Isolation and Identification of Fungi Type From Juice of Cabbage Waste as Probiotic Agency

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ABSTRACT

The aim of research is to identify type of fungi that isolated from fermented juice cabbage waste as a probiotic agent. The research design is descriptive research. The study began by isolating fungi from fermented juice cabbage waste with *Saboroud Glucose Agar (SGA)* media added by 0,1 g/liter tetracycline antibiotic to prevent growth of bacteria and incubated for 72 hours in an incubator at 37 ° C temperature. Purification of fungi colony is done by observing fungal colonies that grewth with different characteristics, isolated and re-inoculated repeatedly until the pure culture was obtained. Fungi identification is done by macroscopic morphology observed in each colony. Morphological observations of fungi include color observation, surface of colony, texture and the edge of the colony. Identification of fungi characteristics was based on identification book. Identification results obtained 2 types of fungis which are first the mold *Rhizopus oryzae (R. oryzae)* and second the yeast *Saccharomyces cerevisiae (S. cerevisiae)*. According to growth characteristic, both of types of that fungis are resistant to acid with the pH range 2-5, then can be made as probiotic agent for poultry. Conclusion of study that this study obtained 2 types of fungis which are mold *Rhizopus oryzae* and yeast *Saccharomyces cerevisiae* that potential as a probiotic agent.

Keywords: waste cabbage, fungi, isolation, identification, probiotic

INTRODUCTION

In 2014 Statistics Agency of Central Java has noted that waste production in Central Java in 1697 units came from traditional market of 37,000 tons / day of which 61.62% is organic waste such as fruit and vegetable waste. The disadvantage of waste that came from traditional market is high content of moisture of 92, 44% which causes it's easily being rotten. Market waste has great potential as a fermentation starter (Utama and Mulyanto, 2008). Cabbage waste is one of the waste from the vegetable market. Cabbage waste consists of the outer shell cabbage that has damaged by the collision so not feasible for sale. Chu et al., (2002) stated that cabbage is a vegetable that is rich in minerals, vitamin C, and fiber and phytochemical. Cabbage processed into pickle, contains several species of microbes such as *Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, Pediococcus pentosaceus, Rhizopus sp* and *Saccharomyces Sp.* (Plengvidhya *et al.,* 2007; Utama *et al.,* 2013)

Several types of fungis have been shown to play a role in fermented of various types of foods and beneficial for animal feed. Fungi is an eukaryotic microorganisms, producing spores, does not has chlorophyll, obtaining nutrients by absorption, reproducing sexually and

asexually, having somatic structures in the form of hyphae, and cell walls composed of chitin and cellulose. Fungis can be divided into two, namely molds and yeasts (Alexopoulus *et al.,* (1996); Dube (1996)). Fungis can be utilized in the cultivation of livestock as a biological controller, probiotic and immunostimulant. Certain fungi that can be used for livestock such as *Rhizopus oryzae, Aspergillus niger, Trichoderma Viridae, Beauveria bassiana, Metarhiziumanisopliae Duddingtonia flagrans* and also can be used to improve animal health and as biological control. The yeast *Saccharomyces cerevisiae* can act as a probiotic and immunostimulant to improve the productivity and health of livestock (Ahmad, ~~R.Z~~, 2005).

Probiotic is a food supplement formed as life microbial that administered into the digestive tract and provide benefits in the gastrointestinal tract of the host (Roberfoid, 2000). Fuller (1992) and Karpinska *et al.* (2001) explained that probiotic is an additional feeds formed in microbial that are beneficial to and affect the host by improving the balance of microorganism in the digestive tract. One of probiotic is *Rhizopus oryzae* and yeast *Sacharomyces cerevisiae.* Administration of *Saccharomyces cerevisiae* as the life microbial into the body will affect the host (poultry, ruminants) through improving the balance of microorganisms in the digestive tract. Administration of probiotics in ruminants will improve cellulolytic bacteria and lactic acid in the digestive tract. In poultry, probiotics will increase the number of beneficial microbes and suppress the harmful microbes by competing to live in the digestive tract. Giving mold *Rhizopus oryzae* on rooster laying hens can increase the productivity of meat, lower fat and cholesterol in meat. *Rhizopus oryzae* is a heterofermentative molds that produce lactic acid from the fermentation process of glucose, xylose and starch (Efremenko, et al*.,* (2006).

Shin *et al.* (1989) stated that *S. cerevisiae* is one of the commonly used microbes as probiotics for livestock, along with bacteria and other fungi such as *Aspergillus niger, Rizhopus oryzae, A. oryzae, Bacillus pumilus, B. centuss, Lactobacillus acidophilus, Saccharomyces crimers, S. Cerevisiae, Streptococcus lactis* and *S. termophilus*. Before *S. cerevisiae* used as a probiotic, first its ability of life to organic acids, bile salts and low pH was tested (Agarwal *et al.,* 2000). Tedesco *et al.* (1994) found a correlation of administrating *S. cerevisiae* against bacteria in rabbits in the way by reducing the number of pathogenic bacteria and increasing the number of aerobic and anaerobic bacteria that beneficial in the gut. Kumprecht *et al.* (1994) that gave a mixture of *S. cerevisiae* with *Streptococcus faecum* in broiler chickens has resulted reducing the population of *Eschericha coli* to 50%. Kompiang (2002) used *S. cerevisiae* in chicken feed and gain a significant increase in body weight. Kumprechtova *et al.* (2000) administered *S. cerevisiae* with dose of 200 g / 100 kg of feed to improve the meat quality and reduce the smell of ammonia in chicken excreta. Another result of the administration of *S. cerevisiae* was increasing weight of the chicken and in vitro was able to suppress the growth *of S. Typhimurium* (Istiana *et al.,* 2002; Gholib *et al.,* 2003). Administration of *S. cerevisiae* to ruminants can improve milk production by 4,3% and increase weight of 8,7%. In sheep that accepted the mixing *S. cerevisiae* with Bioplus in feeds with a dose of 4 g / day (with 1 g *S. cerevisiae* containing 14 x 10^{10} of colony) could increase weight and lower the feed conversion to 6 kg / kg (Ratnaningsih, 2000). Based on research mentioned above, the *S. cerevisiae* and *R. oryzae* can be used as probiotics for livestock.

MATERIALS AND METHODS

Materials used in this study were waste cabbage, molasses, salt, fermented cabbage waste, *Saboroud Glucose Agar (SGA)* media*,* distilled water, NaCl physiological, KOH 10%, *Lactophenol cutton Blue* (LPCB), crystal violet and alcohol 90% . The instruments used were autoclave, oven, scales, stirrer, erlenmeyer glass, beakers, petri dishes, test tubes,

spatulas, pH meter, measure pipette, needle ose, object glass, tweezers, cotton, microscopes, calipers, fire bunsen, test tube rack, rubber, tissue, markers, label names, and scissors.

The study began by cutting the cabbage waste as smooth as possible, then put it in a blender, added salt 8% and molasses 6.7% , then fermented in a sealed container for 6 days (Utama *et al.,* 2013). The fermented result then harvested and isolated by taking fermented cabbage waste as much as 1g, then put into a test tube containing 9 ml of NaCl physiological. Thereafter 1 ml of each dilution put in a petri dish and poured with *Saboroud Glucose Agar* (SGA) medium which has been added with 0,1 g/liter tetrasiclin antibiotic, which is used to prevent bacterial growth. Furthermore incubated at 37°C for 72 hours in an incubator. Purification of fungi colony is done by observing fungal colonies that grewth with different characteristics, isolated and re-inoculated repeatedly until the pure culture was obtained. Fungi identification is done by macroscopic morphology observed in each colony. Morphological observations of fungi include color observation , surface of colony, texture and the edge of the colony. Identified the characteristics of fungi are matched based on the morphological characteristics and identification book (Pham et al., 2012).

RESULTS AND DISCUSSION

The fungi which were isolated from fermented juice cabbage waste were obtained as 2 isolates. The selected isolates consist of one type of mold and one type of yeast. The mold isolate can be seen in Figure 1 and the yeast isolate in Figure 2.

Figure 1. Mold IsolateThe

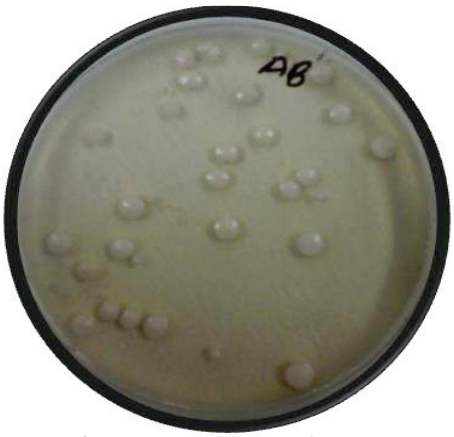

Figure 2. Yeast Isolate

result of identification based on the microscopic from each fungi isolate, was obtained the figure of fungi isolate as shown in Figure 3 and 4.

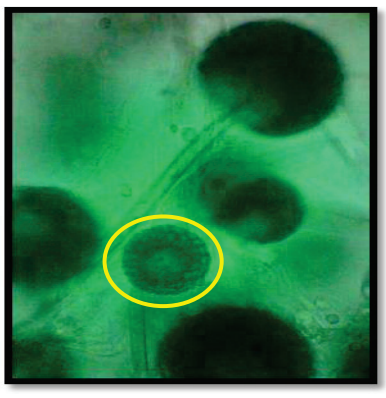

Figure 3. *Rhizopus oryzae* Isolate

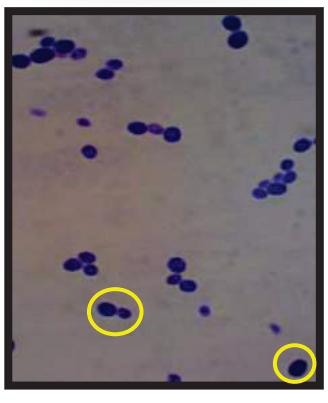

Figure 4. *Saccharomyces cerevisiae* Isolate

Molds isolate (Figure 3) identified the species *Rhizopus oryzae.* The characteristics of the macroscopic *Rhizopus oryzae* isolated on SGA medium at 3 days of age with incubation temperature of 37 ° C were appeared whitish color then grayish brown due to brown color of sporangiofor and blackish brown color of sporangia. The surface being vined because it has rhizoid and surface texture of the hair, on the other hand the colonies was white. Edge or margin of the colony can not be determined because of *Rhizopus oryzae* was growing very fast on the second day after inoculation with the diameter of 2 cm, 7,1 cm on the third day and the fourth day already meet the petri dish. *Rhizopus oryzae* did not have concentric circles and did not produce exudates. From the microscopic characteristics obtained were having rhizoid, sporangia round to semi-round shaped and blackish brown. Columella round shaped, sporangiophores irregularly shaped, there was a round and elliptical and has a line on the surface.

Yeast isolate (Figure 4) identified the species *Saccharomyces cerevisiae*. The macroscopic characteristics of *Saccharomyces cerevisiae* isolate on SGA medium at 3 days with an incubation temperature of 37 ° C were white, round / oval shaped, unicellular with 5-10 micron size. *Saccharomyces cerevisiae* is the yeast of the family *saccharomycetaceae*. *Saccharomycetaceae* family is the family of the order *saccharomycetales* yeast that reproduces by forming buds. When breed, yeasts will divide itself and produce buds that has multipolar germinate. During the process of asexual reproduction, a new buds grow from yeast to certain conditions and when it reaches adult size, it would apart away from the parent cell. Spores size is 5-10 µ in diameter. *S. cerevisiae* is the unicellular yeast cell, which breed rapid by sexually and asexually. Breeding through buds multipolar sprout and buds can occur on the entire surface of the cell wall. Sexual reproduction will forms Askospora in the askus. In one askus usually there are four askospores in various forms. This yeast have microscopic morphological characteristics which were forming spherical, oval, cylindrical, oval or ovoid short and long blastospores (spores) influenced by strain (Elliot, 1994; Dube, 1996). Based on the amount of cells, yeasts categorized into three, namely cells with 3.50-10 µ x 5-19 µ size, cells with 3-8 µ x 4-18 µ size, and cells with 2.50-7 µ x 4.50-18 µ size. Other than that, there was a filamentous cell on the spores with more than 30 µ size and has pseudomiselium. Macroscopic morphology showed colonies were round, white, beige, gray to brownish, sparkling until dull colony surface, slick, with soft texture (Lodder, 1970; Barnet *et al.* 2000).

CONCLUSION

This study obtained 2 types of fungis which are Mold *Rhizopus oryzae* and Yeast *Saccharomyces cerevisiae* that potential as a probiotic agent.

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