NATURAL MEDIUM FOR GROWING OF ENDOPHYTIC BACTERIA FROM SOLANACEAE IN MALANG-INDONESIA

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ABSTRACT

Endophytic bacteria are important microorganisms having potential as biocontrol agents for many pathogens. Until now, the growth of it always uses semi-synthetic or synthetic medium so it was difficult to be used by farmers in the field and it was expensive to have its propagation as biocontrol agents. Based on the problem, this research will study the natural medium as propagation medium of Endophytic bacteria. It had natural ingredients such as soybean, chicken broth, egg, worms, snail, sorghum and they were easy to get by farmers. This study used endophytic bacteria from Solanaceae in Malang- Indonesia. Four isolates of endophytic bacteria were grown in agar and liquid medium with ingredients of corn flour, soybean flour, sorghum flour, snail flour, and worm flour. There is no difference in the incubation period, color, shape, and surface colony. The population in medium with snail flour ingredients at a concentration of 10^7 cfu/ml is the highest and snail flour is the best medium for growing endophytic bacteria.

Keywords: natural medium, endophytic bacteria

INTRODUCTION

Endophytic bacteria have been found in every plant, where they colonize the internal tissues of their host plant and can form a range of different relationships including symbiotic, mutualistic and commensalistic. Endophytic bacteria can also be beneficial to their host by producing natural products that be potential use agriculture, having been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Christy and Sudha, 2014; Kobayashi and Palumbo, 2000).

The growth of it is influenced by the medium and growth factors. Growth factors are required in small amounts by cells because they fulfill specific roles in biosynthesis. The need for a growth factor results from either a blocked or missing metabolic pathway in the cells and can be added to culture media that are used to grow bacteria. The type of the culture medium used depends on the purpose, whether liquid or solid medium. The culture medium may be classified into several categories depending on their composition or use. Chemical (synthetic) medium is one in which the exact chemical composition is known and the semi-synthetic medium is one in which the exact chemical constitution of the medium is not known (Todar, 2012). The growth of bacteria also influenced by the diversity of nutritional types found among bacteria like inorganic salt, carbon, inorganic nitrogen, amino acid, and vitamins (Goyal, 2007) but the medium is usually expensive.

Endophytic bacteria as biocontrol agents of pathogens in Malang-Indonesia need a growing medium with a composition that is easy to use for propagation by farmers in the field and also cheap. Based on the problem, we used mediums from natural materials such as corn flour, soybean flour, sorghum flour, snail flour, and worm flour. All of them contain growth factors such as organic and inorganic elements, vitamins, and other elements (Ahmad, 2002; Syatrawati, 2008).

The object of this experiment is to study the natural medium for growing of endophytic bacteria from Solanaceae in Malang-Indonesia.

MATERIAL AND METHOD

Isolates of endophytic bacteria

Endophytic bacteria used for the experiment was a personal collection Dr. Arika Purnawati. Bacteria culture was purified on NA (Difco) : 8 g / l, pH 7.0, at 24 hours age is used for experiments.

Natural Medium

Corn flour. Corn was shelled then cleaned, dried (1-2 days) at 50^0C until having 15-18% moisture content. Provision of flour was done using a sieve size of 50 mesh. **Soybean flour.** Soybean was washed with clean water flow until it is clean. It was then soaked in water (4 hours) while squeezed to clean the epidermis. Soybean then was washed again with water and drained (15 minutes). It was dried (1-2 days), roasted (10-15 minutes) and ground into the flour. **Sorghum flour**. Sorghum seeds were cleaned then dried to 20% moisture content and soaked in water (8 hours) and drained until 16% moisture content. **Snails flour**. Snails fresh meat was removed from the shell then dried through drying in the sun (3 days) or dried using a dryer until having 14% moisture content. After the snail meat was dried, it then was milled using a grinder into flour. **Worm flour.** Worms were washed then boiled in boiling water (3 minutes) then drained. After the leak, the worms were cut into pieces of 1 cm and being washed again. The pieces were dried using an oven at 500C (4 hours) and mashed using a mortar into flour. **Achatina flour**. Achatina was allowed to stand for 2 days and 2 nights, added with salt and stirred (15 minutes) then drained (15 minutes). It was then washed and boiled (20 minutes), drained, and dried. The process was repeated once again: washing and boiling (20 minutes), draining, and drying. The end result was then sliced and ground into flour.

Natural medium test for growing. Before undergoing the experiments, each flour type was weighed as much as 20 g/l. The medium was divided into liquid medium without added agar and solid medium with added agar 20 g/l. Then the medium was sterilized using autoclave at 1210C, 1.5 atm (15 minutes) and then as much as 10 ml was poured into a Petri dish. Endophytic bacteria colonies were inoculated to the medium then incubated at 28^0C (48 hours). The observation was performed to investigate incubation period, color, shape, surface colony and the population of bacteria on natural medium using dilution technique at a concentration of 10^7 cfu/ml, at 24, 48, 72, and 96 hours.

The experiments used completely randomized design using 2 factors which were: the type of medium (liquid and solid) and the medium ingredients (corn flour, soybean flour, sorghum flour, snails flour, worm flour, and achatina flour) and with 3 replications. The data is then analyzed statistically using LSD 5%.

RESULTS

According to the experiment, the incubation period was 24 hours, whereas the colony bacteria on all natural medium were white in color, having round shape with shiny surface (Fig 1) with the population on each natural medium is shown in (Fig 2)

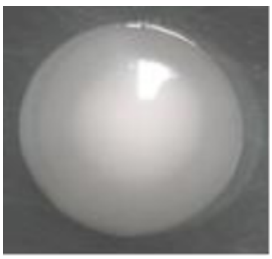

Figure-1. Microscopic colony of endophytic bacteria in natural medium (p.4.5x)

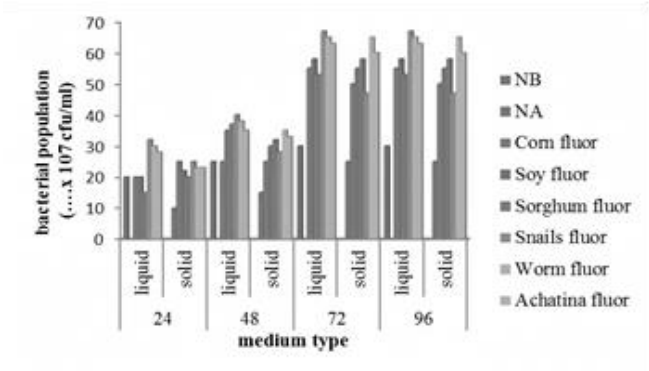

Figure-2. Population of endophytic bacteria in natural medium

According to Fig 2, the population of endophytic bacteria in the solid and liquid mediums at 10^7 cfu/ml is the highest on natural medium with ingredients of snail flour ($7.5x10^6$ cfu/ml) and ($1.1x10^5$ cfu/ml), respectively. The result was followed by the natural mediums with ingredients of soybean flour, corn flour, sorghum flour, worm flour and achatina flour. The population (cfu/ml) in solid natural medium are $6.9x10^6$, $6.7x10^6$, $6.5x10^6$, $7.0x10^6$, $7.4x10^6$, respectively, while in liquid medium are $1.05x10^5$, $1.03x10^5$, $1.02x10^5$, $1.07x10^5$, $1.06x10^5$, respectively. The population in the natural medium is higher than that of in control medium Nutrient Agar (NA) which is $6.0x10^6$ cfu/ml, and in Nutrient Broth (NB) which is $1.0x10^5$ cfu/ml. The difference of bacterial population in the natural medium is significant than control medium, so the bacterial population in the liquid medium is higher than that of solid medium for all natural mediums.

Beside that result, at the 24th hour, for all solid and liquid natural mediums, there was an increase of population and it increased significantly at 48th, but at 72nd there was no increase of population until at 96th.

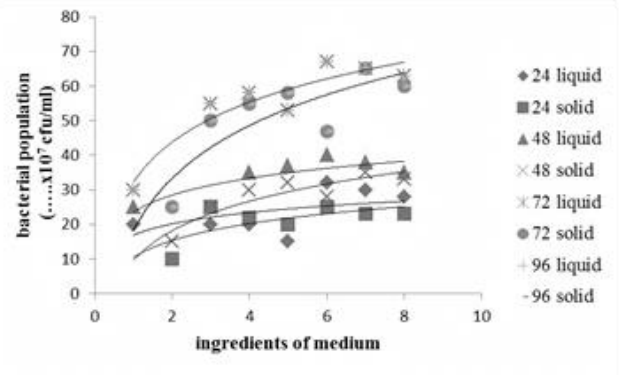

Figure-3. Curve of growing endophytic bacteria

Fig 3 shows that the bacterial growth followed the pattern of the logarithmic curve and the curve is divided into four phases: which are lag phase, log phase, stationary phase and death phase. In Lag phase (24 hours), the bacteria began to divide and perform the metabolic activity. In Log phase or exponential phase (48 hours), the mass and volume of the bacteria were increased. During this period, the growth was balanced and the speed increased. In Stationary phase (72 hours), the population was declining because the number of dead cells increased until a number of the living cells division were equal to the number of dying cells, so the number of living cells is constant, as there was no growing. In Death phase (96 hours), there was an increase in the speed of cell death until the number of living cells decreased. Despite this decline, the number of living cells was not zero; a specified minimum number of microbial cells will remain for a very long time in the medium.

DISCUSSIONS

The incubation period and colony bacteria in all of the natural mediums so in control medium are not different because nutrition in the mediums did not affect the incubation period and colony. The population of endophytic bacteria is different in various media because it was influenced by the nutritional content in the medium. The population of endophytic bacteria in the liquid medium is higher than that of solid medium because in the liquid medium the whole bacteria were mixed with the liquid medium thus affecting the acceleration of cells division and resulted in high population. Todar (2012) stated that the growth of bacteria is affected by major elements (C, H, O, N, S. P, K, Mg, Fe, Ca, Mn) and trace elements (Zn, Co, Cu, Mo). Razzak (2009) stated that bacteria will grow best on agar plates where air readily diffuse into the colony or on a liquid medium that are shaken. Todar (2012) stated that there are other factors such as temperature, pH, and water activity that affected the bacteria growth.

The research conducted by Chikere and Udochukwu (2014) mentioned the use of Nutrient Agar (NA) and Plate Count Agar (PCA) can cause an increase in the counts of endophytic bacteria and in incubation time. The counts obtained in NA were higher than that of PCA followed by the soil extract agar (SEA) which had the lowest count and a longer bacteria lag phase due to the low nutrient content in SEA. The Plate count agar formed more

distinct colonies than that of NA and SEA. During the incubation period, colonies still appeared on the NA even on the 7th day. These characteristics of the NA to support a higher number of colonies for a long period of time could be attributed to its nutrient composition. Typical growth curve of the bacteria population can be divided into lag phase, exponential phase (log phase), stationary phase and death phase. In Lag phase: When a microbial population is inoculated into a fresh medium, growth usually does not begin immediately but only after a period of time called the lag phase, which may be brief or extended, depending on the history of culture and growth conditions. In Exponential Phase: This is the phase of the bacterial growth curve in which the bacterial cell numbers doubles during each unit time period. When the cell number from such as experiment is graphed on arithmetic coordinates as a function of elapsed time, one obtains a curve with a constantly increasing slope. The rate of increase in cell number is slow initially but in a later stage the cell numbers increase explosively. The rate of exponential growth varies between bacterial genera (i.e. Genetic characteristics of bacteria) and is also influenced by environmental conditions. In Stationary Phase: exponential growth cannot occur indefinitely because the essential nutrients of the culture medium are used and waste products of organisms were built up in the environment. In stationary phase, there is no net increase or decrease in cell number. The cells functions, such as energy metabolism and some biosynthetic processes, continue on. In Death Phase**:** the bacterial population reaches the stationary phase and the cells may start dying. Cell death may be due to cell lysis and this is also an exponential process but is much slower than that of exponential growth (microbeonline.com, 2013*).*

CONCLUSIONS

From the experiment, in all solid and liquid mediums, there is no difference in the incubation period and colony, whereas the population in agar and liquid medium with the ingredient of snail flour at a concentration 10^7 cfu/ml is the highest.

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