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RESEARCH ARTICLE

The Making of Probiotic Using Corn as Growing Media and Micromineral as Additive

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ABSTRACT

The purpose of this study was to determine the growth of *Trichoderma*sp. used corn as growing media with the addition of micromineral as a probiotic. This study was designed based on a completely randomized design with four treatments and six replications. The treatment used are P0 = corn media + *Trichoderma* sp., P1 = corn media + *Trichoderma* sp. + 1% premix, P2 = corn media + *Trichoderma* sp. + 2% premix, and P3 = corn media + *Trichoderma* sp. + 3% premix. Making probiotics used corn growing media with the addition of micromineral (premix) had no significant effect (P> 0.05) on the growth of *Trichoderma* sp. Spores, but the highest number of spores was treated with the addition of 1% premix (P1) with spores 2,12 × 10^{10} cfu / g media. The treatment without the addition of premix (P0) was treated with the least amount of spores compared to other treatments with a number of spores of 1.75 × 10^{10} cfu/g media. The degree of acidity (pH) of probiotics with the addition of micromineral (premix) had a significant effect (P <0.05) on probiotics without the addition of micromineral. It was concluded that the addition of micromineral (premix) could increase the growth of *Trichoderma* sp. but if were excessive it would reduce the growth of *Trichoderma* sp. Spores.

Keywords- : Corn, Premix, Probiotics, *Trichoderma*sp.

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INTRODUCTION

The utilization of antibiotic (antibiotic growth promoters or known as AGP) has been banned in various countries including Indonesia, because the utilization of antibiotic caused pollution and residues for consumer. The alternative for antibiotic is the probiotic.

Probiotic is beneficial microba used as supplementary feed. Microba in probiotic could improve the balance of microorganism in the digestive tract (Fuller, 1989; Collins and Gibson, 1999). The recommended concentration of prebiotic as supplementary food is approximately 10^8 cfu/kg f feed (Haryati, 2011). One of microorganisms that could evoke the growth of pathogenic migroorganism is *Trichoderma* sp. Harman *et al*. (2004) stated that *Trichoderma* sp. is a very interactive free-living mushroom in the roots, soil and leaves, and produces various kinds of antibiotic substances and is antagonistic to other fungi.

Gusnawaty *et al*. (2017) mentions that *Trichoderma* sp. could grow on various media. *Trichoderma* sp. mostly grow on bran and corn because the nutrients in bran and corn fulfill the nutrients required. Khalil and Anwar (2006) stated that the nutritional value of corn is mainy determine by its NFE (Nitrogen Free Extract), which is around 80% in dry matter and low crude fiber content (around 3%). Corn contains about 70% starch and 2% sugar, while in young corn it is around 3%. Nutrient content of milled corn is 8.9% Crude Protein, 4.0% Etanol Extract, 2.2% Crude Fiber, 1.7% ash, and 68.6 NFE (Sartika *et al*., 2018).

Fungi growth in fermentation medium is influenced by nutrients that are exist in the media or also added to the substrate. The nutrients needed by fungi consists of C element, N element and minerals with certain comparisons (Safaria *et al*., 2013). These inorganic minerals are divided into two classes, namely macromineral and micromineral (Beyer and Wilson, 2000). Physiologically, the most important macromineral is Ca, K, Na and Mg, and similarly for Fe, Cu and Zn as microminerals (Silvera and Rohan, 2007; Barroso *et al*., 2009). Micromineral or micro minerals are minerals that are needed in very small amounts and are generally found in tissues with very small concentrations (Arifin, 2008). One of the additive which has micro mineral element is premix. Premix is a mixture of several micro-iniments with ingredients of the diluents and their presentation is mixed into the ration. Proteins in the premix are in the form of amino acids mixed with minerals and multivitamins (Saputra *et al*., 2016).

This study aims to determine the growth of *Trichoderma* sp. using corn as growing media with the addition of micromineral as a probiotic.

MATERIAL AND METHOD

Location and Time

This study was conducted at the Animal Feed and Nutrition Laboratory and Biotechnology Laboratory at Politeknik Pembangunan Pertanian Malang (POLBANGTAN) which was held in March 2019.

Tools and Materials

he tools used in this study were stationery, cameras, drop pipettes, micropipets, analytical scales, 10 ml measuring cups, Bunsen, test tubes, test tube racks, microscopes, autoclaves, Haemocytometers, handcounters, spatulas and matches. The material used is corn, *Trichoderma* sp. second filial (F2), aquades, premix (micromineral), label paper, plastic bags, sterilized markers, 70% alcohol, glove, masks, and tissues.

Methods

This study used a completely randomized design (CRD) method, which consists of four treatments and six replications. The

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treatment is P0 = corn media + *Trichoderma* sp., P1 = corn media + *Trichoderma* sp. + 1% premix, P2 = corn media + *Trichoderma* sp. + 2% premix, and P3 = corn media + *Trichoderma* sp. + 3% premix.

Trichoderma sp. Fungus inoculum is the collection of the Animal Feed and Nutrition Laboratory in Politeknik Pembangunan Pertanian Malang which has been revealed to be the second filial. Probiotic production begins with washing the corn media and then aerating it. After that, the corn was put into heat-resistant plastic with a weight of 100 g each, then sterilized using an autoclave with a temperature of 121^0C for 15 minutes. The corn media is cooled then mix the premix according to the treatment. Inoculation of *Trichoderma* sp. into the media. Each plastic was coded according to treatment and incubated for seven days.

Observed Variable

Observed variable included physical quality with a pH test using a pH meter and the number of spores using the haemocytometer test. Measurements of acidity (pH) were carried out after probiotics were incubated for seven days. Conidia density calculated by taking 1 ml of the conidia suspension and placing it slowly in the calculated field to fill the canal using a micro pipette, then let it stand so that the position is stable and close with a cover glass, then calculate the conidia density in the calculated box in 5 fields view (Juliana *et al*., 2017). The number of spores is calculated using the following formula (Gabriel and Riyatno, 1989; Uruilal *et al*., 2012) :

$$C = \frac{t}{n \, x \, 0.250)} \, x \, 10^6$$

Information :

C = Konidia density per ml of solution

t = Total conidia in the sample box observed

n = Number of sample boxes (5 large boxes x 16 small boxes)

0.250 = Correction factor using a small scale sample box on the haemocytometer.

Data Analysis

Data gained from observation was analyzed using Variation Analysis (ANOVA), one way ANOVA using IBM SPSS Statistics 20 Software.

RESULT AND DISCUSSION

pH of Probiotic

Most microbes could grow well at pH between 6.5-7.5. While *Trichoderma* lives in a pH range of 4-8 (Daning and Karunia, 2018). Uruilal *et al*. (2014) suggested that pH affects the growth of *Trichoderma* sp. on the soil and low pH gives a better influence.

Based on the result of research, the making of probiotic within 7 days that has been analyzed by Analysis of Variants (ANOVA), it was shown that the medium of corn + 1% *Trichoderma* sp. (PO) was significantly different (P <0.05) with corn media + 1% *Trichoderma* sp. + 1% premix (P1), corn media + 1% *Trichoderma* sp. + 2% premix (P2), corn media + 1% *Trichoderma* sp. + 3% premix (P3). Uruilal *et al*. (2014) suggested that the activity of antagonistic fungi such as *Trichoderma* sp. only driven by acidic conditions. This proves *Trichoderma* sp. could grow and develop on corn media with the addition of minerals.

The Amount of *Trichoderma* sp. Spores

Growth of *Trichoderma* sp. was characterized by the appearance of fungi in white, yellow, or green colour. This is consistent with the opinion of Juliana *et al* (2017) who stated that*Trichoderma* sp. colony initially appeared in white and then green colour. Microscopically the *Trichoderma* sp. could be seen in the form of conidia which has oval form (Figure 1). Based on the results of the observations in Table 2, it can be seen that the average number of *Trichoderma* sp. spores not significantly different (P> 0.05) in each

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treatment. The highest number of spores was treated with the addition of 1% premix (P1) with the number of spores of 2.12 × 10^{10}cfu / g media. The treatment without the addition of premix (P0) has the least amount of spores compared to other treatments with number of spores of 1.75 × 10^{10} cfu / g media.

Trichoderma sp. could grow well on media with the addition of micromineral. Zembayashi (1974); Supriyati *et al*. (2000) reported that by adding minerals in vitro increased microbial activity. Treatment with the addition of 2% premix (P2) and 3% (P3) decreased spore growth. Excessive use of micromineral can reduce spore growth. This is consistent with the opinion of Arora (1989) that selenium in normal amounts can stimulate sitesa microbial protein but if given excessively, it will inhibit microbial protein synthesis.

CONCLUSION

Probiotics from *Trichoderma* sp. could be used as alternative of antibiotics. Making probiotics using corn as growing media with the addition of micromineral (premix) could increase the growth of *Trichoderma* sp. spores. This probiotic also qualified to be used as poultry supplementary feed because it contains 2.12 × 10^{10} cfu / g.

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Table-1: The average of probiotics

Composition of probiotics mixture	pH probiotics
P0 (corn media + *Trichoderma*sp. 1%)	5,3[b]
P1 (corn media + 1% *Trichoderma*sp. + 1 % premix)	5,0[a]
P2 (corn media + 1% *Trichoderma*sp. + 2 % premix)	5,0[a]
P3 (corn media + 1% *Trichoderma*sp. + 3 % premix)	5,0[a]

Note: different superscripts in the same column show significant differences (P<0,05)

Table-2: The average of *Trichoderma* sp. spores on multiplication media

Composition of probiotics mixture	Average of *Trichoderma* sp. spores (10^8/g media)
P0 (corn media + *Trichoderma*sp. 1%)	175,00[a]
P1 (corn media + 1% *Trichoderma*sp. + 1 % premix)	212,50[a]
P2 (corn media + 1% *Trichoderma*sp. + 2 % premix)	192,50[a]
P3 (corn media + 1% *Trichoderma*sp. + 3 % premix)	184,17[a]

Note: Superscript in the same column shows no significant difference (P>0,05)

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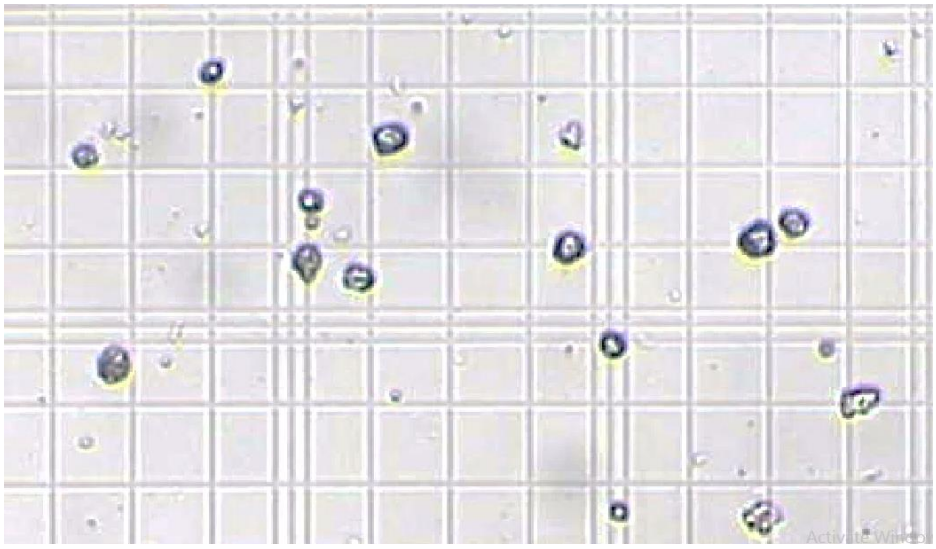

Figure-1: *Trichoderma* sp. spores