

BS-006

STUDIES ON SPECIES DIVERSITY AND GROWTH RATE OF MOLD IN MUSK LIME PICKLE (*Citrofortunella microcarpa*) FOOD SOCIETY OF MELAYU

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ABSTRACT

This research aims to study the diversity of species, growth rate and colony number of mold growth in Musk Lime Pickle in various storage time. The research method is Non Factorial Experiment, complete random sampling (RAL). The results indicated that in Musk Lime Pickle, there are 6 molds such as : *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus tamari*, *Fusarium solani*, *Mucor mucedo*, and *Penicillium digitatum*. The duration of storage decreased the growth rate of mold, and the diversity of mold is not increase. The mold that growth more is *Aspergillus fumigatus* and *Penicillium digitatum*, while the least is *Mucor mucedo* and *Fusarium solani*. The species of mold found in more number in the storage of one week and the few number without treatment.

Keywords : *Diversity, Growth Rate, Musk Lime Pickle*

INTRODUCTION

The food consists of protein, fat, carbohydrates, vitamins, and minerals. Generally, the food is a good medium for the growth of various microorganisms. In favorable physical state, especially in the temperature range 7°C-60°C, microorganisms will grow and cause changes in the appearance, taste, smell, and other properties on the food (irianto, 2006)

Change caused by microorganisms in food is not limited to the formation of decomposition alone, but can also be a product of microbial synthesis. Some microorganisms form a pigment that changes the color of the food. There also may synthesize polysaccharides and produce mucus in or on foods such as pickled foods.

Pickle is a traditional fermented foods are quite favored by most people of Indonesia. Pickles comes from the word "Achar" in Hindi or "Pickle" in English, has the meaning of a side dish mixed with various herbs that have a sour or spicy flavor. There are various types of pickles are often used as a side dish. Pickles are often served at traditional events and religious holidays (Anonymous, 2007).

Basically pickle is a food that is treated by given of vinegar, salt, sugar and mixed with other spices. Giving vinegar, sugar, salt and spices that not only add flavor to food, but also serves as a preservative. Acidic environment can inhibit the growth of some microorganisms

that are not acid resistant. Although the administration of vinegar can maintain the condition of pickles, but just like other foods, pickles can also be contaminated (Supardi and Sukanto, 1999).

The preliminary study of contamination in the event has been done on Pickled Cucumbers. The results obtained indicate that Cucumbers are made into pickles can also be contaminated by various types of fungi. Therefore, the authors wanted to examine the diversity and growth of mold in pickled foods, in this case made pickle is Lemons Kasturi Pickle with various of storage period treatment.

In Lemons Kasturi Pickle is likely contaminated period can last longer than cucumbers pickle, this is due to the manufacturing process through the heating process in advance, using spices, vinegar and lime sour musk which naturally will slow the growth of microorganisms contaminants. Therefore, the authors wanted to see whether there is a difference in the type of mold that grows on cucumber pickle with Lemons Kasturi pickle.

Contamination of food can occur since the early post-harvest, processing time, and storage period. Contamination can be caused by bacteria, viruses, and fungi. Contamination caused by fungi commonly found in foods, because reduce the substrates of food into compounds that can be used as a food source for the mold.

In addition to causing a change in color, flavor and texture to the pickle that has been contaminated, very high humidity can also help the growth of mold to produce mycotoxins. Mycotoxins are fungal metabolic compounds that are toxic and can harm human health (Gandjar et al., 2006). Therefore, the authors wanted to hold explorative experiment by isolating fungi-mold on musk lime pickle with various of the storage period treatment, observe the rate of growth of mold and identify the types of mold that could potentially produce mycotoxins.

METHODOLOGY

This research was conducted at the Laboratory of Biological Science UNIMED. When the study carried out for 8 months starting in May 2011 to December 2011. The sample is mold found on Pickled Lemons using PDA medium that has been provided. Determining the type of mold is done with the "purposive sampling" (intentional footage) in each treatment in musk lime pickle (*Citrofortunella microcarpa*) Each type of mold identification and calculated the rate of growth and the number of colonies microscopically by microscope observation.

Non-factorial experimental research methods, completely randomized design (CRD) with 5 level storage treatment and 5 replications. Level storage treatments: 0 week, 1 week, 2 weeks, 3 weeks, and 4 weeks. The parameters observed, among others; diversity, growth rate, and the number of colonies of each type. The method used in the isolation of fungi are planting

dilution method. Samples were transferred to the PDA medium and then incubated at 27°C for 6 weeks. Analysis of the data used are variants of Non Factorial Analysis and counting the number of colonies.

RESULTS AND DISCUSSION

Results of research on diversity both of species and rate of growth from mold that grows on lime pickle with various storage times and then isolated on PDA (Potato Dextrose Agar) with an incubation period of 6 weeks, the obtained data is the result of the diversity from types in Table 1.

Table 1. Type of Kapang that Growing in Kasturi with Pickled Lemons with treatment of Various Storage Time

Long Storage	Repetition				
	Cup 1	Cup 2	Cup 3	Cup 4	Cup 5
Control	<i>A. niger</i> <i>F. solani</i>	<i>A. niger</i>	<i>A. niger</i> <i>A.fumigatus</i> <i>P. digitatum</i>	<i>A. niger</i>	<i>A. niger</i>
1 week	<i>A. fumigatus</i> <i>A. niger</i>	<i>A. fumigatus</i> <i>A. niger</i> <i>P. digitatum</i>	<i>A. niger</i> <i>F.solani</i>	<i>A. niger</i>	<i>A. niger</i> <i>A. fumigatus</i>
2 weeks	<i>A. tamarii</i>	<i>A. fumigatus</i> <i>P. digitatum</i>	<i>A.fumigatus</i>	<i>A.tamarii</i> <i>F. solani</i>	<i>A. fumigatus</i> <i>F. solani</i> <i>M.mucedo</i>
3 weeks	<i>M.mucedo</i> <i>A.tamarii</i> <i>A.fumigatus</i>	<i>P. digitatum</i> <i>F. solani</i>	<i>F. solani</i> <i>A.tamarii</i>	<i>A. fumigatus</i>	<i>P. digitatum</i>
4 weeks	<i>A. tamarii</i> <i>A.fumigatus</i>	<i>A.fumigatus</i>	<i>A. tamarii</i> <i>P.digitatum</i>	<i>A. fumigatus</i>	<i>A.tamarii</i> <i>A. fumigatus</i> <i>M.mucedo</i>

Based on the data about both of types and growth from mold that grows in Lemons Kasturi Pickle with various storage time treatment, it was found six species of fungi are: *Aspergillus fumigatus*, *Penicillium digitatum*, *Aspergillus tamarii*, *Aspergillus niger*, *Fusarium solani*, and *Mucor mucedo*.

Based on the data of diversity, the storage time period, the diversity of types of mold that grows in a steady state, since each - each type of mold does not grow at the same time, there are some types of mold that is only able to grow in different environmental conditions than other mold. Many types of mold that grows in every treatment is *Aspergillus fumigatus*, *Penicillium digitatum*, and *Fusarium solani*, although in storage for 4 weeks, *Fusarium solani* was not found again. *Aspergillus tamarii* and *Mucor mucedo* only grow in treatment 2, 3, and 4 weeks, while *Aspergillus niger* grown only on storage treatment without (control) and storage of one week, it is because Lemons Kasturi Pickle treated without storage and 1 week still has high acid levels

are 4 -5. growth of *Aspergillus niger* would be better in acidic or low pH (Fardiaz, 1992). Meanwhile, *Aspergillus tamarii* found in storage 2,3, and 4 weeks showed that this fungus can grow and sporulate well at neutral pH conditions are 6.5-7 and 30°C. In the storage period 2, 3 and 4 weeks of acidity on Lemons Kasturi Pickle began to decrease because the environment has been mixed with a compound of the metabolism of fungi that grow first. Furthermore, the growth rate of the data obtained by counting the number of colonies for 6 weeks showed that the storage period, the number of colonies of mold that grows from each kind of diminishing and even grow. The number of colonies on the wane, it is because mold cannot adapt to the environment has changed. These environmental changes may lead to reduced availability of food so that energy needs to be used for the synthesis of various parts of the cell is also reduced.

Aspergillus fumigatus has the most number of colonies, it is because of *Aspergillus fumigatus* is a mold that is able to live in conditions of acid or alkaline environment that is at pH 4-8. *Aspergillus fumigatus* can grow at a temperature of 25°C-35°C and the environment containing a little water (Svein, 2008). This situation according to the temperature at the time of incubation. Meanwhile, at least the number of colonies present in *Mucor mucedo*, this is because the fungus *Mucor mucedo* is often found in food that has been damaged because of its ability to break down the food contained compounds. However, the presence of other fungi were also able to break down food compounds capable of inhibiting the growth of *Mucor mucedo* colony.

Based on the growth rate chart from each of mold in each treatment showed that the longer the incubation period did not affect the increase in the number of colonies on all types of molds. *Penicillium digitatum*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus tamarii* is capable of living and adapt to the acidic environment such as food pickles and circumstances appropriate temperature is 25°C - 35°C support to continue to grow and sporulate. While *Mucor mucedo* and *Fusarium solani* can live in an environment that is closer to neutral pH is 5.5 to 6.5 so that the number of colonies growing on a bit because of certain conditions.

Aspergillus fumigatus, *Aspergillus tamarii*, and *Aspergillus niger* is a mycotoxin-producing fungi that can cause a type of aflatoxin liver cancer. Diseases caused by fungi of the genus *Aspergillus* called Aspergillosis (Wikipedia, 2008). Meanwhile, *Penicillium digitatum* the growth of mold that is found in citrus fruits contaminated. This is due to *Penicillium digitatum* works by generating gas Etilene capable accelerate the ripening process so much faster citrus fruit and soft rot (Makfoeld, 1993).

From the data obtained, it can be identified morphological types both of fungi macroscopic and microscopic.

1. *Aspergillus fumigatus*

Macroscopic observation. Colony color green moss, coarse texture, semi-spherical shape of the colony, colony reverse yellowish white color, colony diameter ranges from 25 mm.

Microscopic observation. Hyphae do not have septum, mycelium bright with bright green to yellowish green (if older), produces asexual spores form conidia and sexual spore form of the ascus. Conidia have a columnar-shaped green color (chain), coarse texture, made up of many cells. Conidiophores long, single and simple, smooth-walled with a slightly rounded shape and colorless. (Makfoeld, 1993).

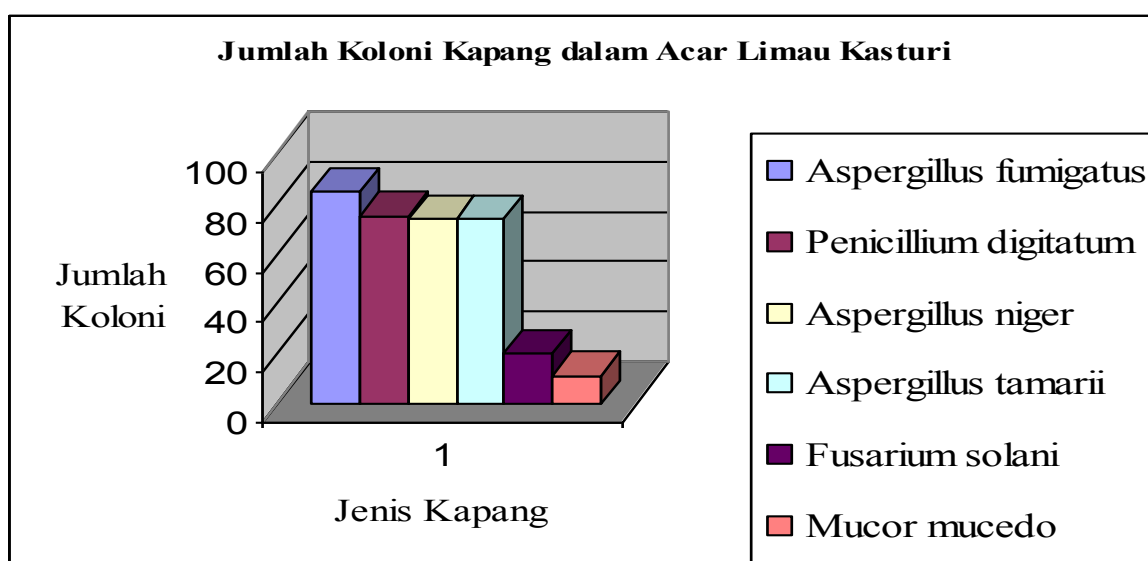


Figure 1. Bar Chart of the number of colonies throughout the mol Pickled Lemons in Kasturi for 6 weeks

2. *Aspergillus niger*

Macroscopic observation. Colony is brownish black, spherical shape, rough texture, tawny reversionary colony, have diameter ranges from 83 mm.

Microscopic observation. Hyphae do not have septum, mycelium cloudy with a blackish brown color, has a form of asexual spores conidia with blackish brown color, globosa shape, rough texture, composed of many cells. Conidiophores long, single, simple and colorless. (Makfoeld, 1993).

3. *Aspergillus tamarii*

Macroscopic observation. Colony is dark brown, spherical colony shape, terkstur coarse, brownish yellow color reversionary colony, the colony diameters ranging from 81 mm.

Microscopic observation. Hyphae do not have septum, mycelium cloudy with a dark brown color, has a form of asexual spores menjari conidia with a yellow brown color, shape

globosa, rough texture, composed of many cells. Single and simple conidiophores, no color. (Makfoeld, 1993).

4. *Fusarium solani*

Macroscopic observation. Colony is bright white, semi-spherical shape of the colony, smooth texture like cotton, white colonies brownish color reverse, ranging from 65 mm diameter colonies.

Microscopic observation. Hyphae have septum, mycelium white light with bright colors, have asexual spores to produce two kinds of conidia, which is divided into makrokonidia and mikrokonidium. Makrokonidia shaped curved long, thin-walled, smooth texture, which deal directly with conidiophores forming sporodokium. Mikrokonidium small round, consisting of one to three cells (Wikipedia, 2008).

5. *Mucor mucedo*

Macroscopic observation. Colony is yellowish white, shape round, smooth texture like cotton, reverse color yellowish white, colony diameter of 75 mm.

Microscopic observation. Hyphae have septum, mycelium bright yellow color, has a sexual spore form of asexual spores form zigospora and sporangiophores, sporangium round, gray to black and filled with sporangiophores. Sporangiofor short, straight and branched to form elongated branching. Sporangiofores is round, has a specific structure in the form of rhizoid (Wikipedia, 2008).

6. *Penicillium digitatum*

Macroscopic observation. Colony color turquoise, semi-spherical shape of the colony, smooth texture, color reverse yellowish white colonies, diameter of about 12 mm.

Microscopic observation. Hyphae have septum, mycelium bright turquoise, has a form of asexual spores conidia in green, penicillus shaped, smooth texture, composed of many cells. Head carrier conidial spore chain, branched conidiophores with branching shape biverticillata.

CONCLUSION

Six types of mold found in musk lime pickle namely: *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus tamarii*, *Fusarium solani*, *Mucor mucedo* and *Penicillium digitatum*. The period of incubation is longer, so growth rate from each of mold type showed varying results. *Penicillium digitatum*, *Aspergillus fumigatus* and *Aspergillus tamarii* continue to increase the number of colonies along the incubation period and peaked at week 6. While *Fusarium solani* and *Mucor mucedo* shows a steady growth rate. *Aspergillus fumigatus* has the most number of

colonies (85 colonies) because of its ability to grow in any condition, while the mold *Mucor mucedo* (11 colonies) have the fewest number of colonies for living with a broken down food and compete with other types of fungi. Statistical analysis showed that the difference in the growth rate of the storage period no significant effect on growth for each type of mold, but the real impact on the number of colonies of *Aspergillus niger*.

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