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The Effect of Mineral Admixtures on Alkali-silica Reaction in Concrete

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Abstract

In this research, antagonistic activities of 30 *Bacillus* species isolated from various fish samples were studied. Isolated *Bacillus* species were analyzed using the agar diffusion method in terms of their general inhibition effects against some food pathogen/contaminant bacteria and lactic acid bacteria isolated from the fish intestinal tracts. Some of these strains exhibited antimicrobial activity against *Bacillus subtilis, Pseudomonas fluorescens, Lactobacillus coryneformis, Lactobacillus plantarum* and *Lactobacillus xylosus.* Furthermore, the inhibitory effects of the lactic acid bacteria on the *Bacillus* isolates were analyzed by using the same method. It was determined that the lactic acid bacteria inhibited *Bacillus* spp. strains at different levels of inhibition zones.

Keywords: *Bacillus, Lactobacillus*, Fishes, Isolation, Antimicrobial.

INTRODUCTION

The antibiotics are widely distributed in the nature, where they play an important role in regulating the microbial population of soil, water, sewage, and compost. The Gram-positive, aerobic, rod-shaped endospore-forming bacteria of the genus *Bacillus* are most impressively produced antibiotics as secondary metabolites [1-3]. Kümmerer [4] is stated that, within the last decade, an increasing number of studies covering antibiotic input, occurrence, fate and effects have been published, but there is still a lack of understanding and knowledge about antibiotics in the aquatic environment despite the numerous studies performed. The increased practice of aquaculture has led to a high number of disease outbreaks with an increasing range of pathogens. Consequently, the extensive use of broad-spectrum antibiotics in aquaculture has led, as in other fields, to drug resistance problems [5]. In recent years, many studies have been determined the antimicrobial properties of strains of *Bacillus* [6-10]. Shelar et al. [11] determined that a bacteriocin produced by the isolate of *B. atrophaeus* JS-2 was showed antimicrobial properties against some Gram-positive and Gram-negative bacteria. In a study, 50 out of the 118 *Bacillus* strains isolated from soil samples exhibited antagonistic activities against at least two or more strain from a panel of pathogenic and non-pathogenic microorganisms [12]. Probiotics may provide an alternative way to reduce the use of antibiotics in aquaculture and simultaneously may avoid the development of antibiotic-resistant bacteria. Most probiotics proposed as biological control agents in aquaculture belong to the lactic acid bacteria family [13]. Most studies have reported that the different strains of *Bacillus* spp. and the lactic acid bacteria found in fish and fish products [14-17].

The aim of this study was to evaluate the antagonistic activity of *Bacillus* spp. strains isolated from the fishes and to determine the antimicrobial activity of fish intestinal lactic acid bacteria on the *Bacillus* isolates.

MATERIALS AND METHODS

Isolation, Identification and Growth Conditions

Fresh fishes (anchovy, bluefish, gray mullet, horse mackerel, red sea bream, and whiting) were procured from fish retail markets in Ankara, TURKEY and packed in iceboxes and transferred to the laboratory within 1 h. Each 1 g of the fish samples (the intestinal digestive tract) was suspended in 9 mL sterile distilled water and shaken vigorously for 2 min. The samples were heated at 60 °C for 60 min in a water bath. Then, the liquid was serially diluted in sterile distilled water, and the dilution from 10^{-1} to 10^{-6} was plated on nutrient agar medium (Oxoid). The plates were incubated at 37±1 °C for 24-48 h [18].

Based on Bergey's Manual [19], the strains were classified using the following criteria: Gram reaction, spore occurrence, growth temperature range (5, 30, 40, 50, 55, 65 °C), pH values of the growth (5.7, 6.8), salt resistance (2, 5, 7, 10 g NaCl/100 mL), catalase activity, lecitinase activity, production of gas from glucose, sugar fermentation (D-glucose, D-mannitol, lactose, sucrose, melibiose, D-xylose, L-arabinose, maltose and salicin, 1 g/100 mL), reduction of nitrate, and gelatin and starch hydrolysis.

The bacterial strains were cultivated in Nutrient Broth (NB) (Oxoid), which contained (per L) 1 g lab-lemco powder, 2 g yeast extract, 5 g peptone and 5 g NaCl. The pH was adjusted to 6.8 with 0.01 M HCl and 0.01 M NaOH. The incubation temperature was maintained at 37±1 °C, and the agitation was maintained at 100 rpm. The cultures were inoculated into broth medium with 2% (v/v) inocula.

Test Microorganisms

Some pathogen/contaminant bacteria and lactic acid bacteria isolated from the intestinal tracts of rainbow trout and mirror carp used in this study were obtained from Culture Collections of the Biotechnology Laboratory at the Department of Biology in Gazi University. The names and codes of the test bacteria are presented in Table 1. The pathogen and contaminant bacteria were activated through incubation in a NB (Oxoid) for 24 h. The lactic acid bacteria were cultured in MRS broth (Oxoid) for 24 h. *Micrococcus flavus* TIM and lactic acid bacteria were incubated at 30±1 °C while the other test bacteria were incubated at 37±1 °C.

Table 1. Names and codes of the test microorganisms.

TEST MICROORGANISMS	
Food Pathogen and Contaminant Bacteria	**Lactic Acid Bacteria**
Bacillus subtilis ATCC 6633	*Lactobacillus casei* HS1
Escherichia coli ATCC 11230	*Lactobacillus coryneformis* HS18
Micrococcus flavus TIM	*Lactobacillus jensenii* HS30
Pseudomonas aeroginosa ATCC 29212	*Lactobacillus plantarum* HC13
Pseudomonas fluorescens RSKK 240	*Lactobacillus xylosus* HC9
Staphylococcus aureus 4-43	-
Yersinia enterolitica ATCC 1501	-

Inhibitory Effect by Agar-Well Diffusion Method

The inhibitory effects of isolates on test bacteria were determined by agar-well diffusion method [20]. Similarly, the inhibitory effects of the lactic acid bacteria on the *Bacillus* isolates were analyzed by using the same method. All the bacteria were incubated at the appropriate temperature and medium for 24 h. Nutrient agar and MRS agar media (20 mL) were poured into each sterile petri dish (100 mm diameter). 100 µL suspensions of target strain cultured for 24 h were spread on the plates, and wells of 6 mm diameter were punched in the agar with a sterile steel borer. The *Bacillus* and lactobacilli cultures were centrifuged at 6000 g for 15 min to remove cell debris. After centrifugation, supernatant samples (100 µL) were filled into the wells of agar plates directly. The inoculated plates were incubated for 24 h at their optimum growth temperatures, and the diameter of the inhibition zone was measured with calipers as mm. The measurements were done from the edge of the zone to the edge of the wall.

RESULTS AND DISCUSSION

Thirty *Bacillus* species were isolated from various fish samples. In the identification tests, 30 *Bacillus* strains were identified as 4 *B. pasteurii*, 3 *B. badius*, 3 *B. circulans*, 3 *B. licheniformis*, 3 *B. megaterium*, 3 *B. thuringiensis*, 2 *B. brevis*, 2 *B. cereus*, 2 *B. sphaericus*, 2 *B. subtilis*, 1 *B. coagulans*, 1 *B. lentus*, and 1 *B. pumilus*. Names of isolated species and their codes are given in Table 2.

Table 2. Isolated species and their codes.

Code	Species
P1, P4, P7	*Bacillus megaterium*
P2, P3, P6, P12	*Bacillus pasteurii*
P5, P16, P17	*Bacillus circulans*
P8, P9	*Bacillus subtilis*
P10	*Bacillus coagulans*
P11, P13	*Bacillus cereus*
P14, P15	*Bacillus sphaericus*
P18	*Bacillus lentus*
P19, P21, P22	*Bacillus badius*
P20, P25, P26	*Bacillus licheniformis*
P23, P29	*Bacillus brevis*
P24	*Bacillus pumilus*
P27, P28, P30	*Bacillus thuringiensis*

This study examined the antagonistic activities of the *Bacillus* spp. strains against the test bacteria. *B. subtilis* P9, *B. pasteurii* P12 and *B. licheniformis* P25 showed an inhibition zone diameter of 5.80 mm, 4.95 mm, and 5.00 mm against *P. fluorescens* RSKK 240, respectively. It was also determined that *B. cereus* P11 (5.05 mm) and *B. thuringiensis* P30 (5.70 mm) had inhibitory effects on *B. subtilis* ATCC 6633 (Table 3).

Table 3. Antimicrobial activity of *Bacillus* strains on some food pathogen/contaminant bacteria**.**

Antibacterial Activity		
Strains	**Tested Bacteria**	
	***B. subtilis* ATCC 6633**	***P. fluorescens* RSKK 240**
B. subtilis P9	-	+++
B. cereus P11	+++	-
B. pasteurii P12	-	++
B. licheniformis P25	-	+++
B. thuringensis P30	+++	-

Antimicrobial activity: zone of inhibition.
-: No effect.
+: Zone width 1-3 mm; ++: Zone width 3-5 mm; +++: Zone width 5-7 mm.

According to Pinchuck et al. [21], probiotic strain *B. subtilis* 3, whose safety was previously demonstrated, is known to have antagonistic properties against species of the family *Enterobacteriaceae* as well as inhibit *Helicob*acter *pylorii*. Binnet [22] reported that *Bacillus* strains isolated from milk displayed antimicrobial activity against *E. coli*, *Y. enterolitica* and *P. aeroginosa*. In the study by Perez et al. [23, 24], *B. subtilis* MIR 15 strain did not show antimicrobial activity against *E. coli*, *M. luteus* and *P. aeroginosa.* Aslim et al. [25] found that *B. megaterium*, *B. subtilis* and *B. thuringiensis* strains were active against *E.*

coli and Y. *enterolitica*. In a study on the antimicrobial activity of 29 *Bacillus* strains isolated from the soil against some tested bacteria, Yilmaz et al. [26] determined only 5 isolates with antimicrobial activity. They concluded that not all the *Bacillus* isolates showed inhibitory effects on *E. coli* ATCC 11230, *M. flavus* TIM, *P. aeroginosa* ATCC 29212, *S. aureus* 4-43 and *Y. enterolitica* ATCC 1501. This study deduced that the isolates used have no inhibitory effects regarding *E. coli* ATCC 11230, *M. flavus* TIM, *P. aeroginosa* ATCC 29212, *S. aureus* 4-43, *Y. enterolitica* ATCC 1501. Oscariz et al. [27] reported that *B. cereus* strain isolated from soil was active against most Gram-positive but not Gram-negative bacteria.

The inhibitory effects of *Bacillus* isolates against lactic acid bacteria are given in Tablo 4. *B. cereus* P13 (3.90 mm), *B. thuringiensis* P27 (4.50 mm) and P28 (4.55 mm) were active against *L. coryneformis* HS18. *B. licheniformis* P25 (2.90 mm) and P26 (3.25 mm) had inhibitory effects on *L. plantarum* HC13. In addition, *B. megaterium* P4 (3.50 mm) and P7 (2.75 mm) showed antimicrobial activity against *L. xylosus* HC9. The other *Bacillus* strains did not show antimicrobial activity against the lactic acid bacteria tested. Kalayli [28] determined that *Bacillus* strains isolated from milk and fermented products did not show any antimicrobial activities against *L. casei*, but the strains inhibited the growth of *L. plantarum*

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Table 4. Antimicrobial activity of *Bacillus* strains on lactic acid bacteria.

Strains	**Antibacterial Activity**		
	Tested Bacteria		
	***L. coryneformis* HS18**	***L. plantarum* HC13**	***L. xylosus* HC9**
B. megaterium P4	-	-	++
B. megaterium P7	-	-	+
B. cereus P13	++	-	-
B. licheniformis P25	-	+	-
B. licheniformis P26	-	++	-
B. thuringensis P27	++	-	-
B. thuringensis P28	++	-	-

Antimicrobial activity: zone of inhibition.
-: No effect.
+: Zone width 1-3 mm; ++: Zone width 3-5 mm; +++: Zone width 5-7 mm.

Additionally in our study, the lactic acid bacteria were assayed for their ability to produce inhibitory substances against the growth of *Bacillus* strains. The results concerning the determination of the antimicrobial effects are presented in Table 5. *L. xylosus* HC9 did not show antimicrobial activity against *B. megaterium* P1, *B. pasteurii* P6 and *B. cereus* P13. *L. casei* HS1 and *L. jensenii* HS30 did not have inhibitory effects on *B. subtilis* P9. The other lactic acid bacteria inhibited the growth of the *Bacillus* strains, at different levels of inhibition zones. The highest antimicrobial activity of lactic acid bacteria (*L. xylosus* HC9) was against *B. megaterium* P4 (5.60 mm) whereas the lowest antimicrobial activity of bacteria (*L. casei* HS1) was against *B thuringiensis* P30 (1.00 mm).

Table 5. Antimicrobial activity of lactic acid bacteria on the *Bacillus* strains.

Lactic Acid Bacteria	**Antibacterial Activity**														
	***Bacillus* strains**														
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
L. casei HS1	++	++	++	+	++	+	++	++	-	+	+	++	++	+	++
L.coryneformis HS18	++	++	++	++	+	++	+++	++	+++	+	++	++	+	+	++
L. jensenii HS30	++	+	+	++	++	+	++	++	-	+	++	++	++	++	++
L.plantarum HC13	++	++	++	++	++	++	++	+	+++	+	++	+++	++	+	++
L. xylosus HC9	-	+	++	+++	+	-	++	+	+	++	+	++	-	+	+

Antimicrobial activity: zone of inhibition.
-: No effect.
+: Zone width 1-3 mm; ++: Zone width 3-5 mm; +++: Zone width 5-7 mm

Table 5 (Continued). Antimicrobial activity of lactic acid bacteria on the *Bacillus* strains.

Lactic Acid Bacteria	**Antibacterial Activity**														
	***Bacillus* strains**														
	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30
L. casei HS1	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+
L. coryneformis HS18	++	+	+	++	+	++	+	++	++	++	++	++	++	++	++
L. jensenii HS30	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+
L. plantarum HC13	++	++	++	+	++	++	+	+	+	+	+	+	+	+	+
L. xylosus HC9	+++	+	+	+	+	+	+	+	+	+	+	+	++	+	+

Antimicrobial activity: zone of inhibition.
-: No effect.
+: Zone width 1-3 mm; ++: Zone width 3-5 mm; +++: Zone width 5-7 mm.

Among the bacteria used as probiotics, lactic acid bacteria have an important role because they are beneficial to human and animal health [29, 30]. Probiotic strains should have desirable antibiotic resistance and sensitivity patterns, be antagonistic toward potentially pathogenic microorganisms, and have metabolic activities beneficial to the well-being of the host [31]. In our study, *L. xylosus* HC9 did not show antimicrobial activity against *B. megaterium* P1, *B. pasteurii* P6 and *B. cereus* P13. *L. casei* HS1, and *L. jensenii* HS30 did not have inhibitory effects on *B. subtilis* P9. The other lactic acid bacteria inhibited the growth of the *Bacillus* strains at different levels of inhibition zones. Katircioğlu [32] reported that the lactic acid bacteria inhibited *Bacillus* spp. at different levels of inhibition zones. Jiranvanichpaisal et al. [33] emphasized the use of *Lactobacillus* sp. as the probiotic bacteria in the giant tiger shrimp (*Penaeus monodon* Fabricus). Inhibiting activity of two *Lactobacillus* sp. against *Vibrio* sp., *E. coli*, *Staphylococcus* sp. and *B. subtilis* was also determined.

In conclusion, *Bacillus* spp. strains isolated from the intestinal tracts of various fishes are antimicrobial activity against some food pathogen/contaminant bacteria. Also, it has been found that lactic acid bacteria isolated from the intestinal tracts of fish have inhibitory effects against *Bacillus* spp. Strains

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