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RESEARCH ARTICLE

CHARACTERIZATION AND SELECTION OF SUPERIOR DRY STARTER MICROBES FOR COCOA BEANS FERMENTATION.

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Abstract

Cocoa farmers use various dry starter microbes to ferment cocoa beans. The quality of fermented cocoa beans is low and different. Characterization and selection of dry starter microbes are needed. There are three types of dry starter microbes which are tested, namely BB-Pasca dry starter microbes, Ragi-1, and Ragi-2. Characterization and selection of dry starter microbes was carried out by fermentation of cocoa bean pulp for 24 hours using dry starter microbes at various temperatures, namely 20°C, 30°C and 40°C. Parameters observed were maximum growth rate of starter microbes, consumption level of reduced sugar by starter microbes, total acid produced, decrease in pH level, and increase in fermentation temperature. Characteristics of BB-Pasca dry starter microbes in fermentation of cocoa bean pulp at 30°C are maximum growth rate at 0.280 hours⁻¹, consumption of reduced sugars 10.8% (w/v), increasing total acid 6.3% (v/v), decrease the pH of 1.4, and increase the fermentation temperature to 3.7°C. Correlation between the parameters observed was more than 0.59. BB-Pasca dry starter microbes were chosen as superior starter.

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Introduction:-

Various cases show that fermented cocoa beans has low quality. Low quality of cocoa beans was characterized by brown color, slaty, the aroma and taste are lacking, the seeds germinate, contaminated with fungal and mycotoxin, and not uniform in quality. Fermented cocoa beans in Indonesia are produced through spontaneous fermentation. Spontaneous fermentation is anabolism and catabolism process of nutrients contained in pulp (sugar, protein, fat, citric acid) by microbes derived from the surrounding environment. The microbial sources of spontaneous fermentation of cocoa beans originate from surrounding air, fermentation location, cocoa pods, knives used by workers, and workers' hands when opening cocoa fruit (Ho et al. 2014; Periera et al. 2013; Papalexandratou et al. 2011; Galvez et al. 2007; Nielsen et al. 2007; Ardhana and Armada. 2003). Spontaneous fermented cocoa beans has some disadvantages such as microbial species are very diverse, microbial inoculation occurs during fermentation, microbial spread in cocoa beans is uneven, the largest inoculums is found on the surface of the pile of cocoa beans, low microbial growth rate, low microbial growth rate, low microbes adaptation, and low ability to produce metabolites (Ho et al. 2014; Periera et al. 2013; Papalexandratou et al. 2011; Galvez et al. 2007; Nielsen et al. 2007; Ardhana and Armada 2003), cocoa beans contaminated with unwanted bacteria, fungi or mycotoxins (Copetti et al. 2012; Copetti et al. 2011; Sanchez-Havas et al. 2008).

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The main problem with spontaneous fermentation is the quality of fermented cocoa beans is not optimal and complicated fermentation process. The quality of fermented cocoa beans is not optimal in color, aroma, taste, slaty, seed germination, and not uniform in the quality (Ho et al. 2014; Periera et al. 2013; Papalexandratou et al. 2011; Galvez et al. 2007; Nielsen et al. 2007; Ardhana & Fleet 2003). Spontaneous fermentation is complicated that is fermentation takes 6-10 days (Ho et al. 2014; Pereira et al. 2013; Papalexandratou et al. 2011; Galvez et al. 2007; Nursalam 2005; Ardhana and Armada 2003); require regular stirring every 12 hours (Santoz et al. 2011; Ardhana and Fleet 2003), every 24 hours (Pereira et al. 2013; Galvez et al. 2007) every 48 hours (Ho et al. 2014; Guehi et al. 2010); need a special place for fermentation (Ho et al. 2014, Guehi et al. 2010; Ardhana and Fleet 2003); and frequent failures (Holzapfel, 2002). Spontaneous fermentation is not optimal therefore need to be improved.

Spontaneous fermentation can be improved by controlled fermentation by using dry starter microbes that have been provided. The benefits of dry starter microbes for fermentation are able to produce quality fermented cocoa beans, shorten fermentation time, reduce fungal contamination, reduce mycotoxin contamination (Copetti et al. 2014; Copetti et al. 2012; Copetti et al. 2011; Sanchez-Hervas et al 2008); and increasing the functional value of chocolate (Ho et al. 2014). The benefits of dry starter microbes are control fermentation by shortening adaptation phase or lag phase, and reducing failure risk (Sandhya et al. 2016). The risk of fermentation failure is damaging fermented materials, pathogens growth, contamination with microbes and the presence of toxins (Holzapfel. 2002).

Dry starter microbes are liquid starter which dried at a certain temperature, solid, and maximum water content of 12%. Such starter is often experiencing a decrease in quality either in the manufacturing process, storage, and transportation due to pressure from environmental conditions. Dry starter microbes are produced by adding a carrier material to maintain viability, stability and simply the application. The carrier material is dry flour which is added when making dry starter microbes. Such carrier has functions to maintain the viability and stability of the starter by protecting microbes from various temperature pressures, moisture content, friction, light, oxygen and others. Another benefit is facilitate transportation, simplify the use, provides nutrition, and facilitates adaptation during application (Blessington et al. 2013; Chan et al. 2011; Vogelmann et al. 2009; Papapostolou et al. 2008; Tsaousai et al. 2008; Kumar and Mishra, 2004; Holzapfel. 2002). Various carrier have been used for dry starter microbes such as milk skim, whey (Koutinas et al. 2009), soy milk (Wang et al. 2004), sugar (Santivarangkna et al. 2008; Tsaousai et al. 2008), corn starch (Chan et al. 2011), nata de coco fiber (Jagannath et al. 2010). The protection mechanism of carrier material is forming a covers layer on the cell to protect from exposure to temperature, heat, light, oxygen, pressure (Chan et al. 2011; Santivarangkna et al. 2008).

Some advantages of dry starter microbes are high viability, compactness, easy storage, easy transportation, easy application, and long life span (Peighambardoust et al. 2011). Dry starter microbes contain important microbes such as yeast, lactic acid bacteria, and acetic acid bacteria (Visitin et al. 2016; Sandhya et al. 2016; Nielsen et al. 2007). Controlled fermentation using dry starter microbes can increase the effectiveness and fermentation efficiency. Production of dry starter guarantees the availability of fermented cocoa starter, high starter population level, high starter adaptation level, and high starter metabolite productivity. Fermentation of cocoa beans with dry starter microbes will be faster, reduce failure risk, and uniform in quality. Various starter microbes for cocoa beans fermentation is include dry BB-Pasca starter (BB-Litbang Pascapanen, 2015) and various Ragi dry starter (Wahyudi et al. 2009), and others.

Superior dry starter microbes are needed to produce qualified and uniform fermented beans. Therefore, the purpose of this study was to obtain superior dry starter microbes and study the characteristics.

Materials And Methods:-

1. Research Materials

Nutrient Broth (NB) and Plate Count Agar (PCA) use as microbial growth media. BB-Pasca dry starter microbes was obtained from the Microbiology Laboratory, Center for Agricultural Postharvest Research and Development, Agricultural Research and Development Agency, Bogor, West Java, Indonesia; Ragi-1 dry starter obtained from Anyar Market, Bogor, West Java, Indonesia and Ragi-2 obtained from Parung Kuda, Sukabumi, West Java, Indonesia. Cocoa fruit is obtained from the cocoa farm of the Industrial Crops and Freshener Research Institute, Plantation Research and Development Center, Sukabumi, West Java, Indonesia.

2. Research Methods

The research was carried out with a completely randomized factorial design, consisting of two factors. First factor is dry starter, consisted of three levels, namely BB-Pasca dry starter, Ragi-1 dry starter and Ragi-2 dry starter. Secondly is a fermentation temperature factor, consisting of 20°C, 30°C and 40°C. Two kilograms of cocoa beans was fermented in plastic in incubator. Observation carried out at hours: 0, 4th, 8th, 12th, 16th, 20th, and 24th. The parameters observed were Total Plate Count (Hoet al. 2014), maximum growth rate of starter microbes, pH (Senayake et al. 1997), total acid (Fardiaz, 1989), reducing sugars (Miller, 1959), and temperature.

Fermented cocoa beans (Ho et al.2014).

Cocoa beans were harvested from the cocoa farm of the Industrial Crops and Freshener Research Institute, Plantation Research and Development Center, Sukabumi, West Java, Indonesia. Cocoa fruit is broiled or stored for 5 days at room temperature. Cocoa fruit is cut using a knife to remove the bean and put in clean plastic. Cocoa beans are stirred using a clean stirrer to evenly mix. As much as 2 kg of cocoa beans are put into aseptic plastic. The plastic tip is tied with loose rubber to keep aeration during fermentation. Cocoa beans were inoculated with prepared starter with a density of 10⁶ cells/mL as much as 20 mL. Cocoa beans are stirred evenly using an aseptic spatula. Cocoa beans were incubated at an incubator temperature of 20°C, 30°C, and 40°C and fermented for 24 hours. Observations were made at hours 0, 4th, 8th, 12th, 16th, 20th and 24th.

Microbes Counting (Ho et al. 2014)

One (1) g of cocoa bean pulp is mixed with 9 ml of distilled water (0.1%), shaken using shaker to form a homogenous suspension. Dilution was carried out using 0.1% of distilled water. A sample of 0.1 mL was taken from each and inoculated on Plate Count Agar medium by duplo, incubated at room temperature for 48 hours.

Maximum growth rate of microbes

The maximum growth rate of dry starter microbes = (ln of microbe population from cocoa beans pulp fermented for 24-hour – ln of microbe population from cocoa beans pulp fermented for 0 hour) at 0) / 24 hours.

Total Acid Analysis (Fardiaz, 1989).

Total acid analysis was carried out by taking 10 g sample of cocoa beans mixed with 100 mL of distilled water, shaken with shaker to obtain a homogenous suspension. A 5 mL supernatant was put into erlenmeyer and 2 drops of phenolphthalein 1% were added. Titration is carried out using 0.1N NaOH solution until it appears pink.

$$\text{Total Titrated Acid} = \frac{\text{mL } 0,1 \text{ N NaOH} \times 100}{\text{gram sample}}$$

pH Measurement (Senayake et al. 1997)

PH analysis follows Senayake et al. 1997. Ten cocoa beans and pulp were shaken with 100 mL of distilled water for 15 minutes. Cocoa beans are separated by adding supernatant. pH was measured using a digital pH meter.

Temperature Measurement

Temperature of fermented cocoa beans is measured by a thermometer. The tip of the thermometer is sterilized using alcohol and then inserted into a pile of fermented cocoa beans.

Analysis of Reducing Sugar (Miller, 1959)

Making of Pb free sample solution

A 2 g of sample was taken and put in a measuring flask 20 mL, added with 10 mL of distilled water. Al(OH)₃ slurry was added drops by drop until there is no more turbidity. Distilled water was added into the flask to the mark and filtered. The filtrate is collected in a 25 mL measuring flask. Excess Pb is removed by adding 8% of Na-phosphate, then distilled water is added until the mark, and then shaken and filtered. This Pb-free filtrate was added with 8% of Na-phosphate and keep remains clear.

Making of standard curves

A 0.2-1.0 mg/mL of standard anhydrous glucose solution is prepared. 1 mL of such solution is taken and put it into a test tube and added with 1 mL of distilled water. A control is made by 2 mL of distilled water. A 3 ml of DNS was added to each reaction tube. The reaction tube is heated in a boiling water bath for 15 minutes and cooled for 20

minutes. The reaction tube is monitored at a wavelength of 575 nm. Glucose standard curve is prepared (mg/mL X absorbance).

Determination of reducing sugars

Standard sugar is diluted with distilled water until reducing sugar content is 0.1 - 1.0 mg/mL. The dilution factor is recorded. A 1 mL of dissolved sample is taken, added with 1 mL of distilled water in a tube and added with 3 mL of DNS solution. Boil for 15 minutes and cooled for 15 minutes. Absorbance is measured at a wavelength of 575 nm. Reduced sugar levels is based on the standard curves and multiplied by the dilution factor.

Results And Discussion:-

Maximum growth rate of starter microbes (hours⁻¹)

Maximum growth rate of dry starter microbes in cocoa pulp medium is diverse which shows differences in the characteristics of growth rates. BB-Pasca dry starter microbes have the highest maximum growth rate, namely 0.280 hours⁻¹ at fermentation temperatures of 30°C, and 0.395 hours⁻¹ at 40°C fermentation temperatures (Table 1). Other dry starter microbes have lower maximum growth rate. Good adaptability of dry starter microbes have shorter lag phase, maximum growth rate, which can reduce fermentation failure risk (Sandhya et al. 2016).

Table 1:-The results of Duncan's Multiple Range Test (DMRT) on the effect of dry starter microbes and fermentation temperature on maximum growth rate of starter microbes in cocoa bean pulp fermentation

Temperature	Control	BB-Pasca	Ragi-1	Ragi-2	Average
20°C	0.070 ^{a (B)}	0.189 ^{a (C)}	0.125 ^{a (B)}	0.154 ^{a (C)}	0.134 ^(C)
30°C	0.224 ^{a (AB)}	0.280 ^{a (B)}	0.254 ^{a (A)}	0.197 ^{a (B)}	0.239 ^(B)
40°C	0.365 ^{b (A)}	0.395 ^{a (A)}	0.334 ^{c (A)}	0.339 ^{c (A)}	0.358 ^(A)
Average	0.220 ^b	0.288 ^a	0.237 ^{ab}	0.230 ^{ab}	

Note: Numbers followed by the same lowercase letter in the same row or the same capital letter in the same column are not significantly different based on the 5% DMRT

BB-Pasca dry starter microbes have better adaptation level, that is better ability to grow maximum with increasing temperatures from 20°C, 30°C and 40°C. Increasing fermentation temperature significantly increases microbe's growth rate of BB-pasca dry starter. At a temperature of 40°C, BB-Pasca dry starter show the highest microbial growth rate, that is significantly different compared to Ragi-1 and Ragi-2. BB-Pasca dry starter microbes have higher maximum growth rate compared to Ragi-1 and Ragi-2 at temperatures of 20°C, 30°C and 40°C. BB-Pasca dry starter microbes have better adaptation to cocoa bean pulp media compared to other starters. From the maximum growth rate parameters, BB-Pasca dry starter microbes are superior compared to Ragi-1 and Ragi-2.

Consumption level of reducing sugars (% w/v)

Dry starter microbes grown in cocoa bean pulp media are able to use sources of sugar in the substrate to produce energy and growth. Sugar sources in the pulp are sucrose, glucose, and fructose (Kadow et al. 2015). Superior dry starter microbes use sugar sources efficiently. Such superior dry starter microbes have better ability to ferment cocoa beans. Table 2 shows BB-Pasca dry starter microbes have the highest ability to utilize sugar, namely 10.800% at 30°C and 10.870% at 40°C (Table 2).

Table 2:-The results of Duncan's Multiple Range Test (DMRT) on the effect of dry starter microbes and fermentation temperature on the consumption level of reducing sugars (% w/v) in cocoa bean pulp fermentation

Temperature	control	BB-Pasca	Ragi-1	Ragi-2	Average
20°C	4.00 ^{c (C)}	5.600 ^{a (B)}	4.890 ^{b (C)}	5.090 ^{b (C)}	4.896 ^(C)
30°C	8.836 ^{b (B)}	10.800 ^{a (A)}	8.886 ^{b (B)}	9.130 ^{b (B)}	9.413 ^(B)
40°C	9.503 ^{c (A)}	10.870 ^{a (A)}	10.400 ^{a (A)}	10.623 ^{ab (A)}	10.349 ^(A)
Average	7.448 ^d	9.090 ^a	8.058 ^c	8.281 ^b	

Note: Numbers followed by the same lowercase letter in the same row or the same capital letter in the same column are not significantly different based on the 5% DMRT

Fermentation temperature affects sugar consumption by dry starter microbes, either BB-Pasca, Ragi-1 and Ragi-2. BB-Pasca dry starter show the most efficient in reducing sugars at temperatures 30°C. At 40°C, sugar consumption

by BB-Pasca dry starter does not increase significantly. This shows that such dry starter use the most efficient substrate at 30°C. The most superior dry starter microbe in term the ability to consume sugar is BB-Pasca.

Level of Total Acid (%)

Dry starter microbes have ability to produce more metabolites than other dry starter. Dry starter microbes are more efficient at converting reduced sugar substrate to organic acid. Organic acids produced will affect total acid concentration in the fermented environment of cocoa bean pulp (Nielsen et al. 2007). Concentration of organic acids affects the fermentation of cocoa beans and the quality of fermented cocoa beans produced.

Table 3:-The results of Duncan's Multiple Range Test (DMRT) on the effect of dry starter microbes and fermentation temperature on total acid level (% v/v) in the fermentation of cocoa bean pulp

Temperature	control	BB-Pasca	Ragi-1	Ragi-2	Average
20°C	2.666 ^{a (B)}	3.000 ^{a (B)}	3.000 ^{a (B)}	3.000 ^{a (B)}	2.916 ^(B)
30°C	3.000 ^{b (B)}	6.333 ^{a (A)}	5.666 ^{a (A)}	6.000 ^{a (A)}	5.250 ^(A)
40°C	5.333 ^{a (A)}	6.000 ^{a (A)}	4.666 ^{a (AB)}	5.666 ^{a (A)}	5.416 ^(A)
Average	3.666 ^b	5.111 ^a	4.444 ^{ab}	4.888 ^a	

Note: Numbers followed by the same lowercase letter in the same row or the same capital letter in the same column are not significantly different based on the 5% DMRT

Superior dry starter microbes are obtained based on microbe's ability to produce organic acids. The amount of organic acid affect on total acid. Total acid influences the microbial growth environment. Cocoa bean fermentation increases the quality of cocoa beans in term of taste, aroma, suppresses microbe's contaminants growth, and reduces bean germination.

Figure 3 shows the total amount of acid produced by dry starter microbes in the fermentation of cocoa bean pulp. Fermentation lasts for 24 hours. The total amount of acid produced by dry starter microbes shows the performance of dry starter microbes. BB-pasca dry starter produce the highest total acid (6.333%) at 30°C. At lower temperatures, namely 20°C, microbial activity of BB-Pasca dry starter has decreased significantly in producing total acid which is equal to 3,000%. However at a temperature of 40°C, activity of BB-Pasca starter experiences a significant increase by 6,000%.

Decrease in pH

Activity of dry starter microbes in cocoa bean pulp media produces organic acids. Organic acids change the acidity level of fermentation environment or pH level (Nielsen et al. 2007; Galves et al. 2007). The pH decrease in fermentation of cocoa bean pulp occurs from 0 to 12th hours of fermentation. After 12th hours of fermentation, pH increases. The pH level at 0 hour is 5.62. Decrease in pH level is used as performance indicator of dry starter microbes in fermentation of cocoa bean pulp. The largest decrease in pH was shown by BB-Pasca dry starter microbes at temperature of 30°C, namely 1,443. The decrease in pH by BB-Pasca dry starter at 40°C is 1,443. Decreasing pH by BB-Pasca dry starter at 30°C and 40°C is not significantly different. Thus the superior dry starter microbes for fermentation of cocoa beans at room temperature (30°C) are BB-Pasca.

Table 4:-The results of Duncan's Multiple Range Test (DMRT) on the effect of dry starter microbes and fermentation temperature on pH Decrease in the fermentation of cocoa bean pulp

Temperature	control	BB-Pasca	Ragi-1	Ragi-2	Average
20°C	0.196 ^{b (C)}	0.370 ^{a (B)}	0.233 ^{b (B)}	0.390 ^{a (B)}	0.297 ^(C)
30°C	0.853 ^{b (B)}	1.443 ^{a (A)}	1.120 ^{b (A)}	1.393 ^{a (A)}	1.202 ^(B)
40°C	1.063 ^{b (A)}	1.443 ^{a (A)}	1.370 ^{a (A)}	1.430 ^{a (A)}	1.324 ^(A)
Average	0.704 ^c	1.083 ^a	0.907 ^b	1.071 ^a	

Note: Numbers followed by the same lowercase letter in the same row or the same capital letter in the same column are not significantly different based on the 5% DMRT

Increased temperature of cocoa bean pulp fermentation

Reaction of alcohol to acetic acid in fermentation of cocoa bean pulp by dry starter microbes is exothermic (Sengun and Karabiyikli, 2011; Nielsen et al. 2007). This reaction increases pulp temperature. Increased temperature can be used as a performance indicator of fermentation by dry starter microbes. With the increase in fermentation temperature, the fermentation of cocoa beans is going well.

Table 5:-The results of Duncan's Multiple Range Test (DMRT) on the effect of dry starter microbes and fermentation temperature on increasing temperature in the fermentation of cocoa bean pulp

Temperature	Control	BB-Pasca	Ragi-1	Ragi-2	Average
20°C	0.633 ^{b(C)}	1.300 ^{a(C)}	0.666 ^{b(C)}	0.733 ^{b(C)}	0.833 ^(C)
30°C	1.200 ^{d(B)}	3.700 ^{a(B)}	2.133 ^{c(B)}	2.966 ^{b(B)}	2.500 ^(B)
40°C	3.200 ^{b(A)}	4.800 ^{a(A)}	4.400 ^{a(A)}	4.066 ^{a(A)}	4.116 ^(A)
Average	1.677 ^c	3.266 ^a	2.400 ^a	2.588 ^b	

Note: Numbers followed by the same lowercase letter in the same row or the same capital letter in the same column are not significantly different based on the 5% DMRT

The level of temperature increase in cocoa bean pulp fermentation shows the performance of dry starter microbes. Increasing temperature varies among dry starter microbes. BB-Pasca dry starter show the highest increasing temperature on fermentation of cocoa bean pulp. Increase in temperature by BB-Pasca dry starter at 30°C and 40°C is 3.7°C and 4.8°C respectively. This shows that the most effective dry starter in increasing temperature is BB-Pasca dry starter.

Correlation between research parameters

Observed parameters in the study were maximum growth rate of starter microbes, consumption level of reducing sugars, increase in organic acids, level of pH decrease and increase in fermentation temperature. Observed parameters have intercorrelation. Interaction between the observed parameters is shown by high correlation value between parameters. Table 6 shows correlation value between the observed parameters and the probability of error (P). Table 6 shows the correlation between parameters from strong to very strong relate. The correlation value is above 0.59.

Table 6:-Correlation value (r) and probability value (P) between observed parameters

	Growth Rate	Reducing Sugar consumption	Increasing total acids	Decreasing pH	Increasing temperature
Growth Rate		r=0.8591 P<0.0001	r=0.5998 P=0.0019	r=0.7698 P<0.0001	r=0.8598 P<0.0001
Reducing sugar consumption	r=0.8591 P<0.0001		r=0.7598 P<0.0001	r=0.9640 P<0.0001	r=0.9075 P<0.0001
Increasing total acids	r=0.5998 P=0.0019	r=0.7589 P<0.0001		r=0.8400 P<0.0001	r=0.8014 P<0.0001
Decreasing pH	r=0.7698 P<0.0001	r=0.9640 P<0.0001	r=0.8400 P<0.0001		r=0.8590 P<0.0001
Increasing temperature	r=0.8598 P<0.0001	r=0.9075 P<0.0001	r=0.8014 P<0.0001	r=0.8859 P<0.0001	

Probability value (P) is probability of data error. Table 6 shows low of probability or low error that is less than 5%, meaning that the probability of data error can be tolerated or accepted.

Discussion:-

BB-pasca dry starter microbes contain yeast, lactic acid bacteria, and acetic acid bacteria (Center for Agricultural Postharvest Research and Development, 2009), while dry Ragi-1 and Ragi-2 contain microbial yeast, acetic acid bacteria, and others bacteria (Dwidjoseputro, 1987). Dry starter microbes grow in the media of cocoa bean pulp. Microbes use nutrients in pulp such as glucose, fructose, sucrose and others (Kadow et al. 2015). Substrate is used as an energy source and cell growth material. Dry starter microbes have different capabilities in utilizing substrate. More efficient microbes will utilize larger substrates. The more efficient use of substrate in the form of sugar shows

that dry starter microbes have advantages compared to other dry starter microbes. In this study, BB-Pasca dry starter has the greatest ability in consuming reducing which is equal to 10.8% (w/v) at 30°C.

Sugar availability is an energy source and building material for microbial cells. Sufficient availability of sugar will support microbial growth. Efficient consumption of reducing sugars will make microbial cell growth more effective. The larger use of sugars will encourage higher microbial cell growth, resulting higher microbial cell densities. Higher cell density shows maximum microbial growth rate. Higher dry starter microbe's growth rates show the superiority of such dry starter. Dry starters which have the highest maximum growth rate are BB-Pasca which are 0.280^{-hours} and 0.395^{-hours} respectively at fermentation temperatures of 30°C and 40°C. Microbes with the highest growth rate will dominate in fermentation environment. Dominant microbes will suppress microbial contaminants, thereby reducing the risk of fermentation failure (Sandhya et al. 2016).

Dominant dry starter microbes in fermentation of cocoa bean pulp utilize larger sugar substrates. The use of a larger substrate produces more organic acids. Amount of organic acid affects the total acid level in fermentation (Nielsen et al. 2007). The larger number of dry starter microbes will produce greater total acidity. Greater total acidity means produce better fermentation of cocoa beans. Thus, level of total acid produced by dry starter microbes is superior parameter for dry starter microbes. Organic acids produce better color and aroma of fermented cocoa beans. Organic acids are beneficial in suppressing microbial contaminants, reducing mycotoxins and reducing seed germination (Ho et al. 2014; Periera et al. 2013; Papalexandratou et al. 2011). The superior dry starter microbes produce organic acids as total acids are BB-Pasca dry starter namely producing total acid 6.33% (v/v) at 30°C.

Increasing of total acid in fermented cocoa bean pulp will decrease pH. Dry starter microbes in the fermentation produce organic acids which reduce pH (Nielsen et al. 2007; Galves et al. 2007). pH value is an indicator of superior dry starter microbes. BB-Pasca dry starter produces the largest total acid, causing the greatest decrease in pH, which is 1.44 at 30°C.

Fermentation of cocoa bean pulp by dry starter microbes produces heat. Changes in alcohol produced by yeast into acetic acid by acetic acid bacteria in aerobic conditions are exothermic (Sengun and Karabiyikli, 2011, Periera et al. 2013). Increased temperature will increase the activity of dry starter microbes in fermentation. Higher temperature increase by dry starter microbes in fermentation is indicated that such starter is superior. BB-Pasca dry starter microbes show the highest temperature increase namely 3.7°C and 4.8°C at 30°C and 40°C respectively. BB-Pasca dry starter microbes were chosen as superior dry starter microbes.

Observed parameters were level of reduced sugar consumption, maximum growth rate, amount of total acid, decrease in pH level, and increase in fermentation temperature. Such parameters have interactions. Interaction value is expressed as a correlation value. Correlation value between parameters is greater than 0.59. This means that all research parameters are strongly correlated. Probability value (P) is level of data error. Table 6 shows the probability value is low, which is less than 5%. This means that probability of data error can be tolerated or accepted.

Conclusion:-

This study found superior dry starter microbes and their characteristics. The superior dry starter microbes for fermentation of cocoa beans are BB-Pasca dry starter. Characteristic of BB-Pasca dry starter at 30°C is show highest maximum growth rate among other dry starters, which is 0.280^{hours⁻¹}, able to use reduced sugar substrate by 10.8% (w/v), increasing total acid concentration by 6.3% (v/v), decreasing pH by 1.4, and able to increase the fermentation temperature by 3.7°C. The correlation value between the research parameters is higher than 0.59.

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