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Research paper



Canna edulis KERR. and spirulina platensis as a prebiotic

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Abstract

Probiotics have benefit on reducing the number of pathogenic bacteria in human the digestive system. One way to keep the amount of probiotics in the human body is to consume prebiotics. One source of prebiotic food is red C. edulis Kerr because contain starch and fiber. S. platensis is a microalgae having 60-70% protein contain and when combined with flour from red tubers C.edulis Kerr will increase nutritional contain. The research about the potency of red tubers Kerr flour and S. platensis powder as prebiotics has done by growing L. casei rhamnosus in De Mann Rogosa and Sharpe Agar (MRSA) as control and control negative without glucose and several modified MRSA. There were glucose MRSA substituted to C.edulis Kerr (MRSA C), peptone to S. platesis (MRSA P) , glucose and peptone to C.edulis Kerr flour and S. platensis (MRSA CP), and also complete flour composite C. edulis, S. platensis and Acalypha indica powder as additive oresevative substantive (MRSA CPA). Indicator potencial flour of Kerr, S. platensis powder in modified medium as prebiotics by calculating Total Plate Count (TPC) methode and test for anti-pathogen bacteria. Gram Test was conducted to make sure the kind of bacteria. The results showed L. casei rhamnosus grew in MRSA and modified medium. The total population of L. casei rhamnosus in MRSA, MRSA C, MRSA CP and MRSA CPA are not significantly (P< 0,05). MRSA P is the highest total of L. casei rhamnosus and when growing in modified MRSA CP can inhibit the growth of E. coli. Gram test bacteria in clear zone showed positif. It indicate L. casei rhamnosus. C. edulis Kerr and S.platensis in modified MRSA have potential as prebiotics by invitro methodology. A. indica in MRSA CPA did not inhibit growth of L. casei rhamnosus.

Keywords: *Probiotics; Prebiotics; Lactobacillus Bacteria; MRSA Modified*

1. Introduction

Probiotic is the microorganisme living in human intestine giving benevite to health (Dommels et al. 2009) such as antiinflamation, antitumor, antihypertension (Toma & Pokrotnieks, 2006). The strains of probiotic are Lactobacillus Bacteria, Bifidobacterium dan Enterococcus (Sari 2012). Prebiotic is medium to stimulate and support growth quality of probiotic. Prebiotic in human intestine cannot digest and absorb, namely oligosaccharide fibers. Consumption oligosaccharide in daily intake can help health condition (Antarini, 2011).

Canna edulis Kerr is a plant of which tubers production can be found from red and white. It contains carbohydrate 70 % including fibers, a lot of total solid solution (TSS) 7.45% (Krisnayudha 2007). TSS is consisted sucrosa, rafinosa, fructose and oligosacharida such as fruktooligosakarida 4.8% (Muchtadi 2010). Based Pramudito (2014) research 100 grams tubers containing 95.00 calory; protein 1.00 g; fat 0.11 ; calsium 21.00 g; phosphor 70.00 g; Fe 1.90 mg; vitamin B1 0.10 mg; vitamin C 10.00 mg and water 75.00g. Noriko and Swandari (2017) Protein in C. edulis Kerr in tubers can be inceasing by mixed up with Spirulina platensis to form Cannalina flour (P 00201508315). It is increasing of immune system respon (Christwardana et al. 2013). Natural preservative substances in Cannalina flour is Acalypha indica . The research showed that cannalina flour from white tubers has potencal as prebiotic. The red tubers was needed to test to know its prebiotic potencal as a medium to support L. casei rhamnosus growth, included the capacity of L. casei rhamnosus to compete E. coli growth.

2. Methodology

The research was conducted in Microbiology Laboratory of Al Azhar Indonesia University within 3 months in 2017. E. coli was found from Siloam Hospital of and L. casei rhamnosus from Microbiology Laboratory of Agryculture Faculty, Bogor Agryculture Institute. The research design used Randomized Block Design Experiment which divided in 6 groups. There were MRSA medium as a positive control, MRSA without glucose as negative control, and many modified in medium cultures namely MRSA without glucose was substituted with C.edulis Kerr flour, MRSA without pepton to S.platensis, MRSA without glucose and pepton to C. edulis Kerr and S. platensis.

Procedures of the research was divided 6 steps .The first steps of the research was made Cannalina flour. Tuber from red C. edulis Kerr was washed by fresh water and minced ±1 mm and continued by oven drying 55 o C within 20 hours and blended dan mixed with S. platensis and A. indica powder. Second step E. coli and L. casei rhamnosus 1 ose were inoculated in 10 mL NaCl fisiologis 0.85% and continued to dilute 〖10〗^(-5) 〖 until 10〗^(-7) twice in each other (duplo) and put in medium. E. coli medium cultured was NA 14 gr in 500 ml aquadest continued to incubate in 37 oC within 48 hours.

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Third step was cultured L. casei rhamnosus in several dilution by using streak plate methode in petridish contained sterilized 32.5 gr MRSA in 500 mL aquadest. Ingredient of MRSA medium without glucose are 20 gr red flour from red tubers C. edulis Kerr, 10 g protease pepton, 8 g Beef extract, 5 g Yeast extract, 2 g amonium sitrat, 1 ml Tween, 20 g Na-asetat, 0.58 g MgSO4, 0.28 g MnSO4, 2 g K2HPO4, and 10 gr gelatin, and dissolved in1 liter aquadest. Formulation MRSA without pepton using S.platensis to substitution 10 gr spirulina powder , 8 gr Beef extract, 5 gr Yeast extract, 2 gr amonium sitrat, 1 ml Tween, 20 gr Na-asetat, 0.58 gr $MgSO_4$, , 0.28 gr $MnSO_4$, 2 gr K_2HPO_4, 20 gr glukosa, dan 10 gr gelation. The others formulation medium were added C.edulis Kerr and S. platensis to substitution glucose and pepton and medium with A. indica 0.03 g for 1 liter. All af medium with HCl 1% atau NaOH 1% to increasing and decreasing pH 6.4-6.6 and continued to sterilized in autoclaf 121 ^oC for 15 minutes. L. casei rhamnosus cultured in petridish contained 10 mL medium and incubated 37 ^oC within 48 hours.

Table 1: Composition of L. Casei Rhamnosus in Modified

Ingredient	Medium					
	MRSA	MRSAC	MRSAP	SMRSACCP	MRSACPA	Non Glucose
Peptone [10gr/L]	√	√	–	–	–	√
Beef Extract [8gr/L]	√	√	√	√	√	√
Yeast Extract [5gr/L]	√	√	√	√	√	√
Ammonium Citrate [2gr/L]	√	√	√	√	√	√
Sodium acetat [5gr/L]	√	√	√	√	√	√
MgSO4 [0,2gr/L]	√	√	√	√	√	√
MnSO4 [0,05/L]	√	√	√	√	√	√
Dipottassium Phosphate [2gr/L]	√	√	√	√	√	√
Agar [12gr/L]	√	√	√	√	√	√
Tween [2ml/L]	√	√	√	√	√	√
Glukosa [20gr/L]	√	–	√	–	–	–
C. edulis Kerr[20gr/L]	–	√	–	√	√	–
Spirulina [10gr/L]	–	–	√	√	√	–
A. ndica [0,03gr/L]	–	–	–	–	√	–
Aquades [1 L]	√	√	√	√	√	√

The fourth steps were potencial Test C. edulis Kerr as prebiotic by counted colonies of L. casei rhamnosus and usually will find 30 - 300 colonies. Total Plate Count (TPC) methode (Yunita et al. 2015).

Total population (CFU/mL)= Total Colonies x 1/ dilution factor x Total volume bacteria which was planted (0.1mL)

The fifth steps was test of anti pathogen microba activity of L. casei rhamnosus (Maunatin & Khanifa 2012). L. casei rhamnosus 2-3 ose inoculated in 10 mL MRSB and incubated within 2 days in 37 °C. E. coli 1 ose incubated in 10 mL NB in 37 °C. Control and modified medium MRSA 50 mL mixed with 25 µL E. coli and poured 25 mL 〖(10〗^8 CFU/mL) to petridish. After medium ossified made well diameter 6 mm with using blue tip pipet 1 mL Total bacteria was minimal tolerance limite to human infection. L. casei rhamnosus 50 µL droped to the well and incubated 37 °C for 2 days. Indicated of capability to inhibit E coli growth showed by clear zone appear around the well (Rachmawati et al. 2005).

Gram positive and negative Test (Yusmarini et al. 2009)

Bacteria sample from clear zona and outer was tested washed by alcohol 70% and drying in fire of the bunsen and continued with coloring with 3 drops cristal violet in I minute. After drying put 2 drops Lugol in 1 minutes and washed in water and drying after that continued to droped 3 drops alkohol 70% within 30 seconds. Put 3 drops safranin within 20 seconds. Bakteri Gram positif will show the purple colour and gram negatif the red colour (Kholisoh 2016) in microscope enlarger 100 x with assited by immerse oil. Data analysis with two way univariate and post hoc test IBM SPSS Statistic 19

3. Result and discussion

Out of 121.5 gr wet C.edulis Kerr flour only get 80.3 gr dry flour with 10.09% water concentration (Figure 1), It means the flour contains 30% water. Based on Purwaningsih et al. (2013) beside water the flour contain ash 3.25%, lipid 0.43%, protein 2.34%, fibers 5.12% carbohydrate 70.36% .

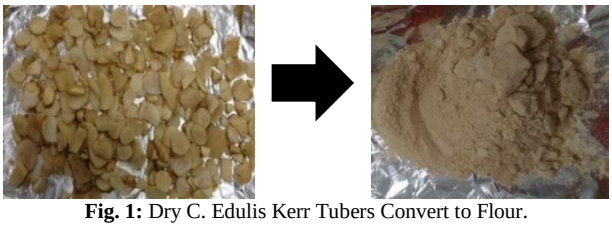


Fig. 1: Dry C. Edulis Kerr Tubers Convert to Flour.

(Martini 2013) said decreassing of water in the flour can inhibit microorganism growth. Richana dan Sunarti (2004) said C. edulis Kerr contain 78.9% carbohydrate and Krisnayudha (2007) explain fruktoolioligosaccharide which can be fermentate by Lactobacillus bacteria (LAB)

Potencial Test C. edulis Kerr as Prebiotic

Lactobacillus casei rhamnosus growth in MRSA, MRSA C, MRSA P, MRSA CP, and MRSA CPA. Total LAB each ather 6.12 x 10^7 CFU/mL, 3.57 x 10^7CFU/mL, 7.12 x 10^7 CFU/mL, 5.07 x 10^7 CFU/mL, 4.48 x 10^7CFU/mL In negative control Lactobacillus casei rhamnosus did not grow caused no glocosa in its containing (Figure 2 and Graph 1)

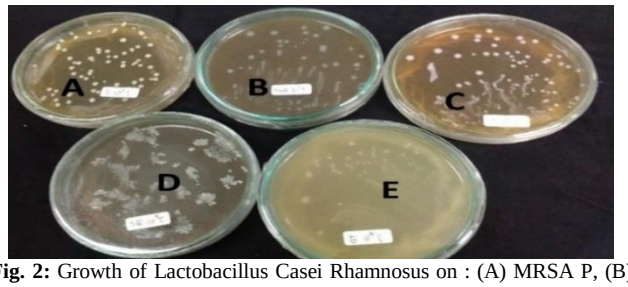
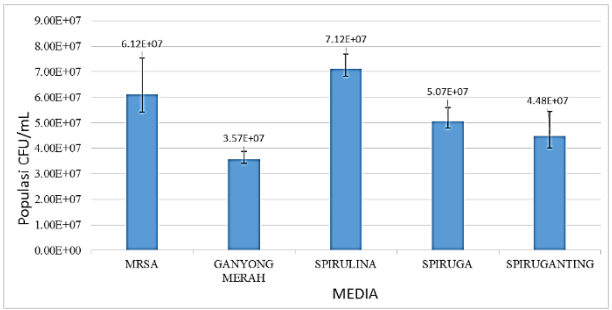


Fig. 2: Growth of Lactobacillus Casei Rhamnosus on : (A) MRSA P, (B) MRSA PC, (C) Media MRSA, (D) MRSACPA, (E) MRSAC.

Based on Graph 1. The series of medium showed total L. casei rhamnosus from the higest to the lowest are MRSA P, MRSA , MRSA CP, MRSA CPA, and MRSA C

MRSA P is the higest capacity medium to support growth of L. casei rhamnosus. Statistic analysis showed MRSA P significant P ≤ 0.05 is the higest suport growth L. casei rhamnosus compared with other medium . It is caused S. platensis contain protein 60-71% (Widianingsih et al.2008) and amino acid such as metionine, sistine, lisine dan tryptophan and fatty acid palmitic, oleat, linoleat. The other reason Spirulina sp. can substitute pepton as source of protein in MRSA, betakaroten as precursor to vitamin A, vitamin B and 3- 7% mineral (Chriswardana et al. 2013). Glucosa in S. platensis is the first substances which consumed by bacteria in medium culture (Tamime & Robinson 2007).



Graph. 1: Total Bactery in Several Growth Medium (CFU/Ml).

Total L. casei rhamnosus in MRSA C, MRSA CP and MRSA CPA lower than MRSA P caused bacteria had to hydrolyzed carbohydrate to glucose, fruktosa dan mannose. Spirulina sp contain 18% carbohydrate such us glucose, rhamnosa, mannosa, xylosa and galactosa. Actually glucose in C. edulis Kerr red tubers only 10%. (Krisnayudha 2007). Other reason red tubers of C. edulis Kerr contain flavonoid which has function to destruct bacteria wall, lisosom and mikrosom L. acidophilus (Fatmala 2015). Hidroksil in flavonoid will change organic substances and give effect toxic to bacteria (Sabir 2005). Herdiansyah (2016) report the kind of flavonoid are isoflavon, dihidroflavonol dan flavonol. The lower total L. casei rhamnosus ocured in MRSA CPA and, It caused A. indica contain antibacterial substances (Pambudi et al. 2014) that give effect to inhibit growth of L. casei rhamnosus. MRSA did not indicate significant difference with all of modified medium except MRS P. It showed C. edulis Kerr and S. platensis have potensial as prebiotic. Growth of L. casei rhamnosus based on statistica not significant $P \geq 0.05$ between kontrol positif (MRSA) and MRSA P and MRSA C. MRSA is standart growth medium for probiotic bacteria (table 2)

Table 2: Function Ingredient MRSA for Grosth of Bacteria (Sutarman 2000)

Ingredient	Function
Peptone	Source of amino acid and micronutrien
Beef Extract	Source of organic base
Yeast Extract	Source of vitamin B- complex to stimulate bacteria growth
Ammonium Citrate	Source of nitrogen organic
Sodium acetat	pH stabilized for growth of bacteria
MgSO4	Source of metal anorganic
MnSO4	Source of Sulphur for biosynthesis amino acid
Dipottassium Phosphate	Source of phosphor to synthesis nucleic acid
Tween	To help nutrition absorbtion
Agar	For medium to compact and solid
Glukosa	Source of energy
Aquadest	Media solution

L. casei rhamnosus which planted in MRSA CP formed clear zona diameter 190 mm average. It indicated L. casei rhamnosus inhibited growth of E. coli (Fig. 3). Based on Priyatmoko (2008) diameter clear zone > 20 mm the higest inhibition capasity. Clear zone caused by L. casei rhamosus produced metabolite secunder (Cardici and Citak, 2005). Accumulation of metabolite secunder were lactic acid, etanol, acetic acid, carbondioxide and bacteriosin (Delgado et al. 2001), carbohydrate fermentation (Rachmawati et al. 2005) and flavonoid (Primasari, 2015) Bacteriosin has capacity to penetrate bacteria membrane and change permeability anda also inhibiting protein, nucleic acid, enzyme synthesis intracellular and metabolisme (Pelezar et al. 2008).

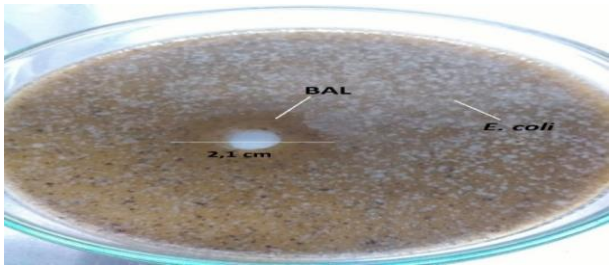


Fig. 3: Inhibition Activity L. Casei Rhamnosus to Patogen E. Coli.

Gram Test

The gram positive bacteria is coming from clear zone in medium but negative gram from the outer. Bacteria gram positif is L.casei rhamnosus having peptidoglican in its wall. Gram negative is E. coli having lipid in its wall (Fitri & Yasmin 2011). Peptidoglican mantenance bounding between christal violet and iodium, but in gram negative bacteria do not made this reaction.

4. Conclusion

Red tubers of C. edulis Kerr, S. platensis and both combination have potensial as prebiotic included to inhibit E. coli growth with invitro methodology. A. indica does not inhibit L. casei rhamnosus growth. Gram positif test showed by l. csei rhamnosus and E. coli gram negative

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