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## Use of *Lactobacillus plantarum* fermentation products in bread-making to prevent *Bacillus subtilis* ropy spoilage

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### Abstract

Four fermentation products (FPs) of the lactic acid bacterium *Lactobacillus plantarum* ITM21B were screened for their anti-*Bacillus* activity *in vitro* and in bread-making trials. Results of the storage tests performed with loaves prepared with an FP or calcium propionate demonstrated that after 5 days at 30 °C, gross spoilage was evident in only the control loaves, which contained *Bacillus subtilis* at numbers of about 10<sup>8</sup> cfu/g. The highest inhibitory activity was shown by DM-FP obtained by growing *L. plantarum* in a defined medium (DM). Significantly, this medium contained an amino acceptor of the amino acid transamination, namely  $\alpha$ -ketoglutaric acid, and an amino acid pool. With loaves prepared using the DM acid mixture which simulated the DM-FP composition, the same reduction of ropy spoilage as with DM FP was obtained after 3 days, while the efficacy of the mixture decreased after 7 days. This result suggests the potential involvement of some unknown metabolites in the inhibitory activity of DM-FP. In baked products made with flour based media (M1-FP, M2-FP, M3-FP), no ropy symptoms were noticeable after 3 days storage although a considerable *Bacillus* count was detected. DM-FP was as effective as calcium propionate (0.3% w/w, based on flour mass) in prolonging the *Bacillus* free-shell life of yeast-fermented bread for 7 days. © 2008 Elsevier B.V. All rights reserved.

**Keywords:** Bread shelf-life; Ropy spoilage; *Lactobacillus plantarum*; *Bacillus subtilis*; Fermentation products; Organic acids; Bread-making

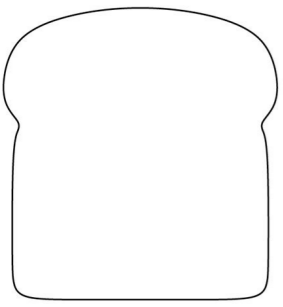
### 1. Introduction

Preservatives are commonly used in breads because economic losses from bread spoilage caused by bacteria or by moulds are substantial. Ropy spoilage is caused mainly by *Bacillus subtilis* and *Bacillus licheniformis*, the spores of which contaminate raw materials such as flour, bread improvers, yeast, etc., and survive baking temperatures (Rosenqvist and Hansen, 1995). Ropy spoilage in bread is first detected by an odour similar to that of pineapple. Later, the crumb becomes discoloured, soft and sticky to the touch, which makes the bread inedible. The deterioration of bread texture is due to slime being formed as a result of the combined effect of the proteolytic and amylolytic enzymes produced by some *Bacillus* strains that results in slime formation (Viljoen and von Holy, 1997; Sonnikova et al., 2003). The full extent of losses caused by ropy spoilage of bread is difficult to

quantify, because the condition is often misidentified as sour or rotten spoilage caused by failed dough leavening or an insufficient bake. Consumption of ropy bread may cause illness if bacteria are present at 2 × 10<sup>8</sup> cfu/g (Kramer and Gilbert, 1989; Rosenqvist and Hansen, 1995). Ropiness can develop very rapidly under warm and humid conditions, so it is a common problem in the warm climates of Mediterranean countries, Africa and Australia (Vojsey and Hammond, 1993). Our research was prompted by reports of ropy spoilage from several Italian bakeries during the summers and the autumns of the years from 2004 to 2006.

*Bacillus* spore numbers can be controlled by ensuring raw material quality, good sanitation and cooling of production and storage environments (Viljoen and von Holy, 1997). Spore germination and growth in bread can be inhibited by chemical preservatives such as propionic and acetic acids, although the current trend is to reduce the levels of these substances (Pattison et al., 2004; Marin et al., 2002). Acetic acid adversely affects the organoleptic quality of baked products, while propionic acid has been reported to cause irritability, restlessness, inattention and sleep disturbance in some children (Dangate and Ruben, 2002;

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### Study on fungi associated with spoilage of bread

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#### Abstract

Bread is one of the staple foods in the world and it is recognized as a semi perishable commodity. Usually the spoilage of bread is due to improper storage. The fungi associated with the spoilage of bread were studied. Mold spoilage of bread is due to post processing contamination. Twenty bread samples were collected from different shops in Jaffna. The fungi involved during spoilage were isolated and identified based on the cultural and morphological characteristics using the standard keys available. Initially the molds namely *Mucor* sp and *Rhizopus* sp were found to be the cause of bread spoilage. This was followed by *Aspergillus* sp and *Penicillium* sp. In this study *Rhizopus* sp was found to be the most common fungus during the spoilage of bread.

**Keywords:** Bread, spoilage, *Mucor* sp, *Rhizopus* sp.

#### Introduction

Bakery products, like bread has become an important staple food in many countries. Cereals and bakery products serve as a valuable source of nutrients in the diet of many people. They provide most of our food calories. Bakery products provide nutrients such as carbohydrates, proteins, lipids vitamins and minerals. A variety of bakery products are available in the market. Earlier bakery products were considered as a sick man's diet or poor man's diet. It has now become the essential food item for a vast majority of the whole population. Bread is made by mixing flour, salt, yeast and other ingredients which is followed by baking. The basic process involves mixing the above ingredients until the flour is made into dough. The dough is baked into a loaf. The dough is made in such a way that will rise easily and be able to give a bread of good quality to the consumer. Yeast is used in the dough which releases CO<sub>2</sub> and the bread becomes spongy. Earlier airborne yeasts were used in making

bread. This was done by keeping the dough exposed to air for sometime before baking. But the technology has improved the bread making to a greater extent in which high energy mixing is involved.

Usually the mold spoilage of bread is due to post processing contamination. Bread loaves fresh out of the oven are free of molds or mold spores due to their thermal inactivation during the baking process (Pone and Tsen, 1978). Bread becomes contaminated after baking, from the mold spores present in the atmosphere surrounding loaves during cooling, slicing, packaging and storage. Most common source of microbial spoilage is due to mold growth. According to the previous studies (Banwart, 2004) bread molds like *Mucor* and *Rhizopus* are found to grow first during bread spoilage. This is followed by some other fungi like *Aspergillus*, *Penicillium* and *Fusarium* sp.

## Identification, aflatoxin content, and antagonistic test of spoilage fungi in bread to *Aspergillus niger*

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**Abstract.** *Fendiyanto MH, Satrio RD. 2020. Identification, aflatoxin content, and antagonistic test of spoilage fungi in bread to Aspergillus niger. Bioteknologi 17: 60-66.* The major problem in shelf storage life in bread is contaminated by molds fungi and its mycotoxins. The growth of the spoilage fungi indicates the bread has structural damage. Infection from this fungus will affect the shelf life of the bread to be shorter. Contaminated bread will cause influenced human health in the future. One of the many solutions to overcome the fungi-contaminated of bread is the use of biological agents. *Aspergillus niger* has the ability as a biological agent in suppressing food-destroying pathogens in many foods including bread as well. However, there were few reports about the antagonistic test in bread particularly between *A.niger* as biological control agent to spoilage fungi. Therefore, this study aimed to analyze the growth antagonistic test of *A. niger* against food spoilage fungi, expecting the storage life of bread can be extended and avoid mycotoxins contamination. The antagonistic test was in the form of a test that utilizes the properties of microorganisms that grow faster than pathogens or produce antibiotic compounds. The methods used in this study included isolation and identification of fungi, water content analysis, aflatoxin test, and antagonistic test. Interestingly, the antagonist test results showed *A. niger* can inhibit the growth of spoilage fungi on bread. In conclusion, the species of fungus with the highest inhibition value was *Hypophybia burtonii*, while the lowest was *Saccharomyces cerevisiae*. This finding indicates that *A.niger* can be used as a biological control in extending bread shelf storage in the future.

**Keywords:** antagonist test, *Aspergillus niger*, spoilage fungi

### INTRODUCTION

The success of global development is determined by the availability of quality human resources (HR) which has strong physical, strong minds, and excellent health in addition to mastery of science and technology. The quality of a country's human resources will be low if there is malnutrition so that many people whose health is disturbed and their intelligence decreases. Therefore, the main step in creating superior human resources is by improving the quality of nutrition. The continued impact of the decline in the nutritional status or health of these nutrient vulnerable groups in the long term will reduce the quality of Indonesia's human resources (Syarif 1997). FAO classifies Indonesia as a low income and food shortage country. The number of poor people in Indonesia is close to 50 million, of which 33.7 million live in rural areas (BPS 2001). Fulfilling food needs for energy sources in activities. Food sources of energy provide energy and stamina, consuming these foods will increase vitality so that it is possible to live longer, not too easily, increase immunity and help keep the body fit and healthy (Marshall 2005).

Carbohydrates are one of the most common macronutrients and the cheapest source of calories (Handley and Sallem 2002). These nutrients are the main source of calories for almost all of the world's population, especially for residents of developing countries.

Carbohydrates have an important role in determining the characteristics of food ingredients such as taste, color, and texture. Whereas in the body, carbohydrates are useful for preventing ketosis, breaking down excessive body protein, losing minerals, and for helping metabolize fat and protein (Witarno 2008). It is recommended that carbohydrate food be consumed by 6-11 servings per day, for example, food sources of carbohydrates, namely bread, rice, cereals, and pasta (Astawan and Kasih 2008).

Bread is a group of carbohydrate foods that should be consumed by about one-third of the diet. Bread, like pasta, potatoes, rice, and other cereals, is the best food source of energy. These foods also contain B complex vitamins to release energy from food and maintain a healthy digestive and nervous system. The recommended amount of carbohydrate foods is 6 or more servings per day (Marshall 2005). Bread is made from wheat flour that is spread with yeast and baked. Now bread has become one of the staple foods for Indonesians, even among teenagers and children. The position of this food is starting to shift rice as the main source of carbohydrates because of its nutritional content that is not inferior to rice or noodles.

Bread is a flour product that is easily damaged, especially due to fungi attack. The types of fungi that often contaminate food are mold and yeast, but the more dominant ones are molds. Fungi are microbes consisting of more than one cell in the form of fine threads called

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C. Spoilage Signs eÅÅÅ eÅ eÅ eÅ Souring eÅÅÅ Production of acid e.g. sour milk from production of lactic acid eÅÅ eÅ Gas formation eÅÅÅ Meat becomes spongy eÅÅÅ Swollen or bubbling packages and cans 18. eÅ eÅ Spoilage of crustaceans (shrimp, lobsters, crabs and crayfish) is similar, but these products have some CHO (0.5%) and more free amino acids so spoilage can occur more rapidly. - results in increased CO2 levels and thus get a longer shelf life. eÅ eÅ These organisms may metabolize citrate, lactose, pyruvate or lactic acid and produce butyrate or acetate plus CO2 or H2 gas which eÅÅÅblows eÅÅÅ the cheese. eÅ eÅ Rhizopus stolonifer is the common bread mold, and other species from this genus spoil cereals and other baked goods. 56. eÅ eÅ some G- enzymes, thermotolerant G+ bacteria and spores survive eÅ eÅ Psychotropic Bacillus spp. 70. eÅ eÅ eÅ eÅ While the bacteria itself may or may not be harmful, the waste products may be unpleasant to taste or may even be harmful to one's health. -Cooked products have a higher pH (>6.0) which may allow growth of G- facultative anaerobic pathogens like Yersinia enterocolitica. c. Stable or non perishable foods. eÅ eÅ Food spoilage can be the result of: eÅÅÅ insect damage eÅÅÅ physical injury eÅÅÅ enzymatic degradation eÅÅÅ microbial activity 5. B. 19. eÅ eÅ Pasteurization kills most G- (incl. 26. 3. Uncooked or under-cooked animal flesh that spoils is typically quite toxic, and consumption can result in serious illness or death. 28. Meat spoilage eÅÅÅ 2- Bacillus and clostridia (e.g. C. Examples: potatoes, apples and nuts. Meat Spoilage eÅ eÅ Processed meats (hot dogs, sausage and luncheon meats) eÅ eÅ These products are composed of a variety of blended ingredients, any of iserpnoc( enrac alled otnemaroiireted II ehc eraton etnasseretni Å ... enrac alled otnemaroiireted II .S2H o 2O2H id noitU dorP laiborcim ad otasuac gnienerG .only len etnatropmi 'Aip otnemaroiireted id otiveil li 'A adlavnoC adidnac eÅ ~å eÅ eÅ SENIV .35 .21 .elaicifrepus affum alled aticserc al ennat otnemaroiireted id imsinagrorcim ied etrap roiggam al ecсібini elas otla da )3( ossab Hp )2( WA ossab )1( itarutam iggamroF eÅ ~å eÅ .36 )6,5-8 .1 ( hp wol- Hp- erudrev id OHC 'Aip e .itturf ied otnemaroiireted len irettab ied itnatropmi 'Aip onos effum e itiveil .azneugsnoc id .6 onгдаug o .Åtidimu id atidrep- ocisif enoizacifnaiP .)%1

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