soil from the asymptomatic plant (HAS), and symptomatic plant (HSS), flooding-stressed soil from the asymptomatic plant (FSA) and symptomatic plant (FSS), *Phytophthora* spp-infected soil from the asymptomatic plant (PSA) and symptomatic plant (PSS). Means followed by the same letter are not significantly different using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. ▯ : no template control, ■ : soil from asymptomatic plant, ▨ : Soil from symptomatic plant

Our research highlights a case study on analyzing the *acd*S gene abundance in the rhizospheric soil under three stressors: herbicide, flooding, and *Phytophthora* spp. that hit the pineapple plantation. The interpretation of the gene abundance based on quantification cycle (Cq) values in the qPCR assay. The Cq values tell us how many cycles it took to detect a target gene from samples, such as the *acd*S gene. The lower Cq values (typically below 29 cycles) mean a high number of *acd*S sequences, while the higher Cq values (above 38 cycles) indicate a lower amount of *acd*S sequences (Oswald, 2023). Although extensive research has been carried out on ACC deaminase-producing rhizobacteria, reports on *acd*S gene abundance still need to be available, especially in Indonesia.

In general, the *acd*S abundance in non-stressed soil (NSA) was significantly higher than those of some stressed soils (HSA, HSS, and PSS) but not in those of PSA soil and flooding-stressed soils (FSA and FSS) (Figure 2**)**. Correspondingly, a previous study by Keshri et al. (2015) reported that some genes like *nif*H and *cbb*L have the highest abundance in non-stressed soil than those of abiotic stressors like salinity, alkalinity, sodicity, and poor nutrient soils. The poor nutrient, such as higher total phosphorus in stress soils (Table 1), apparently corresponds to lower PGPR degrading insoluble phosphorus (Ratnaningsih et al., 2023) and relates to lower ability to produce organic acids (Prijambada et al., 2009), which leads to lower *acd*S gene abundance. Enzyme activities involved in nutrient cycling are also essential for the availability of nutrients in the soil (Aziz et al., 2018). It revealed that the herbicide in HSA and HSS soil and *Phytophthora* spp in PSS soil firmly lower the *acd*S abundance. It means that the herbicide containing the active compound alters gene abundance. Even though certain herbicides can stimulate the growth of soil microbes, others exhibit a repressive effect. For example, Lo (2010) reports that butachlor-type herbicide allows a stabilized population of nitrogen-fixing bacteria simultaneously in a different type of soil. Differently, *Phytophthora* spp in PSA soil could not diminish the *acd*S abundance due to rhizobacterial domination. This result found that the beneficial rhizobacterial domination significantly defends the pineapple plant from fungal pathogens. This result also corresponds to a previous study by Gamalero and Glick (2015) that related rhizobacteria may reduce the colonization of pathogens.

Interestingly, flooding does not affect the abundance. It may be valid since some previous studies found that flooding facilitates anoxic conditions and provides anaerobic microbes such as methanogens and other archaea to develop but suppresses aerobes (Jackson and Colmer, 2005; Bodelier *et al*., 2012). The healthy plant in this study is associated with the ACC deaminase gene (*acd*S), which expresses rhizobacteria and is tolerant to flooding, which has the same effect on plants in non-stressed soil. It indicates a higher gene expression in PGPR associated with healthy plants. Flooded plants grown under inoculation with ACC deaminase gene (*acd*S)-expressing rhizobacteria were taller and greener (Grichko and Glick, 2001). However, Stromberger et al. (2017) reported that drought (up to 54% irrigation) increases the relative abundance of the *acd*S gene in the rhizospheric soil of the RonL winter wheat cultivar. On the other hand, the Ripper winter wheat cultivar also has shown drought tolerance but has a lower abundance of the *acd*S gene, which indicates the gene was genotype-dependent.

Soil from asymptomatic plants tends to have lower Cq values than those of symptomatic plants in the same stressor, indicating that the rhizospheric soil of asymptomatic plants has a higher amount of *acd*S-containing PGPR populating rhizosphere. A specific result showed that the *acd*S abundance was not significantly different between soil from asymptomatic and symptomatic plants (HSA-HSS and FSA-FSS), except in PSA-PSS soil (Figure 2). In this case, *Phytophthora* spp has more impact on altering the rhizobacterial population and it leads to *acd*S abundance alteration. *Phytophthora* spp dominates rhizobacteria in PSS soil and it leads to a lower abundance of *acd*S gene than that of in PSA soil. This result corresponds to when invasive pathogens dominate the rhizosphere, the microbial population will reduce, and the plant becomes diseased. The plant in PSS soil turns into "stress ethylene," which is unfavorable to a lower amount of ACC deaminase-producing rhizobacteria to promote root/shoot proliferation (Singh *et al*., 2015). It also confirmed that suppressive soils contain a higher amount of antagonistic microorganisms than conducive soils, such as actinomycetes, aerobic spore-forming bacteria, and fungi, which may reduce pathogen load in root plants (Duvenhage *et al*., 1991; Syed-Ab-Rahman *et al*., 2019). The present study finds that a higher abundance of *the acd*S gene in PSA and non-stressed soils from asymptomatic plants indicates a higher percentage of those rhizobacteria expressing ACC deaminase, and leads to suppression of pathogen-induced ethylene production catalyzing its precursor, the ACC. As the plant ethylene declines, the ACC deaminase-

producing rhizobacteria produce IAA to promote plant growth in the vegetative growth stage and facilitate stress tolerance to the healthy plant host (Grichko & Glick, 2001; Bal et al., 2013; Barnawal et al., 2017).

The *acd*S abundance in HSA, HSS, and PSS soil was lower than that of other soils. However, the *acd*S abundance was similar among NSA, FSA, and FSS soil. Therefore, the existence of *acd*S gene-encoding rhizobacteria is established by selective stressors. It was also supported that selective pressure determined microbial community, notably caused by the natural environment and nutrient heterogeneity (Zhou et al. (2002). The lower copy number of *the acd*S gene in HSA, HSS, and PSS soil provides evidence that ecological habitats are deteriorated by the herbicide and pathogen load compared with other types of soil. Therefore, future investigations strongly suggest the proper use of herbicides and the application of plant ethylene suppressors and growth promoters, such as the ACC deaminase-expressing rhizobacteria as bio-inoculants. The latter offers an attractive way of managing pineapple plantation in a practical land-use way.

However, this study has a limitation that should be improved. In this case, the Cq value of the NTC sample appeared in cycle 32.49, which coincides with the HSS sample in cycle 31.47, causing the result of these two treatments to be unfavorable. It is due to non-specific amplification and primer-dimer association. However, this result is strongly valid since herbicides in HSS soil suppress the growth of soil microbes and are not significantly different from *acd*S abundance in both samples. In addition, the purity of the HSS DNA sample was below 1.8 but 1.692 instead (Table 2), which indicates humic substances from soil, acidic phenol, and or protein may contaminate the qPCR reaction. Thus, obtaining an excellent reaction optimization in the qPCR run and or DNA purity of the HSS sample to avoid primer-dimer and non-specific amplification must be conducted well in future investigations.

CONCLUSIONS

This study highlights the abundance of *the* genes for acids from the rhizospheric soil of the pineapple plant, which is exposed to proper herbicide, flooding, and *Phytophthora* spp stressors. Our study concludes that selective stressors such as herbicide and *Phytophthora* spp tend to reduce *acd*S gene abundance. At the same time, flooding makes no difference to *acd*S gene abundance to some extent compared to that of control. This result recommends using proper herbicide and bio-inoculants containing *acd*S genes as biocontrol agents against *Phytophthora* spp infection. This should be applied in future land resource management, especially in pineapple plantation. For future studies, quantifying transcript (mRNA) level would give more substantial results, in addition to the abundance of the *acd*S genes (DNA), which reflects the total bacterial *acd*S gene expression in the rhizosphere. There is also a need to assess bacterial population in the rhizosphere. Therefore, a better further study on this relationship would be interesting.

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