CONFERENCE PROCEEDINGS
BIOTECHNOLOGY

Cation Ion Respknses of Sporobolus Virginicus Halophyte Gradd to NaCl and Na2SO4 in the Greenhouse Conditions

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Abstract

Understanding the salt resistant mechanisms used by halophytic members of the *Sporobolus virginicus* with salt stress contributes to the knowledge necessary to agricultural development. The effect of excessive salt concentrations on plants results in osmotic stress and creates an ionic imbalance due to the accumulation of toxic ions. In present study, extreme NaCl and Na_2SO_4 (400-800 mM) were applied to assay the cation ion content. *S. virginicus* showed the capability to reduce the excessive cation ion using salt secretion in the leaf tissues as an alternative channel to reduce ion toxicity. In 600 mM of NaCl concentration showed highest cation ion content comparing to control between Na^+ and Ca^+ which showed 28 times and 15 times higher respectively while same concentration of Na^2So^4 showed lowest which showed 9 times and 4 times higher respectively. Interestingly, among salt concentrations, Na^+ content in salt stressed *S. virginicus* treated with 800 mM NaCl and Na_2SO_4 salt concentration was dramatically decreased 45% as compared to 600 mM NaCl but, higher than 600 mM Na_2SO_4 salt concentration by 54 %. As *S. virginicus* is halophytes plant which able to secrete excess salt out of the cell by using secretion system to avoid toxicity builds up which caused by high accumulation of Na+. The excess Na+ in the cell leads to imbalance of osmotic pressure and nutrition imbalance affected to whole plant regulation. Thus, salt secretion system was alternative way to reduce Na^+ content in *S. virginicus* grown under extreme saline condition as well as to avoid toxicity build up. Moreover, K^+ showed no significantly different against high salt concentration, leading to the loss of K^+ balance and nutrition imbalance as Na^+/ K^+ ratio. In salt affected soil, the levels of K^+ in plant were reduced in accordance with the antagonism between Na^+ and K^+. Excess Na^+ in the plant tissue leads to the loss of potassium due to membrane depolarization by sodium ion

Keywords: Salt tolerance; ion uptake; Sporobolus virginicus; halophyt

Introduction

Soil salinization is an important growth limiting factor for most plant. Around 20% of the irrigated lands and one-third of the world arable soil are affected by a progressive salinization. Salinity in soil or water is one of the major stresses and, especially in arid and semi-arid regions, can severely limit crop production (Glenn et al, 1999).

Plants are often subjected to periods of soil and atmospheric water deficits during their life cycle as well as, in many areas of the globe, to high soil salinity. It is estimated that > 6% of the world's land and 30% of the world's irrigated areas already suffer from salinity problems (Unesco Water Portal, 2007). It affects the plant growth directly through its interaction with metabolic rates and pathways with in the plants [Gebauer et al.,2004].

A great deal of research into salinity tolerance of plants, mainly on water relations, photosynthesis, and accumulation of various inorganic ions and organic metabolites , the metabolic sites at which salt stress damages plants and, conversely, the adaptive mechanisms utilized by plants to survive saline stress are still not well understood. This is partly due to the fact that the mechanisms of salt tolerance are so complex that variation occurs not only amongst species but, in many cases, also among cultivars within a single species (Hasegawa et al., 2010).

Salinization of soils is divided into primary and secondary, depending on the origin of salinity. Natural accumulation of salts for a longtime is primary salinity, such as accumulation of sea salt, which was brought by winds or water, and release of salts through the natural erosion process of rocks. Secondary soil salinization is caused by human activity (Rouphael 2006). The most widespread examples of secondary soil salinization is the use of

irrigation in agriculture. Soil salinity causes the excessive accumulation of ions of salts soluble in water, such as sodium (Na+), chlorine (Cl–), calcium (Ca2+), magnesium (Mg2+), sulfate (SO2-4), and bicarbonate (HCO3–), in soil which affects the growth and development of plants.

Materials and Methods

Plant materials and growth conditions

Sporobolus virginicus were cultivated on plastic tray. After 14 days cultivating, the plants were treat with three different salt concentrations. Firstly, the concentration of NaCl will vary into three salt concentration conditions which are 400 mmol m^{-3}, 600 mmol m^{-3} and 800 mmol m^{-3}. This treatment is held on salt stress condition. Secondly, the concentration of Na2SO4 will vary into three salt concentration conditions which are 400 mmol m-3, 600 mmol m^{-3} and 800 mmol m^{-3}. This treatment is held on salt and acid stress condition. Thirdly, tab water was used as a control.

Cation Ion content measurement

Leaf samples were collected from the plants of each treatment. The samples were cleaned with distilled water and deionized water respectively. Dry barely leaves were grind in a mortar. The homogenate was centrifuge at 1200 rpm for 8 minutes. The samples were dilute into 2 set. First, control set. The clear solution was put into deionized water for 1:70 ratios. Second, sample set. The 1:70 ratios of clear solution and deionized water were mixed by using vortex mixer. Then, the 1:70 ratios between previous solution and deionize water was made. The diluted sample were filtrated into 2 ml vial by 0.45 um filter. The sample were measured to determine ion content (Na+, K+ and Ca2+) by using HPLC, IC PaK Cation M/D 3.9x150 mm column.

Statistical analysis

Data were analyzed using R statistic 2.13.0 for Windows. All results are given as means. The Least Significant Difference was used to calculate the significance of differences between results. Different superscript letters in each compound of the table indicate significance at $P < 0.05$ level.

Result and Discussion

Measuring of the cation ion content is the most commonly utilized technique at present for commercial and research purpose in order to measure maximum ion holding capacity in halophytes plant. Cation ion content provided direct measure of the among of Na+, K+ and Ca2+ separately that present in stem and leaves part of plant sample.

Na^+ Content

Under the saline condition Na+ content was significantly increased directly relating to high salt concentration until the certain level of salt concentration then, amount of Na+ were decreased. Interestingly, among salt concentrations, Na+ content in salt stressed *Sporobolus virginicus* treated with 800 mMNaCl salt concentrations was dramatically decreased 45.7 % as compared to 600 mMNaCl salt concentration (Table. 1) which were 58.03 mg g-1 DW and 31.48 mg g-1 DW respectively. Moreover, among salt concentrations, Na+ content of the stressed *Sporobolus virginicus* with 800 mMNa2SO4 salt concentration was dramatically increased by 56 % compared to 600 mMNa2SO4 salt concentration which it once decreased 24 % from 400 mMNa2SO4 salt concentration. In addition, Na+ content of the stressed *Sporobolus virginicus* with 600 mM salt concentration of both NaCl and Na2SO4 was significantly different between each other. In 600 mMNa2SO4 salt concentration showed Na+ content significantly lower than 600 mMNaCl by 64.34 % .The effect of sulphate toxicity that cause the nutrition imbalance led to the fluctuation of Na+ content as showed in table 1. It's clear that nutrition imbalance or stress didn't cause by only one factor of Na+ content but it's also involve with So_4^{2-} that take place in the solution (Vieira Santos et al. 2000).

Na+/K+ ratio

The K^+ shown no significantly different against high salt concentration, leading to the loss of K^+ balance and nutrition imbalance as Na^+/ K^+ ratio (Table. 1) (Rus et al,2004). In salt affected soil, the levels of K in plant were reduced in accordance with the antagonism between Na and K (Tabosa, 2000). Excess Na ion in the plant tissue leads to the loss of potassium due to membrane depolarization by sodium ions (Nargis et al, 2013).

Salt secretion system

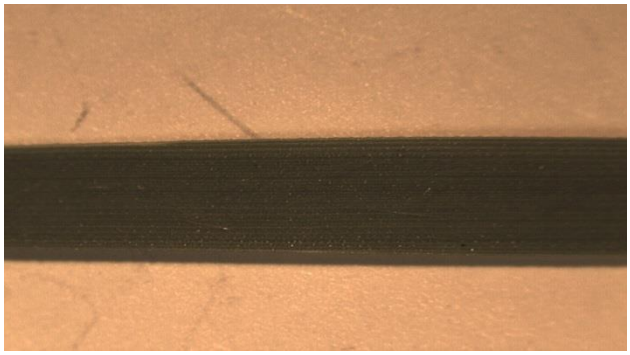
The amount of salt crystal on leaf was directly related to amount of Na^+ content. According to the observation of salt crystal under microscope on the leaves, it's obvious seen by visual eyes that the amount of salt crystal on 600 mMNaCl salt concentration which showed highest accumulation of Na^+ secreted salt crystal less amount comparing to same salt concentration in 600 $mMNa_2SO_4$ (Figure. 1). As *Sporobolus virginicus* is halophytes plant which able to secrete excessive salt out of the cell by using secretion system to avoid toxicity builds up which caused by high accumulation of Na^+. The excess Na^+ in the cell leads to imbalance of osmotic pressure and nutrition imbalance affected to whole plant regulation (Feng Ding. 2012). Thus, salt secretion system was alternative way to help *Sporobolus virginicus* to reduce Na+ content in extreme saline condition as well as to avoid toxicity build up (Nargis NAZ et al, 2013).

Table 1: Leaves cation ion concentration and Na^+/K^+ ratio in *Sporobolus virginicus* grown under 0, 400 mM, 600 mM and 800 mM NaCl and Na_2So_4 salt for 14 days.

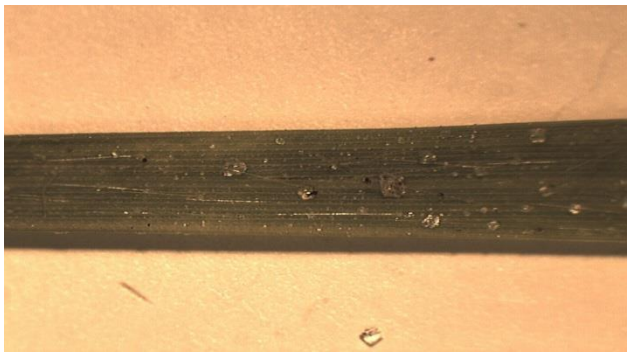
Parameter	Salt conc.	Ions concentrations mmol.g^{-1}DW	
		NaCl	Na_2So_4
Na^+ content	Control	$1.99^{D}\pm 1.02$	
	400 mM	$27.66^{BC}\pm0.81$	$27.46B^{C}\pm2.01$
	600 mM	$58.03^{A}\pm0.75$	$20.69^{C}\pm3.29$
	800 mM	$31.48^{B}\pm4.13$	$32.35^{B}\pm5.07$
Ca^{2+} content	Control	$1.16^{C}\pm0.15$	
	400 mM	$7.31^{BC}\pm1.43$	$9.72^{B}\pm1.52$
	600 mM	$19.14^{A}\pm5.07$	$6.33^{BC}\pm2.02$
	800 mM	$8.33^{BC}\pm1.10$	$9.40^{B}\pm2.49$
K^+ content	Control	$3.31^{A}\pm0.30$	
	400 mM	$4.61^{A}\pm0.61$	$5.68^{A}\pm0.98$
	600 mM	$5.30^{A}\pm1.04$	$3.15^{A}\pm0.34$
	800 mM	$4.13^{A}\pm0.97$	$4.83^{A}\pm1.39$
Na^+/K^+ ratio	Control	$0.37^{C}\pm 0.37$	
	400 mM	$0.92^{AB}\pm 0.92$	$1.06^{BC}\pm 1.06$
	600 mM	$2.63^{A}\pm 2.63$	$1.72^{AB}\pm 1.72$
	800 mM	$2.70^{AB}\pm 2.70$	$1.31^{AB}\pm 1.31$

(Mean ± SE, n=6). Different letters in parameters represent significant difference at $p < 0.05$ by Least Significant Difference (LSD).

A)



B)



C)

Figure 1: Salt crystal secretes on the leaf of *Sporobolus virginicus* grown under control (A) 600mM NaCl (B) and 600mM NaCl (C) for 14 days.

Conclusions

Accumulation of sodium ions was toxic to the cell which leads to imbalance of osmotic pressure and nutrition imbalance which affected to the whole plant regulation. Under the saline condition Na+ content was significantly increased directly relating to high salt concentration until the certain level of salt concentration then, amount of Na+ were decreased by salt gland which responsible to salt secretion system of halophyte plants. Thus, salt secretion system was alternative way to reduce Na ion content in *Sporobolus virginicus* grown under extreme saline condition as well as to avoid toxicity build up. (Liphschitz and Waisel, 1982). Moreover, not only one factor from Na^+ affected to nutrition imbalance or stress which obvious seen that So_4^{2-} also involved that created salt acid condition to the plants which take place in the solution concurrently with Na^+ availability that may involve in Na_2So_4 treatments.

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Isolation and Identification of Palm Oil and Soybean Oil Degrading Bacteria from Bio-extract

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Abstract

Vegetable oils are substances that cause water problem. They released into the environment with wastewater derived from the food processing industry, restaurants and kitchens. They can cause problem in environment due to their physical properties. One method to solve this problem is the use of bio-extract. Bio-extract derived from the fermentation of vegetable and fruits residues with sugar. It contain an organic substances and microorganisms that can degrade oils. The isolation and identification of bacteria from bio-extract which can degrade palm oil and soy bean oil is the aim of this research. The isolation of microorganisms was carried out, using M9 liquid media enriched with 1% (v/v) soybean or 1% (v/v) palm oil added with 2 ml bio-extract. The samples were screened for lipid degrading organisms using serial dilution and spread onto the M9 agar plates enriched with 0.2% (v/v) soybean oil or palm oil and 0.5X PCA media enriched with 0.2% (v/v) palm oil or soybean oil. Thirty microorganisms were isolated from bio-extract that showed ability to degrade palm oil and/ or soybean oil. The bacteria categories in three groups, the first groups including Pcp3y, Pcp16y, M9p10yy, M9p5y, M9p16y, M9p15y, M9p8y, in palm oil, , and M9p16y, Pcp16y, Pcp17y M9p5y and M9p8y in soybean oil. The second groups of bacteria including M9p10yy, M9p15y in soybean, and Pcp17y in palm oil. The first group and second group degrade oils so, these group select as good bacteria due to degrade palm oil and soybean oil. The third groups of bacteria did not degrade oils. So, seven strains of bacteria were selected according to the size of clear zone in soybean and palm oil containing agar plate. The selected bacteria strains were identified using, morphological and physiological characteristics according to the method of "Bergey's manual of determinative bacteriology". The bacteria can be categorized in to three groups. The first group consist of three strains, they are gram positive, rod shape, oxidase negative. The second group consist two strains they are gram negative, rod shape and fermented glucose. The third group consist two strains, they are gram negative, rod shape, and did not fermented glucose.

Keywords: Bio-extract, palm oil, soybean oil, degrade, wastewater

Introduction

Fat, oil and grease (FOGs) is one group of substances use to determine water quality. Fat, oil and grease (FOGs) can be released into the environment with wastewater derived from the food processing industry, restaurants and kitchens or by accidental spill of oils. If they were not treated, they can cause adverse effect in environment due to their physical properties. In natural environment FOGs can cause the covering of animals and plants with oil, reduction of oxygen transfer rate and high biological oxygen demand (BOD) in wastewater (Cipinyte et al., 2009) . On the other hand, accumulation of FOGs in wastewater treatment systems lead to blockage of drainpipes, appearance of unpleasant odors and erosion of sewer pipes (Cipinyte et al., 2009).The main components of FOGs are animal fats and vegetable oil. They may also contain a mixture of glycerol and free fatty acids whenever hydrolysis has taken place (Cammarota, 2006).

FOGs can form oil film in water surfaces, preventing the circulation of oxygen from air into water and leading to the death of many forms of aquatic lives. Collections of oil droplets and other particles present in wastewater can also block water drainage lines. Generally, FOGs are partially recovered from wastewater by air flotation and the remaining residues are treated by physical and chemical method (Mongkolthanaruk , 2002). Then the floated lipid wastes are usually discarded by sanitary landfill dumping, however, this also pollutes the environment (Mongkolthanaruk, 2002). Many microorganisms that are capable of degrading FOGs isolated from soil and water. These genera of bacteria such as *Bacillus, Pseudomonas, Burkholderia, Acinetobacte, Escherichia* and fungi are known to degrade olive oil and tributyrin (Mongkolthanaruk, 2002).

Bio-extract derived from the fermentation of vegetable and fruits residues with sugar is a brownish liquid. It contain an organic substances and effective microorganism. Bio-extract or bio-fermented solution is also called as effective microorganisms (EM). There are different microorganisms in bio-extract such as Lactic acid bacteria, yeast, and Lipolitic bacteria (Kamla, 2007). The benefits of bio- extract have been studied and

one application of bio-extract is to be used in wastewater treatment. Research has shown that EM cultures was effective in purifying wastewaters and sewage effluents (Kamla, 2007).

Lipases (EC 3.1.1.3) are the most important groups of biocatalysts for biotechnological application. They catalyze the hydrolysis and
synthesis of esters from glycerol and long chain fatty acid (Dey et al., 2014). Microorganism have been found produce emulsifying agents or bio-surfactant to help solubilize lipid (Van Dyke et al., 2009). Lipolytic enzymes play an important role in the turnover of the water-insoluble compounds, it can break down lipid. The aim of this study is to isolate bacteria which are able to degrade the vegetable oils from the bio-extract for wastewater treatment application.

Materials and methods

In this experiment our sample is commercial bio-extract purchased from Royal project shop.

Screening and isolation of bacteria

$$\text{Specify activity} = \frac{\left(\frac{\text{ml titrant}}{min}\right) x\ molarity\ of\ tirant\ x1000}{(amount\ of\ protein)/1000ug}$$

Two ml of bio-extract were add to M9 liquid media which enrichment with 1% (v/v) soybean or 1% (v/v) palm oil. Incubated it for one week at room temperature. The enriched sample were screened for lipid degrading organisms using serial dilution and spreaded onto the M9 agar plates and 0.5x PCA plates, These components are provided in the M9 media for 100 ml "M9 salts (5 X)", 20 ml, glucose 20% 2 ml, 1 M Mg SO4 200 µl, 1 M CaCl2 10 µl, H2 O 78 ml. 0.5x PCA media is half concentration of normal PCA. These component are for one litter of 0.5xPCA, tryptone 2.5 g, yeast 1.25g, glucose 0.5g, agar 15g, and water.

Drop plate method was used for measure the size of clear zone, 0.5x PCA media with 0.2% (v/v) palm oil or soybean oil, for selecting the biggest clear zone, for this part prepared 0.5x PCB broth for palm oil and/or soybean oil and PCB for tributyrin. 0.5x PCB broth enriched with 0.2% (v/v) palm oil or soybean oil, then the bacteria inoculated into them, after 48 hours measured the OD 600, then prepared 0.5x PCA media which enrichment with 0.2% (v/v) soybean or 0.2% (v/v) palm oil, also prepare media that contain 1% (v/v) tributyrin as a control, another method was streak method as a selection best strain of bacteria for this condition 0.5xPCA enriched with 0.2% (v/v) palm oil or soybean oil, then the bacteria streak into the plate.

Identification of bacteria

Gram's staining was carried out in order to primarily classify the bacteria. From 48 hours slant cultures, stained the cells using Gram's stain and observed the colonies under the microscope.

To identify the isolated strains, all strains were characterized by classical tests according to
''Bergey's Manual of Systematic Bacteriology''. These tests included: cytochrome oxidase production, catalase production, glucose fermentation, voges-proskauer (vp), motility, gelatin liquefaction, starch hydrolyses, arginine hydrolase, oxygen requirements, pigment production in F agar, and Na^+ required for growing.

Lipase Assay

Preparation of crude enzyme extract

Lipase assay was modified from Dey et al (2014). The bacteria were grown in Erlenmeyer with 50 ml of 0.5xPCB media contain 1% (v/v) palm oil as substrate, the flasks incubated in shaker incubator with 120 rpm agitation at 300 C for 24 hours. Then the cell harvested by centrifugation at 5000 for 15 minute. The supernatant was stored at -200 C for enzyme activity

The crude enzyme were centrifuged at 5000 for 15 minute, then add 1ml of 0.1M Tris-HCl buffer (pH 8), 50 mM KCl, 200 µl Tween 80, 1 ml palm oil and 1 ml of culture supernatant, mixed well on shaker incubator with 60 rpm for 3 hours at 300 C. Then added 3 ml ethanol, the mixture was shaken vigorously to stop the enzyme reaction and the emulsion break down, after that the titration was done with 10 mM NaOH, using phenolphthalein indicator. One unit of lipase activity was amount of enzyme required to liberate 1 µM equivalent of free fatty acid per minute. Specific activity was as units of lipase activity per milligram protein. Specific activity was measured by using following formula

Protein assay

Protein assay was done according to the Bradford method (1976), using the BSA protein to made standard curve, information used for calculating enzyme specific activity.

Result and discussion

Screening and isolation of bacteria

The bacteria isolation was carried out, 18 strains were from M9 media and 12 strains were from PCA media that made clear zone, these were included 15 strains of bacteria and 15 strains of yeast. The result of Statistical Analysis System (SAS) showed, 15 strains of bacteria showed significant difference ($P< 0.05$) in the ability to degrade three kind of oil, (palm oil, soybean oil or tributyrin according to the size of clear zone. All of the bacteria can degrade tributyrin, but 8 of them have ability to degrade palm oil. The bacteria showed the ability to degrade palm oil better than soy bean oil. The result of clear zone showed in table 1. The bacteria which isolated from bio-extract categories in three groups in drop plate method. The first groups include, Pcp3y, Pcp16y, M9p10yy, M9p5y, M9p16y, M9p15y, M9p8y, in palm oil, , and M9p16y, Pcp16y, Pcp17y M9p5y and M9p8y in soybean oil according the size of clear zone are the same, these bacteria make biggest size of clear zone in palm oil and soybean oil.

The second groups of bacteria include, M9p10yy, M9p15y in soybean, and Pcp17y in palm oil, the size of clear zone not significant different. But the size of clear zone are less than first groups.

The third groups of bacteria include, M9p10ycs, M9p10ycb, Pcp4y, M9p3y, M9p4y, Pcp5y, and M9p8w2 these groups of bacteria did not degrade soybean oil or palm oil in drop plate method.

7 strains showed the best ability to degrade palm oil and soybean oil, namely M9p5y, M9p16y, M9p8y, Pcp16y, M9p10yy, Pcp17y, and Pcp16y. In this research the bacteria shown the ability to degrade soybean oil and palm oil, they were isolated from bio-extract. In previous research bio-extract showed the ability to reduce amount of oil in wastewater (Chiu P, 2012). Research showed the bacteria which isolated from soil, such as Bacillus, Pseudomonas, Burkholderia, Acinetobacte, Escherichia and fungi can degraded Olive oil and tributyrin (Matsumiya et al., 2007).

Identification of selected bacteria

Gram staining

Slant cultures were taken after 48 hours, the stained cells using Gram's stain and observed the colony under the microscope. The selective bacteria were two groups, gram positive, rod shapes, and gram negative, rod shape. M9p10yy, M9p5y and Pcp16 were gram positive rod shape and M9p16y, M9p8y, Pcp17y and M9p15y were gram negative rod shape

Biochemical test

The biochemical test was carried out according to the guide line from Bergey's manual of determinative bacteriology (Bergey's manual, 1957). The table 1. Shows biochemical characteristic of bacteria

Table: 1 The size of clear zone for 0.5x PCA + (0.2% v/v) Palm oil and 0.5 PCA + (0.2% v/v) Soybean oil drop method

	0.5 PCA + Palm oil	**0.5 PCA + Soybean oil**
Name	Drop method	Drop method
	size of clear zone (mm)	Size of clear zone (mm)
1.m9p8y	0.14 ± 0.05^{becd}	0.12 ± 0.03^{bc}
2.m9p16y	0.13 ± 0.06^{bc}	0.14 ± 0.01^{bc}
3.pcp16y	0.18 ± 0.02^{ab}	0.13 ± 0.04^{bc}
4.m9p15y	0.15 ± 0.00^{bc}	0.03 ± 0.00^{d}
5.pcp17y	0.06 ± 0.00^{kij}	0.14 ± 0.02^{bc}
6.m9p5y	0.19 ± 0.06^{bc}	0.14 ± 0.06^{bc}
7.m9p10yy	0.16 ± 0.03^{bc}	0.02 ± 0.01^{d}
8.pcp5y	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
9.m9p8w2	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
10.m9p4y	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
11.pcp4y	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
12.pcp3y	0.21 ± 0.00^{a}	0.00 ± 0.00^{e}
13.m9p3y	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}

Table 2: Biochemical characterization of seven isolated bacteria from bio-extract

Test	M9p5y	Pcp16y	M9p10yy	M9p16y	M9p8y	M9p15y	Pcp17y
Gram test	+ Rod	+ Rod	+ Rod	- Rod	- Rod	- Rod	- Rod
Oxidase	-	-	-	+	+	+	+
Catalase	+	+	+	+	+	+	+
VP	na	na	na	+	+	na	na
Glucose	-	-	-	+	+	-	-
Motility	-	-	-	-	-	+	+
Gelatin	-	-	-	-	-	-	-
Arginine	na	na	na	na	na	-	-
Spore	-	-	-	-	-	-	-
Na^+ required	na	na	na	-	-	na	na
Oxygen require	+	+	+	+	+	+	+
Fluorcent	na	na	na	na	na	-	-
Starch	+	+	+	na	na	+	+

Note: + positive reaction, - negative reaction, na, not analyzed

In this study, seven strains of bacteria selected and identification was carried out, for more identification molecular identification is necessary. Physical and biochemical test was carried out to identify bacteria, as a result showed these bacteria categorized in three groups, the first groups (M9p5y, Pcp16y, and M9p10yy) are gram positive and rod shapes bacteria they might be *Corynebacterium spp*, because as a result shows this genus of bacteria are non-motile and straight to slightly, they widely distributed in different ecological such as soil, vegetables, sewage, and skin (Burkovski, 2008). The other groups (M9p8y, and M9p16y) might be belong to the *Aeromonas spp*. because of their characteristics, they are gram negative, rod shapes and fermented glucose, the other characteristic that made it different from another groups they can grow

in 0% NaCl but did not grow in 7% NaCl and also is VP positive, they are found in aquatic environment (Abbott, 2003). The third groups of bacteria are M9p15y and Pcp17y, accordingly the biochemical test results shows these groups of bacteria might be *Pseudomonas spp*. the genus *Pseudomonas* is gram negative rod shape, and aerobic that are wide-spread throughout nature and ecologically significant they are found in soil, fresh water, and marine environments and in plant (Uğur et al., 2012*).* There are some species of Pseudomonas exhibit activity of bioremediation and biocontrol agents in seawater
(Tripathy et al., 2006). The aerobic degradation of FOGs in wastewater by active microbial cultures is a common and one of the most efficient practices of biological treatment (Cipinyte et al., 2009).

Lipase activity

The crude enzyme was extracted, and the lipase assay was carried out according the method of titration, using palm oil as a substrate at 30^0 C. The result shows seven strains produced lipase, statistical analyses system shown all of seven strains no significantly different, they have ability to produce lipase the figure 1. Shows the maximum enzyme specific activity is (22.66 ± 5.9 U/µg). In previous research by Cypinyt *et al*. (2009), shown the bacteria isolated from the environment polluted with various lipid have potential to lipase activity, the best lipase activity was from *Enterobacter aerogenes* and *Arthrobacter sp.* In this research M9p5y have shown the best activity to produce lipase.

The table 2 showed the amount of protein for 7 strains of selected bacteria, as the table shown amount of protein in M9p10yy is higher than the other strains (19.11 ± 3.4 µg) but enzyme specific activity is 13.94 ± 1.2 u/ µg despite M9p10yy produced more protein but protein is not lipase because enzyme specific activity is less than another. In this research bacteria from bio-extract have shown ability to produce lipase.

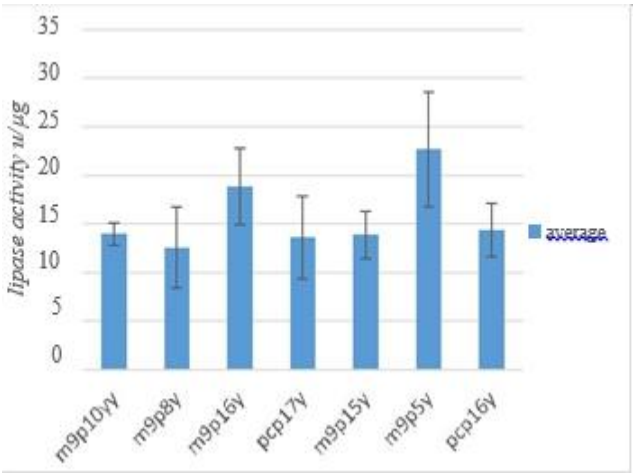

Figure 1. Specific activity for seven strains of bacteria

Table 2: Average amount of protein and enzyme specific activity for seven strains of bacteria

Conclusion

Thirty candidate colonies were isolated from M9 and 0.5x PCA media enriched by bio-extract, 15 strains were bacteria, these bacteria degrade tributyrin, 7 strains selected as the best bacteria including M9p5y, M9p8y, Pcp16y, Pcp17y, M9p10yy, M9p15y, and M9p16y, due to the size of clear zone to degrade palm oil and soybean oil. The result obtained in this study shows that enzyme activity of these bacteria has proved that bacteria have potential candidate for industrial application the highest lipase specific activity is (22.66 ± 5.9) and the lowest is (12.57 ± 4.2). The bio-extract has proved to have potential to use for degrading of palm oil and soybean oil in wastewater.

name	average amount of protein (µg)	average of enzyme specific activity (u/ µg)
m9p10yy	19.11 ± 3.4	13.94 ± 1.2
m9p8y	17.90 ± 1.2	12.57 ± 4.2
m9p16y	14.17 ± 0.9	18.86 ± 4.0
pcp17y	18.06 ± 8.2	13.58 ± 4.2
m9p15y	15.81 ± 1.6	13.88 ± 2.4
m9p5y	12.14 ± 6.8	22.66 ± 5.9
pcp16y	18.13 ± 3.3	14.35 ± 2.7

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