100-) ANTIFUNGAL ÖZELLIKLERE SAHIP LAKTIK ASIT BAKTERILERI KULLANILARAK BEYAZ EKMEKTE KÜFLENMENIN ENGELLENMESI VE RAF ÖMRÜNÜN UZATILMASI

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ÖZET

Ekşi hamur; kendiliğinden fermente olabilen, ekmeğin besleyici değerini, duyusal özelliklerini ve raf ömrünü geliştirmek için kullanılan geleneksel bir ürün olmasının yanı sıra fonksiyonel ve teknolojik özelliklere sahip laktik asit bakterilerinin bir kaynağı olarak gösterilmektedir. Laktik asit bakterilerinin, biyo-koruyucu etkisi uzun süredir bilinen bir fonksiyon olmakla birlikte bu etki laktik asit, asetik asit ve hidrojen peroksit oluşturmalarıyla yakından ilişkilidir. Ekmek ürünlerinin bozulmasının ana etmenini küfler oluşturmaktadır. Laktik asit bakterilerinin fırıncılık ürünlerinde küf gelişimini inhibe ettiği bilinen fonksiyonları arasında yer almaktadır. Bu çalışmanın amacı, 12 farklı LAB suşlarının antifungal aktivitesi belirlemek ve antifungal özelliğe sahip bakteriler ile beyaz ekşi hamur ekmeği hazırlayıp, küflenmenin engellenmesi ve raf ömrünün uzatılmasını sağlamaktır. İn vitro antifungal analiz sonucunda 6 bakterinin antifungal aktiviteye sahip olduğu belirlenmiş ve aralarından iki laktik asit bakterisi ile maya starteri olarak HM3 (*S.pastorium*) kullanılarak üç farklı ekşi hamur hazırlanmıştır. Fermentasyon süresince 0. ve 24. saatlerde ekşi hamur örneklerinde pH, titrasyon asitliği, mikrobiyolojik analiz belirlenmiştir. Daha sonra bu ekşi hamurlardan ekmek yapılarak, kontrollü küflendirilmesi takip edilmiş ve *L. brevis- L.plantarum* ile hazırlanan ekmekte en iyi sonuçlar elde edilmiştir. Sonuç olarak, bazı laktik asit bakterilerinin, ekmekte biyo koruyucu olarak küf gelişimini engelleyici fonksiyon gösterdiği tespit edilmiştir.

Anahtar Kelimeler: Ekşi hamur, LAB, Antifungal aktivite, Beyaz ekmek

Prevention of White Bread Mould Spoilage and Shelf-Life Extension of White Bread by using Lactic Acid Bacteria with Antifungal Properties

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ABSTRACT

Sourdough; It is a traditional product that can be fermented spontaneously and used to improve the nutritive value, sensory properties and shelf life of the bread. It is also shown as a source of lactic acid bacteria with functional and technological properties. The bioprotective effect of lactic acid bacteria is a known long-lasting function. This effect is closely related to the formation of lactic acid, acetic acid and hydrogen peroxide. Molds are the main factor in the deterioration of bread products. Lactic acid bacteria are among the known functions of inhibiting mold

growth in bakery products. The aim of this study is to determine the antifungal activity of 12 different LAB strains and to prepare white sourdough bread with lactic acid bacteria which have to antifungal properties, to prevent mold and to extension shelf life of bread. As a result of in vitro antifungal analysis, 6 of bacteria were found to have antifungal activity and three different sourdoughs were prepared by using two lactic acid bacteria and HM3 (*S. pastorium*) as yeast starter. In sourdough samples at 0 and 24 hours during fermentation were determined pH, titration acidity, microbiological analysis. Later, making bread from this sourdough, controlled mould was followed and the best results were obtained in bread prepared with *L. brevis- L. plantarum*. As a result, it has been found that some lactic acid bacteria have to function as a bioprotective agent preventing mold growth in bread.

Keywords: Sourdough, LAB, Antifungal activity, White bread.

Introductıon

Today, economical loss caused by bread mold is in large quantities and various methods such as the addition of chemical preservatives, packaging methods, use of sourdough are tried to prevent these losses. The long-term carcinogenic effects of chemicals such as calcium propionate, sorbic and benzoic acid salts, which are commonly used, have a negative impact on the consumer. For this reason, the use of sourdough, an alternative method for bread production, increases consumer demand as it is a natural method as well as improving textural and aromatic properties (Yan et al., 2016; Axel et al., 2016). The inhibitory effect of lactic acid bacteria (LAB) obtained from sourdough on molds is one of the popular topics recently studied. The complex microbial structure of the sourdough is largely composed of *Lactobacillus* species, and the antifungal effect of LAB-produced metabolites is reported by severel researchers belirtilmektedir (Gerez et al., 2009; Garofalo et al., 2012; Hassan et al., 2014; Rizzello et al., 2011; Oranusi et al., 2013; Blagojev et al., 2012). These metabolites include carboxylic acids, fatty acids, organic acids. It is also stated in the literature that antifungal peptides produced by some lactic acid bacteria are effective on inhibiting mold growth (Rouse et al., 2007; Rizzello et al., 2011). 3-phenyllactic acid and 4-hydroxyphenyllactic acid are expressed to have synergistic effect with other compounds besides antifungal effect (Mu et al., 2012; Lavermicocca et al., 2000). Axel et al. (2016) reported that *L. amylovorus* is the bacterium that exhibits the best antifungal activity against the *Fusarium* mold in sourdoughs made with three different *Lactobacillus* species. In another study, it was reported that the moldiness period of the *Penicilium* strain was similar when compared to the sour dough bread prepared with *L. plantarum* and the chemical preservative (0.25% CP) bread (Yan et al., 2016). It is known that lactic acid bacteria reduce the formation of mycotoxins produced by molds besides inhibiting the growth of molds. Gomah and Zohri (2014) reported that *L. paracasei* and *L. rhamnosus* species reduced the level of fumonisin B1 toxin produced by the *Fusarium* strain by 76.5%. Similarly, some lactic acid bacteria significantly reduce the formation of

aflatoxin B1 produced by *Aspergillus flavus* (Gomah et al., 2009).

The aim of our study is to determine the antifungal properties of 12 different lactic acid bacteria against 4 different molds (*Penicillium carneum, Aspergillus flavus, Aspergillus niger, Alternaria*) and examine the results by making bread with antifungal bacteria. After the microbiological count, pH and titration acidity of the maked sourdoughs were measured, breads were prepared. Lastly, controlled mold were followed by putting mold on bread slices.

Materıals And Methods

1)Bacterial strains and growth conditions

Bacterial strains used in this study were isolated from traditional Turkish sour dough. All strains were grown in MRS broth for 24 h at 37 ° C under anaerobic conditions.

2)Determination of in vitro Antifungal activities of sourdough isolates

Sourdough isolates were grown were grown at 37 ° C for 24 h with 1% inoculation in 10 mL of MRS broth. The suspensions were adjusted to 10^7 colony-forming unit (CFU)/ml and following the overnight growth, two 5-µl cellspots of LAB strains were placed on the MRS agar plates and plates were incubated anaerobically for 48 h at 37 °C. 10 ml of soft malt extract agar (2% glucose, 0.8% agar) containing 10^4 cfu / ml molds were inoculated on lactic acid bacteria and incubated for 2-3 days at 25 ° C and the antifungal activity was measured as clear zones of inhibition around the bacterial spots (Mauch et al. (2010).

3) Preparation of sourdough samples

Sourdoughs were prepared using the combination of lactic acid bacteria and starter yeast use. Doughs were prepared by mixing 100 g flour, 75 g water, 0.2 g lactic acid bacteria-1, 0.2 g lactic acid bacteria-2, 0.2 g yeast (starter) and fermented for 24 h at 30 ° C. LAB and yeast cell counts of sourdough samples were determined at 0 and 24 h of fermentation and were measured total titratable acidity (TTA) and pH values of the fermented sourdough.

4) Microbiological analysis in sourdough samples

10 g of the sourdough samples were homogenized the stomacherde in 90 ml physiological saline (FTS) and serial dilutions were prepared and plated onto MRS agar.

5) Determination of pH and acidity of sourdough samples

The pH and the total titratable acidity of the sourdoughs were determined according to the method given by Erbaş (2003) using the (WTW Inolab 7110) pH meter. A 5g sample was homogenized in 50 ml of water and suspension was titrated with a 0.1 N NaOH to a pH of 8.1.The TTA was expressed as the % lactic acid.

6) Making of sourdough bread

The dough was kneaded using 220 g flour, 110 g sourdough, 60 g water, 3 g salt, 2.75 g gluten. After shaping, fermentation was allowed for 2 hours. Then, sourdough bread was made.

7) Controlled mold of breads

Each slice of bread was inoculated with 10 µl of 10^6 spores per millilitre. Tests were made in parallel and incubated at 25° C. Colony diameter was measured on the fourth (Suhr and Nielsen, 2003).

Results and Discussion

1)Determination of in vitro Antifungal activities of sourdough isolates

The most common cause of degradation in bakery products is fungal growth. Mycotoxins threaten the health of the population. Also, It causes economic loss (Legan 1993). The antifungal activities of LAB isolated from sourdough against *A. niger, A. flavus, P. carneum,* which play an important role in the mold of bread, are shown in **Table 1**. While all strains

were ineffective against the *P.carneum* and *A.alternata* mold, 59E1B4-Lb.plantarum and 39LB2-Lb.plantarum strains were the most inhibitory to *A. niger,* A.flavus. Inhibitory effect of 78STB4-*L. numerensis* strain was observed to be inadequate for 4 mold species. Our results clearly demonstrated the antifungal effects of LABs isolated from sourdough.

Table 1. Antifungal activity of sourdough LAB

Types of Bacteria	*Penicillium carneum*	*Aspergillus flavus*	*Aspergillus niger*	*Alternaria*
4(34TB6N)-*Lb.crustorum*	-	8,0±0,0	6,0±0,0	-
7(39CB11)-*Lb.paracasei*	-	-	-	-
13(34TB1M)-*Lb.numerensis*	-	8,0±0,0	6,0±0,0	-
17(34BB5)-*Lb.koreensis*	-	-	2,0±0,0	-
11(59E1B4)-*Lb.plantarum*	-	8,0±0,0	10,0±0,0	-
21(37KB2)-*W.cibaria*	-	-	8,0±0,0	-
22(28C1B3)-*Lb.brevis*	-	2,0±0,0	12,0±0,0	-
24(61TB8)-*Lb.paracasei*	-	-	6,0±0,0	-
29(78STB4)-*L.numerensis*	-	-	-	-
31(34TB2M)-*Lb.brevis*	-	6,0±0,0	2,0±0,0	-
33(59O1B2)-*Lb.paralimentarius*	-	4,0±0,0	2,0±0,0	-
39LB2-*Lb.plantarum*	-	8,0±0,0	10,0±0,0	-

Lavermicocca et al., (2000) of *L. plantarum 21B* reported that they inhibited and showed a wide range of antifungal properties, such as *Eurotium repens, Empetrum rubrum, Penicillium corylophilum, P. roqueforti, P. expansum, Endomyces fibuliger, A. niger, A. flavus, Monilia sitophila and Fusarium graminearum.*

2)Microbiological analysis in sourdough samples

Changes in LAB and yeast counts were determined during the fermentation period at the end of 0 and 24 hours in the sour dough samples **(Tablo 2)**. A triple combination of 39LB2-28C1B3-HM3 was used as a control example. At the beginning, *Lactobacillus* and yeast values were close to each other, while the highest Lactobacillus growth level at the end of 24 hours was 39LB2-28C1B3-HM3 combination. Also, It was observed that the lowest yeast growth was again the combination 39LB2-28C1B3-HM3. It was determined that the yeast growth was highest and accordingly the lowest LAB development was 2 number dough. The positive effect of the bread on sour dough quality has been the subject of many studies to focus on the complex reactions occurring during the microbial metabolism of lactic acid bacteria and yeasts and their interaction with the endogenous enzymatic activity of the raw material (De Vuyst and Neysens, 2005; Galle, 2014).

Coda et al. (2018), it was found that the sourdough they prepared using LAB was 6.04 ± 0.05 log cob / g at the beginning and 9.16 ± 0.04 log cob / g after 24 hours.

3) pH and TTA in sourdough samples

Changes in pH and TTA (as the % lactic acid) at 0 and 24 h of sourdough samples in the study are given in **Table 3**. In our results, it is observed that of the dough at the 24th hour a significant decrease in the acidity. The most pronounced pH drop was observed in the control dough with the combination 39LB2-28C1B3-HM3, while the combination doughs 1, 2

and 3 showed similar pH value. It was determined that acidity increases with decreases in pH and these values changed between 1.18-1.31% at the end of 24 hours.

Table 2. Results of lactic acid bacteria and yeast at 0 and 24 h (cfu / g)

Sample Code	0th		24th	
	Lactobacilli	Yeast	Lactobacilli	Yeast
1(22-11-HM3 coded)	7.93±0.33	5.00±0.03	9.34±0.01	7.17±0.03
2(4-31-HM3 coded)	7.72±0.03	-	9.14±0.06	7.50±0.01
3(13-33-HM3 coded)	7.91±0.01	5.20±0.01	9.25±0.05	7.25±0.01
Control(39LB2-28C1B3-HM3)	7.98±0.33	5.78±0.01	9.44±0.05	7.08±0.03

Meroth et al. (2002) examined the pH values by making 4 different sourdoughs with 3 different LAB strains and commercial yeast combinations as starters.

The results indicated that the C coded sourdough had a pH value of 3.4 to 3.6 and the lowest pH value in this dough. Harth et al. (2018) showed that at the end of 24 hours of laboratory sourdough, TF1-coded dough dropped of pH from 6.1 to 3.9 and pH of TF2-coded dough dropped from 6.0 to 4.6. In another study, Dertli et al. (2016) found that the pH of the sourdough at the end of fermentation varied between 3.37-3.95.

Table 3. pH and TTA values of sourdough samples (% lactic acid)

Sample Code	0th		24th	
	pH	TTA	pH	TTA
1(22-11-HM3 coded)	5.94±0.007	0.17±0.00	3.46±0.007	1.25±0.00
2(4-31-HM3 coded)	6.00±0.01	0.18±0.00	3.46±0.03	1.31±0.00
3(13-33-HM3 coded)	5.27±0.007	0.29±0.00	3.46±0.007	1.26±0.00
Control(39LB2-28C1B3-HM3)	5.55±0.01	0.17±0.00	3.39±0.03	1.18±0.00

by

4) Controlled mold of breads

In Table 5, it was observed that the controlled mold zones of the sour dough breads were close to each other. Fungal contamination of bakery products is affected by several factors such as product type, additives, fermentation sources, size, packaging. Bakery products can be protected from mold spores each by using sour dough with antifungal activity or destroying spores contaminating the products using fungal inhibitors such as atmospheres or other packaging techniques, ethanol and propionic, sorbic, benzoic and acetic acids, and certain salts korunabilir (Legan 1993, Rocken 1996). Only a few studies have reported antifungal activity of lactic acid bacteria. The increase in shelf-life of sourdoughs has been attributed to organic acids initially produced by lactic acid bacteria (Spicher 1983, Spicher 1984).

Table 5. Zone diameters of molds in sour dough breads controlled mouldy (mm)

Sample Code	*Penicillium carneum*	*Aspergillus flavus*	*Aspergillus niger*	*Alternaria*
1.dough bread	-	14,0±0.00	15,0±0.00	-
2.dough bread	-	15,0±0.00	15,0±0.00	-
3.dough bread	-	14,0±0.00	15,0±0.00	8,5±0.00
Commercial yeast bread	-	17,0±0.00	15,0±0.00	10,0±0.00
Control(39LB2-28C1B3-HM3)	-	14,0±0.00	15,0±0.00	-

Conclusion

As a result, 6 of the 12 lactic acid bacteria in this study were found to have antifungal properties. In sourdough breads made with these antifungal bacteria, zone formation for *A. flavus, A. niger* and *Alternaria* was observed in 4 days, but no zone formation was observed in *Penicillium.* On the 3rd day, zone formation was observed in breads prepared with commercial yeast and *L. numerensis-L. paralimentarius*, while bread containing *L. plantarum-L. brevis* was later forming zone. The results of the in vitro antifungal analysis and the results of controlled mould on bread were similar.

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