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A study on bread mould spoilage by using lactic acid bacteria and yeast with antifungal properties

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Abstract
Bread is the most important staple food in the Western world and it is generally viewed as a perishable commodity, The presence of mould, another concern is the potential mycotoxins production that may cause public health problems Lactic acid bacteria (LAB) as bio preservation organisms are of particular interest: they have been used for centuries as starter cultures in the food industry and are able to produce different kind of bioactive molecules that inhibit fungal spoilage. The efficacy of antifungal LAB as bio-preservative in bread manufacture industry is quite relevant. The inhibitory activity against molds. Thus, Lactic acid has the potential to improve the shelf-life of bread under normal circumstances. The use of LAB as protective cultures may possess several advantages over the use of purified bacteriocin; the cultures may serve as the source of bacteriocin as well as a broad range of other antimicrobials including organic acids, carbon dioxide, ethanol, hydrogen peroxide, and diacetyl These microorganisms may also exhibit antimicrobial activity against spoilage microorganisms, thus increasing shelf life. Therefore, LAB can be used as protective cultures to inhibit pathogens and/or prolong the shelf life of foods, or they may be useful as biosanitizer to reduce or eliminate colonization of pathogens in an environment. The proper choice of LAB (either alone or in combination with other hurdles), as well as the selection and development of strains with best performance for each particular food, may greatly reinforce the competitiveness of bio preservation methods in the food industry.

Keywords: bread spoilage, lactic acid bacteria, antifungal activity

1. Introduction
Bread is one of the most important staple foods in the world and can be spoiled by many moulds, of which Penicillium species are by far the most common. However, the dominant spoilage flora varies with the type of bread and the storage temperature. Fungal spoilage is the main cause of substantial economic losses in packaged bakery products and might also be regarded as sources of mycotoxins, involving public health problems (Brashears and Durre, 1999) [1]. In this context, LAB may be considered as an alternative for bio-conservation. In this study but limited applications of the antifungal strains in baking have been reported (Allende *et al*. 2007) [6] were used in the formulation of a mixed starter culture and used together with *S. cerevisiae* (commercial yeast) in bread elaboration.
Spoilage of bakery products is mainly due to fungal growth; the major species involved are Aspergillus, Fusarium, and Penicillium. In addition to the great economic losses derived from the presence of mould, another concern is the potential mycotoxin production that may cause public health problems (Brashears *et al*. 1998) [7]. Contamination by moulds can be prevented by irradiating the goods with infrared rays or microwaves, by using modified atmospheres during packaging, or by adding chemical preservatives such as propionic acid (Braun *et al*. 1974) [8]. In recent years, bio-preservation (the use of microorganisms and/or their metabolites to prevent spoilage and to extend the shelf life of foods) (Calderon *et al*. 2002) [5] has gained increasing interest due to consumers' demands. Lactic acid bacteria (LAB) as bio-preservation organisms are of particular interest: they have been used for centuries as starter cultures in the food industry and are able to produce different kind of bioactive molecules, such as organic acids, fatty acids, hydrogen peroxide and bacteriocin.
Lactic acid bacteria (LAB) are a heterogeneous group of bacteria found widely in nature. They colonize the gastrointestinal and urogenital tracts of humans and animals, and are present in foods such as dairy products, fermented meats, fruits and vegetables. LAB is also intentionally added to several probiotic products because of their potential health benefits. Many LAB species are generally recognized as safe (GRAS), and several LAB species have received a Qualified Presumption of Safety (QPS) status given by European Food Safety Authority (EFSA) When a food or an ingredient is unfamiliar, consumers tend to amplify the risk; conversely, their perception of risk declines with familiar foods or ingredients (Carla *et al*. 2009) [3]. Consumers are increasingly conscious of food contents and are more frequently reading labels; when they encounter names of additives that are difficult to pronounce and not familiar to them, they perceive these substances to present a higher risk (Corsetti *et al*. 2005). LAB has the potential to provide a "clean label" desired by many consumers, and may represent a useful and effective strategy to prevent or reduce the incidence of pathogens, thus improving food safety and consumer health
Research on LAB has shown that a number of natural antimicrobials produced by these Microorganisms are capable of playing an expanding role in meeting the increasing Consumer demand for "clean labels" on their food products. The time seems opportune For increased commercial evaluation of LAB in potential novel roles as bio preservatives And biosnitizers Lactic Acid Bacteria (LAB) is a diverse group of beneficial bacteria which have been inadvertently used by

mankind for thousands of years. They have been used as starter cultures for preparation of an array of fermented dairy products which include yoghurt, cheese, buttermilk, kefir and many products indigenous to various regions of the world. The preparation of these products has been documented in archaic texts of various regions such as ancient Iraq (De Angelis *et al*. 2003) [3]. The starter cultures not only provide peculiar taste to fermented products, but also serve to extend the shelf-life of the product mainly by the conversion of lactose to lactic acid.

2. Materials and methods

2.1 Sample collection

Both refined flour [350g] and wheat flour [350g] was added with LAB mix culture and [15-20g] fresh yeast and 5mg salt for texture and moisture luke warm milk [5-10ml] and another batch of bread samples without adding LAB mix culture for control and then Baked at 180˚C in oven. All bread samples were kept at room Temperature.

2.2 Preparation of samples

Potato Dextrose Agar (PDA) which is a common medium to grow fungi was used in this study. Thirty grams of PDA was dissolved in 100ml distilled water. Then this medium was autoclaved at 121˚C for 15 mins. After sterilization, it was allowed to cool down to about 50˚C. About 20 ml of the medium was poured into each sterilized petri dish. The PDA medium in petri dish was allowed to solidify. One-gram bread sample was mixed with distilled water and a homogenate was prepared. Dilution plate method was carried out to enumerate the fungi, The working surface was sterilized using ethanol. 1ml was taken from the above homogenate. Serial dilution was done at the recommended dilution rate *i.e* 1:10 (1+9). Dilution was done using saline water. Aliquots are drawn for dilution within one min because fungal spores sediment quickly than bacteria (Beuchat, 1992) [9]. Dilutions up to 10-4 were carried out. 0.1 ml of the inoculum was added on the surface of the PDA medium and spreaded evenly over the surface using a sterile spreader (bent glass rod). The plates were incubated in an upright position at 30˚C for 4-5 days.

The same procedure was carried out for all the samples. The fungal count was recorded. The different types of colonies were used as inocula to obtain pure cultures by sub culturing in PDA. A small portion of each sub-cultured colony was cut using a sterile scalpel. It was placed on a sterile glass slide using sterile forceps. The slide was covered with a cover slip and placed in a petri dish. Similar procedure was carried out for other fungal colonies as well. These petri dishes were left at 30˚C for 5 days. The cover slips were taken with forceps and placed on slides containing cotton blue. The excess stain was removed and observed under the microscope. The morphology ie shape, structure of conidia, conidiophores, pigmentation, shape of sporangia, sporangiophores were recorded. The identification was based on the standard keys available.

3. Results and discussion

After 7 days of incubation period, the range of fungal count was 7-10 x 10^4 colonies per plate on average. Fungal growth was not observed during the first two days in all the bread samples. But fungal growth was observed in all the samples from the fourth day. The fungal count increased with the days of storage. Table 1 shows the isolated fungi based on the cultural and morphological characteristics. From the studies of Table 2 and 3 we found that those bread samples having activated with LAB mix culture was able to withstand fungal spoilage until the 4th day. In Figure 1 shows Fungal isolates concentration present in the bread sample (wheat flour and refined flour). Is too higher than compared to Figure 2 Fungal isolates concentration present in the bread sample (wheat flour and refined flour + LAB mix culture).its because of the antifungal activity of LAB. Lactic and acetic acids, the main products of the fermentation of carbohydrates by LAB prevent the further growth of fungus species. Also the frequency occurrence of fungi were 40% difference in *Mucor* sp., and 20% in *Rhizopus* sp. compared to control bread sample which doesn't contain LAB mix culture. Its promises great storage and shelf life extent ability. Also it's healthier and it can be kept for a longer duration without fungal spoilage

This study revealed that there is contaminant in bread that may pose a biohazard to consumers. The high prevalence of *Rhizopus* and *Mucor* sp. in this study agrees that the organisms should not be taken lightly because is associated with food poisoning or intoxication. Research on LAB has shown that a number of natural antimicrobials produced by these microorganisms are capable of playing an expanding role in meeting the increasing consumer demand for "clean labels" on their food products. The time seems opportune for increased commercial evaluation of LAB in potential novel roles as bio preservatives and biosanitizer. Anti-microbial of *Lactobacillus* inhibits *Aspergillus niger, Mucor, Rhizopus, Penicillium* contaminants from flour, yeast and other sources. These metabolites may also exhibit some activity against spores, which may pass into a vegetative form. If these metabolites are heat stable, they may inhibit pathogen organisms contaminated through packaging and marketing and pore germination after baking.

Table 1: Cultural and morphological characteristics of identified fungi's

Fungal isolate	Cultural characteristics	Morphological characteristics
Mucor sp.	Large white colonies which turns into black later.	Erect sporangiophores are formed. Sporangiophore swells at the tip to form sporangia which are globular shaped. Columella is present.
Rhizopus sp.	White cottony mycelia, with black dots and covers the entire surface	Sporangiospores are produced inside a spherical sporangium. Columella is present on the top of the sporagiophore. Root-like rhizoids are found.
Penicillium sp.	Fast-growing colonies in green colour with dense conidia	Branched conidiophores with chains of conidia looks like a brush.
Aspergillus sp.	Yellow or yellowish green colonies with distinct margin	Conidiophores arise from a footcell. Club shaped vesicles at top of the conidiophores. Conidia are found in chains.

Table 2: Frequency of occurrence of fungi in bread sample (refined and wheat flour)

Day	Fungal isolates	Fungus frequency (%)
1-3	No growth	0
4	*Mucor* sp	70
5	*Rhizopus* sp	80
6	*Aspergillus* sp	20
7	*Penicillium* sp	15

Total no of samples=10, % frequency = (No of occurrence of fungi/Total No of samples) x 100

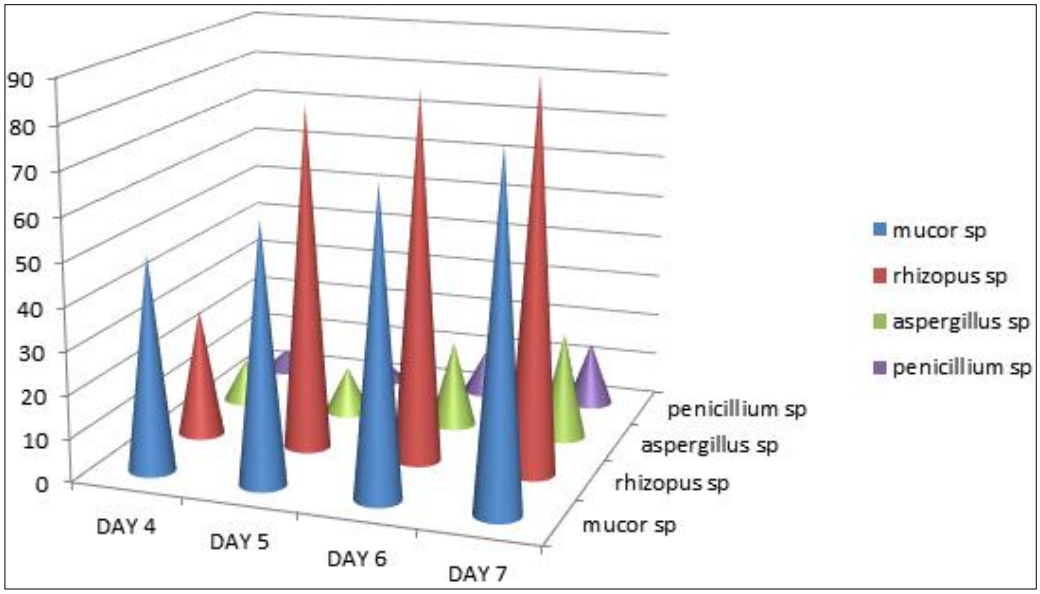

Fig 1: Fungal isolates concentration present in the bread sample (wheat flour and refined flour)

Table 3: Frequency of occurrence of fungi in bread sample (refined and wheat flour + LAB mix culture)

Day	Fungal isolates	Fungus Frequency (%)
1-3	No growth	0
4	*Mucor* sp	30
5	*Rhizopus* sp	60
6	*Aspergillus* sp	20
7	*Penicillium* sp	15

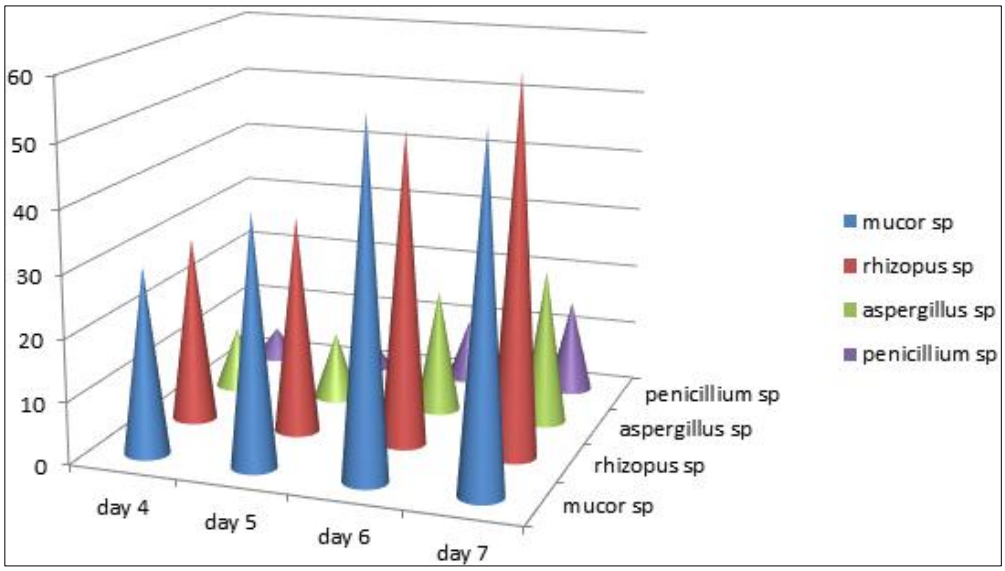

Fig 2: Fungal isolates concentration present in the bread sample (wheat flour and refined flour + LAB mix culture).

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