USE OF RED BEANS (*Phaseolus vulgaris* L) AS A SOURCE OF PROTEIN SUBSTITUTE FOR SOYBEAN (*Glycine max*) IN THE MEDIA BROWN JELLY FOR *Neisseria gonorrhoeae* GROWTH

Eem Hayati[1], *Iis Kurniati*[1], *Yeni Wahyuni*[1]

[1]Department of Medical Laboratory, Poltekkes Kemenkes Bandung, Jalan Babakan Loa Cimahi Utara , Indonesia. ZIP code 40514

Corresponding author: eem.hayati@yahoo.co.com

Abstract. **Background**: Protein is one group of macronutrient materials that play a more important role in the formation of biomolecules than the energy source. However, if the bacteria is lack of energy, then this protein can also be used as an energy source. Protein in the side dishes are found in animal products derived from various types of egg meat, fish and milk, then in nuts such as, soybeans, red beans, green peas, peanuts. Based on the determination of protein content in red beans using the Kjeldhal method On every 100 g of red beans (*Phaseolus vulgaris L*) contains protein nutrients (23 g) and carbohydrates (61 g), fat (1.5 g), Calcium (260 mg), phosphorus (260 mg), sodium (15 mg).

Methods : On laboratory tests for *N.gonorrhoeae* one of them is with growth media, the sample is isolated on special media, for example Chocolate agar medium, Thayer Martin or Modified Thayer Martin media. *N.gonorrhoeae* is best grown on media containing complex organic substances such as heated blood, hemin, protein and in air spaces containing 5% CO_2. This study aims to determine whether red beans (*Phaseolus vulgaris L*) can be used as a source of soy substitute protein (*Glycine max*) that can grow *N.gonorrhoeae.* This study used One Way Anova Statistical Test to know the average difference between the experimental and control groups.

Results : The results of colony growth analysis in this study can be concluded that Chocolate Agar agar using Red Beans can be used as an alternative medium of growth of *N.gonorrhoeae* and concentration

Conclusion : Red Beans can be used as an alternative medium of growth of *N.gonorrhoeae* The minimum effective concentration of red beans to grow *N.gonorrhoeae* is 5.2 grams.

Keywords: Protein, *Phaseolus vulgaris L*, *Glycine max*, Chocolate agar, *N.gonorrhoeae.*

Introduction

Neisseria gonorrhoeae is a gram negative bacterium, nonmotilic, not spore-shaped, colonizing to form a diplococcus, or single (monococcal). This bacterium causes gonorrhea or gonorrhea. This disease is a sexually transmitted infection (STI) that often occurs and humans are the only natural host. (Pelczar, MJ. (2009).

According to the World Health Organization (WHO) data in 1999 there were 62 million cases of gonorrhea in the world that were ranked third in all cases of sexually transmitted infections (STIs). In 2012 there were 78.3 million new disease sufferers caused by N.gonorrhoeae throughout the world. In Indonesia, the Ministry of Health in 2011 conducted a survey with the name of integrated biological and behavioral methods, which stated that 4,339 people were infected with gonorrhea. (Irianto, 2014)

One of the laboratory tests for N.gonorrhoeae is growth media, samples were isolated on special media, for example Agar Chocolate, Thayer Martin or Modified Thayer Martin media (Soemarno 1987)

N.gonorrhoeae is best grown on media containing complex organic substances such as heated

blood, hemin, protein and in air chambers containing 5% CO2.

Protein is one group of macronutrient materials that play a more important role in the formation of biomolecules than energy sources. However, if the bacteria are lacking in energy, this protein can also be used as an energy source. The protein in side dishes found in animal products comes from various types of eggs, fish and milk, then on nuts such as soybeans, kidney beans, green beans, peanuts. (Hagerman, AE. 2002)

Based on the determination of protein content in red beans using the Kjeldhal method In every 100 g of red beans (Phaseolus vulgaris L) contains protein nutrients (23 g) and carbohydrates (61 g), fat (1.5 g), calcium (260 mg), phosphorus (260 mg), sodium (15 mg).

In this research, Tryptic Soy Agar (base on making Chocolate Agar), Soybean (Soy) on Tryptic Soy media was used to be replaced with red beans, then N.gonorrhoeae was grown and incubated for 48 hours. The diameter, number, and characteristics of the colony are assessed on this media.

In Trypticase Soy Agar media has a composition of 15 g casein, 5 g soybean, 5 g Sodium chloride, 15 g agar and 5% blood in, Aquadest 1000 ml.

The blood cells used are lysed so that erythrocyte cells release intracellular materials such as hemoglobin, hemin and coenzin Nicotinamide Adenine Dinucleotida (NAD), in the procedure for making agar media after the blood is heated again using a waterbath at 80-90oC for 10-15 minutes until it's brown.

On the principle of TSA, Casein and peptone soybeans provide nitrogen, amino acids, and peptides needed for bacterial growth. Dextrose is a source of energy, sodium chloride supplies important electrolytes and maintains an osmotic balance and agar as a compactor agent (Jawet, 1995).

Trypticase Soy Agar (TSA) is a growth medium used for isolating and cultivating various types of aerobic bacteria. This medium is used for a variety of purposes which include maintenance of aquaculture stocks, isolation of various species of bacteria, and as a basis for seeding including blood.

In a previous study from Maharani (2011), a study was conducted on media to optimize Haemophilus influenza in ACTSA media (Chocolate Agar with Trypticase Soy Agar) with ACAN (Order chocolate with Agar nutrient) as a control. After 48 hours incubation, the diameter of H. influenza colonies in ACTSA was significantly greater than that of ACAN. The conclusion is that chocolate from blood with TSA (ACTSA) is feasible as an alternative growth medium for H. influenza.

In this study red beans were used as a substitute for soy protein in Chocolate Agar media, based on the protein content of 23 grams of kidney beans per 100 grams of red beans.

In the preliminary test the use of kidney beans with a concentration of 2.6% as a substitute for soybean protein in Chocolate Agar Media showed that N.gonorrhoeae can grow well on the media so that chocolate uses red beans.

This study aims to determine the minimum concentration of red beans (Phaseolus vulgaris L) which is effective as a source of soybean substitute protein (Glycine max) in growing N.gonorrhoeae.

Methods

This research was conducted at the Microbiology Laboratory of the Department of Health Analyst of the Health Ministry of Bandung in the period March 2017 to October 2017. The analysis of the determination of plants (red beans) was carried out at the Bandung Institute of Technology, School of Life Sciences and Technology. The proximate analysis of red bean flour was carried out at the Basic Chemistry Laboratory of the Department of Health Analyst, Ministry of Health, Bandung.

To determine the number of repetitions, the formula Gomez (1996) is used, namely: (t - 1) (r - 1) 20, t is treatment (treatment) in this case there are 4 treatments, namely treatment of modified media with variation of concentration Peanuts (Concentration A, Concentration B, Concentration C), and control group Brown Agar, r is replication (repetition) and 20 is a general degree of greatness.

If it is known that t = 4, the number of repetitions in this study is

(t - 1) (r - 1) 20
(4 - 1) (r - 1) ≥20
3r - 3 ≥20
3r ≥20 + 3 Then r = 23/3 = 7.66 = 8

The results of repetition are 8 times.

N.gonorrhoeae samples were taken from pure strains with turbidity similar to Mc Farland 0.5 and then planted on Chocolate Agar media (Control) and on Chocolate Agar (Experimental) media using red beans.

Sample size (n)
= r x t
= 8 x 4 = 32

So that the size of the study sample is 32 samples

The data used is primary data. Data was collected by looking at the results of the growth of the test bacteria namely N.gonorrhoeae based on the number of bacteria and the diameter of the colony. The research data in the form of the number of bacteria and colony diameter growing on alternative media will be analyzed using the Anova Two Way test (two-way anova) at a

confidence level of 95% or α = 0.05 using the SPSS program.

Results and Discussion

Determination of red beans is done at the ITB School of Life Sciences and Technology (SITH) (attached). The proximate test for red beans is done at the Chemistry Laboratory of the Department of Health Analyst (attached). The N.gonorrhoeae affirmation test included: microscopic examination, breeding, oxidase test, biochemical test and PPNG test (betalactamase test). From the affirmation test positive for N.gonorrhoeae sensitive to Penicillin.

The following is a table of calculation of the amount of N.gonorrhoeae as a test bacterial isolate. That is :

Table 2.
The calculation of the number of N.gonorrhoeae as a test bacterial isolate

Treatmen	Amuont(CFU/ml)
10^{-1}	>300
10^{-2}	>300
10^{-3}	>300
10^{-4}	>300
10^{-5}	125

From table no.2, it was found that N.gonorrhoeae isolates used for the study were at 10-5 = 125 CFU / ml dilutions.

The results of the calculation of the number of colonies in agar media Alternative brown beans with media controls can be seen in the following table.

Table 3.
Research Results Using Pure Strains of N. gonorrhoeae

Replication	***N.gonorrhoeae***							
	Control		Red Beans					
			1,3 g		2,6 g		5,2 g	
	1	2	1	2	1	2	1	2
1	249	1	200	1	220	1	240	1
2	260	1	182	1	210	1	245	1
3	239	1,5	202	1	202	1	239	1
4	212	2	200	1	198	1	250	1
5	230	2	192	1	224	1	235	1
6	242	1,5	208	1	230	1	240	1
7	236	1,5	190	1	212	1	245	1
8	250	1	200	1	200	1	235	1

Information :
1: Filled with data on the number of bacterial colonies (CFU / ml)
2: Filled with data on bacterial diameter (mm)

Control here uses pure strain of N. gonorrhoeae

To find out the role of red beans as a protein source, the data analysis was done using One Way Anova for both the number of colonies and the diameter of the colony. From the ANOVA test shows that there are significant differences between the diameters of colonies planted in the control medium and medium of red beans. After a range test can be concluded that the colony diameter in the control medium is significantly greater than the three concentrations of red beans that are treated. Thus the minimum effective concentration cannot be determined as a substitute for protein. While the concentration of red beans with the number of colonies which is almost the same as the control medium is at a concentration of 5.2 g. thus it can be concluded that the minimum effective concentration of red beans as a substitute for protein growth medium N. gonorrhoeae is 5.2 g.

Table 4.
The average diameter and characteristics of the colonies of N.gonorrhoeae concentration of 1.3 grams of red beans

Replication	Control		Consentration1,3 gram	
	Average Colony diameter mm	Character Colony Scale 1 – 2	Average Colony diameter mm	Character Colony Scale 1 - 2
1	1 mm	1	1 mm	1
2	1mm	1	1mm	1
3	1mm	1	1mm	1
4	1 mm	1	1mm	1
5	1mm	1	1 mm	2
6	1mm	1	1mm	1
7	1mm	1	1mm	1
8	1mm	1	1 mm	1

Table 5.
The scale value of the characteristics of the colony in the variation of the concentration of Red Beans

Value	Information
1	Good = clear, convex, round mucoid colonies
2	Not good = Colonies are clear, flat, piled up, not mucoid

Based on the table above it can be seen that the average colony diameter and colony characteristics in the experimental group concentrating 1.3 grams was almost the same as the control group. Except for the characteristics of colonies on repeating the 5 colonies in the media categorized as not good.

Table 6.
The average diameter and characteristics of N.gonorrhoeae colonies concentrated 2.6 grams of red beans

Replication	control		Consenterati on 2,6 gram	
	Average Colony diameter mm	Karakter Koloni	Average Colony diameter Mm	Character Colony
		Scalae 1 - 2		Skale 1 – 2
1	1 mm	1	1 mm	1
2	1 mm	1	1 mm	1
3	1 mm	1	1 mm	1
4	1 mm	1	1 mm	1
5	1 mm	1	1 mm	1
6	1 mm	1	1 mm	1
7	1 mm	1	1 mm	1
8	1 mm	1	1 mm	1

Table 7.
The scale of the characteristic values of the colonies on the variation in the concentration of Red Beans

Value	Information
1	Good = clear, convex, round mucoid colonies
2	Not good = Colonies are clear, flat, piled up, not mucoid

Based on the table above it can be seen that the average colony diameter and colony characteristics in the experimental group with a concentration of 2.6 grams was almost the same as the control group.

Table 8.
The average diameter and characteristics of the N.gonorrhoeae colonies concentrated 5.2 grams of red beans

Replication	Kontrol		Konsenterasi 5,2 gram	
	Average Colony diameter mm	Character colony	Average Colony diameter mm	Character Colony
		Scale 1 - 2		Scale 1 - 2
1	1 mm	1	1 mm	1
2	1 mm	1	1 mm	1
3	1 mm	1	1 mm	1
4	1 mm	1	1 mm	1
5	1 mm	1	1 mm	1
6	1 mm	1	1 mm	1
7	1 mm	1	1 mm	1
8	1 mm	1	1 mm	1

Table 9.
The scale of the characteristic values of the colonies on the variation in the concentration of Red Beans

Value	Keterangan
1	Good = clear, convex, round mucoid colonies
2	Not good = Colonies are clear, flat, piled up, not mucoid

Based on the table above it can be seen that the average colony diameter and colony characteristics in the experimental group concentrating 5.2 grams was the same as the control group.

Table 10.
Results of Calculation of the Amount of Colonies on Chocolate Agar media with Red beans

Total colony CFU/mL (Control)	Replication	Total coloniy CFU/mL	Total Colony CFU/mL	Total Colony CFU/mL
		1,3 gram	2,6 gram	5,2 gram
98	1	57	71	87
93	2	60	73	89
85	3	61	76	86
90	4	62	70	88
89	5	58	71	89
89	6	59	75	87
89	7	60	74	86
85	8	62	71	87
89	*mean*	59,88	72,63	87,38

Based on the results of observations in the tables and figures above, the average values of each concentration are obtained. At the control concentration the average number of colonies was 89 CFU / mL, the concentration of 1.3 grams was obtained by 59.88 CFU / mL, the concentration of 2.6 grams was obtained by 72.63 CFU / mL and for a concentration of 5.3 grams it was obtained at 87, 38 CFU / mL.

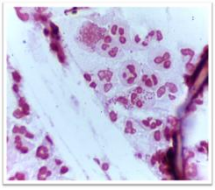
Concentration of drinking red beans for N. gonorrhea growth can be seen at a concentration of 1.3 grams with an average number of colonies of 59.88 CFU / mL while the number of colonies approaching the control can be seen at a concentration of 5.2 grams with an average the number of colonies was 87.38 CFU / mL.

As for knowing whether there is a real difference from each concentration of the use of red beans as a substitute for soybeans in the

media In order for chocolate to grow N.gonorrhoeae then a comparative analysis is carried out with the criteria if the p-value is smaller than α (0.05) then the conclusion is significant differences from each concentration with the determination of hypotheses as follows:
Ho = There is no significant difference from each concentration of red beans as a source of protein substitutes for soybeans in chocolate agar media for the growth of N.gonorrhoeae
Hi = There is a significant difference from each concentration of red beans as a source of protein substitutes for soybeans in chocolate agar media for the growth of N.gonorrhoeae
Decision-making criteria where α = 0.05 or 5%
Reject Ho if p-value <0.05
Accept Hi if p-value> 0.05
From the results of the ANOVA test, it is known that the p-value obtained is 0,000 with a significance level of α = 0.05. Because the p-value is smaller than the significance level α (0,000 <0,05) so that Ho is rejected which means that there are significant differences based on the average number of colonies in the concentration of using red beans for the growth of N.gonorrhoeae.

To see more about the differences in the three concentrations statistically, the following are the results of the post-hoc test using the Duncan method. From the Duncan test, it was seen that the lowest average was at a concentration of 1.3 grams at 59.88 CFU / mL, significantly different from the concentration of 2.6 grams (72.63 CFU / mL), while the highest average was at a concentration of 5.2 gram of 87.38 CFU / mL. And from the test it was seen that the number of colonies that were very close to the control group was a concentration of 5.2 grams (87.38 CFU / mL).


a


b


c


d

Picture :
a. Red bean flour, b. Photograph of the results of microscopic N.gonorrhoeae, c. Calculation of the number of colonies in the media alternative for chocolate, d. Incubate in the wax cap dexatorator on the incubator 37^0 C

This research is an experimental study with the aim to see the growth of N.gonorrhoeae by measuring colony diameter (mm), colony count (CFU / mL) and observing the characteristics of colonies at a minimum concentration of red beans as a source of soybean protein substitutes in Chocolate Agar media.

N.gonorrhoeae is best grown in media that contain complex organic substances such as heated blood, hemin, protein, DNA and in air chambers containing 5% CO2 (3-7%), N.gonorrhoeae is an anaerobic facultative bacteria that can live well with oxygen or without oxygen.
Just like other microorganisms also need food for their needs, microorganisms need organic matter, in order to get food, cells must be able to digest that food, which is to convert complex and large protein, carbohydrate and lipid molecules into simple and small molecules that dissolve immediately so that it can enter the cell. The process of assimilating food is what is called nutrition. (Budianto, 2011)

Red bean seed is a food that has high energy and a potential source of vegetable protein, therefore its role in efforts to improve nutrition is very important. Besides being rich in protein, red bean seeds also have a complete array of essential amino acids. The limiting amino acids in red bean protein are methionine and cysteine with relatively low content, 10.56 and 8.46 mg / 100g. The protein content and amino acid profile in 100 grams of kidney beans from the most are lysine (1323 mg), aspartic acid (1049 mg), leucine (693 mg), glutamic acid (595 mg), arginine (537 mg) , serine (472 mg), phenilalanin (469 mg), valine (454 mg), isoleucine (383 mg), proline (368 mg), threonine (365 mg), alanine (364 mg), glycine (339 mg), methionine (10.56 mg) and cysteine (8.46 mg) (Kay, 1979). in addition to being a source of protein for beans other than as a source of protein as well as a source of minerals. (Sukami, 1979)

In this study the Tryptic Soy Agar (base on making Chocolate Agar) was used in the composition of TSA containing casein, sodium chloride, soybeans and agar. Whereas in alternative media the composition is adjusted to the control composition, only replacing soybeans with red beans as a source of protein in the media.
The results showed that red beans at a concentration of 1.3 grams, 2.6 grams, and 5.2 grams were able to grow N. gonorrhoeae. From the results of the study, the average number of N.gonorrhoeae colonies in the medium for control chocolate was 89. As for the media to make chocolate using red beans at a concentration of

5.2 grams had an average number of colonies of 87.38 (87) then at concentration 2 , 6 grams has an average of 72.63 (73) while the concentration of 1.3 has an average of 59.88 (60). In the calculation of the number of colonies on the media so that chocolate is controlled and so that coklar uses red beans can be seen the number of even the colonies that are closest to control are Media Agar Chocolate uses Red Beans concentrating 5.2 grams, while the average number of colonies is the least can be seen in the concentration of 1.3 grams.

Culture for 48 hours on a medium that is fixed, gonococci colonies are convex, shiny, elevated and mucoid in diameter 1-5 mm in diameter. (Jawetz, 1995). Based on the results of the average measurement of colony diameter on the medium of control chocolate, the average colony diameter is 1 mm, not different from the concentration of 5.2 grams, 2.6 grams and 1.3 grams.

The results of the growth of colonies originating from pure strains obtained the amount more, both in the control and medium of red beans compared with the growth of N. gonorrhoeae isolates from patients. This is because pure strains have adapted to the cultivation media as fertilizers for the growth of N. gonorrhoeae, the Fastidious Broth (FB) medium.

Cultivation / Inoculation of Neisseria gonorrhoeae with the use of Fastidious Broth (FB) was evaluated. FB is proven to be able to support the growth of all strains of N. gonorrhoeae tested in this study without a significant decrease after exponential growth. After 24 hours of incubation at 35 ° C with 5% CO2, the number of colonies of all strains reached more than 108 CFU / mL on FB.

In 1994 Cartwright et al. First use the Fastidious Broth (FB) broth to enrich specimens containing small amounts of bacteria that are difficult to breed, before being cultured on agar media. FB can help the growth of various bacteria (eg Corynebacterium jeikeium, Haemophilus influenzae, Neisseria gonorrhoeae, and Streptococcus pneumoniae) and some organisms, can reduce the incubation time and volume of inoculum needed for detectable growth.

Casein and peptone meat provide amino acids, peptides, and nitrogen substances needed for growth. Yeast extract provides essential B-complex Vitamins and stimulates growth. Sodium chloride is an important electrolyte source and maintains an osmotic balance. Dextrose is an energy source of carbohydrates. L-cysteine improves the recovery of aerobic and microorganisms anaerobic from blood. Tris is a buffer.

Magnesium and iron salts increase aerobic and anaerobic growth. NAD and hemin (factor X) are needed for the growth of difficult-to-breed organisms including H. influenzae. Polysorbate 80 is a source of essential fatty acid nutrients. Agarose is added to block the diffusion of oxygen. Pyridoxal is very important for the growth of many organisms that are difficult to breed.

The composition consists of Casein Peptone (10.0 g), L-Cysteine HCl (0.1 g), Meat Peptone (8.0 g), Sodium Citrate (0.1 g), Sodium Chloride (5.0 g), Magnesium Sulfate (0.05 g), Yeast Extract (5.0 g), Hematin (15.0 mg), Tris (Hydroxymethyl) Aminomethane HCl (2.86 g), Ferrous Sulfate (12.0 mg), Dextrose (2.5 g), Pyridoxal 0.1% (6.0 ml), Tris (Hydroxymethyl) Aminomethane (0.83 g), Polysorbate 80 10% (5.0 mL), Agarose (0.75 g), NAD (1 , 5 mL), Demineralized Water (1000.0 mL), pH 7.2 ± 0.2 @ 25 ° C.

In this study the resulting red bean media has a disadvantage, namely the consistency of the media is less homogeneous and there are more deposits and the consistency of the media becomes denser and harder. This is caused by protein levels in red beans (28.06% b / b) less than soybeans (34.9% b / b). While the levels of red bean carbohydrates are more (53.54% b / b) compared to carbohydrates in soybeans (34.9% b / b). The weakness of cultivation of patients in this study is the presence of both bacterial and fungal contamination. This is because the media used is so that ordinary chocolate is not a modification of Thayer Martin.

Colonies that grow in diameter (± 1 mm) on chocolate agar media (control) and alternative media (red beans) from both the pure strain and N. gonorrhoeae isolates from the patients showed a smaller diameter than the average N.gonorrhoeae colony ideal on agar Thayer Martin chocolate (2-3 mm). This is due to the absence of growth factors in the media so that chocolate (vitamins, carboxylase, DNA and glutamine) in growing N.gonorrhoeae as well as in the Thayer Martin agar (modification of chocolate agar).

In order to add Thayer Martin corn starch also serves to absorb some inhibitor ingredients. The formula is added with trimethoprim to help prevent swarming from Proteus, especially if the sample comes from the patient and also added antibiotics vancomycin which inhibits the growth of normal flora, gram-positive cocci, colystin, gram-negative rods and nystatin prevents the growth of fungi.

Conclusion

1. Red beans (Phaseolus vulgaris L) can be used as a source of soybean substitute protein (Glycine max) which can grow N.gonorrhoeae.
2. The minimum concentration of red beans that is effective for growing N.gonorrhoeae is 5.2 grams.

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