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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

A Detailed Study on Halotolerant Bacteria Isolated from Food Salts Collected from Different Countries

Farklı Ülkelerden Toplanan Gıda Tuzlarından İzole Edilen Tuza Toleranslı Bakteriler Hakkında Detaylı Bir Çalışma

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Abstract
Twenty-five food salts, collected from seven-country, were examined for moisture contents and ash contents, pH values, total numbers of bacteria, and total numbers of halotolerant bacteria. The growth and biochemical reactions of halotolerant bacteria at different NaCl concentrations, pH, and temperature values were investigated. The efficacy of dry-heat sterilization and high-pressure steam sterilization for the inactivation of bacteria was examined using six food salts. Moisture contents, ash contents, and pH values of salts were respectively found as 2.3-3.7%, 95-97%, and 6.37-9.94. Total numbers of bacteria and halotolerant bacteria were detected as between 1-58 CFU/g and 1-40 CFU/g on Modified Nutrient Agar media, respectively. While bacteria were found in twenty salt samples, halotolerant bacteria were detected in seventeen samples. One hundred thirty- eight rod-shaped halotolerant bacteria were isolated from seventeen samples. One hundred eighteen and twenty isolates were found Gram-positive and Gram-negative, respectively. While 131 isolates were motile, seven isolates were observed as non-motile. All isolates grew in both absence of NaCl and presence of 10% NaCl, pH 5, 7, and 9, 24°C, and 37°C. Eighty-six isolates showed different pigmentations. Ammonia production was detected at sixty isolates. Catalase, oxidase, protease, lipase, amylase, caseinase were produced by 138, 83, 84, 48, 92, 100 isolates, respectively. Fifty-seven percent of halotolerant bacteria formed endospores. All bacteria in six salts were killed using both dry-heat sterilization (175ºC for 2 hours) and high-pressure steam sterilization (121ºC for 30 minutes). In conclusion, we suggest using dry-heat sterilization or high-pressure steam sterilization to kill Gram-negative halotolerant bacteria containing endotoxin and Gram-positive halotolerant bacteria with endospores in food salts to prevent food spoilage and foodborne diseases in humans.
Keywords: Halotolerant bacteria, food salt, biochemical tests, enzymatic activities, phenotypic characteristics, sterilization of food salts

Öz
Yedi ülkeden toplanan yirmi beş gıda tuzu nem içeriği, kül içeriği, pH değerleri, toplam bakteri sayısı ve toplam tuza toleranslı bakteri açısından incelenmiştir. Tuza toleranslı bakterilerin farklı NaCl konsantrasyonlarında, pH ve sıcaklık değerlerinde gelişmeleri ve biyokimyasal reaksiyonları araştırılmıştır. Bakterilerin inaktivasyonu için kuru ısı sterilizasyonunun ve yüksek basınçlı buhar sterilizasyonunun etkinliği altı gıda tuzu kullanılarak incelenmiştir. Tuzların nem içerikleri, kül içerikleri ve pH değerleri sırasıyla %2.3-3.7, %95-97 ve 6.37-9.94 olarak bulunmuştur. Modifiye Nutrient Agar besiyerinde toplam bakteri ve halotolerant bakteri sayısı sırasıyla 1-58 KOB/g ve 1-40 KOB/g arasında tespit edilmiştir. Yirmi tuz örneğinde bakteri bulunurken, on yedi örnekte halotolerant bakteri tespit edilmiştir. On yedi örnekten yüz otuz sekiz çubuk şeklinde halotolerant bakteri izole edilmiştir. Yüz on sekiz ve yirmi izolat sırasıyla Gram-pozitif ve Gram-negatif olarak bulunmuştur. 131 izolat hareketli iken, yedi izolat hareketsiz olarak gözlenmiştir. Tüm izolatlar hem NaCl yokluğunda hem de %10 NaCl varlığında, pH 5, 7, ve 9, 24°C ve 37°C'de gelişmiştir. Seksen altı izolat farklı pigmentasyonlar göstermiştir. Altmış izolatta amonyak üretimi tespit edilmiştir. Katalaz, oksidaz, proteaz, lipaz, amilaz ve kazeinaz sırasıyla 138, 83, 84, 48, 92 ve 100 izolat tarafından üretilmiştir. Tuza toleranslı bakterilerin %57'si endospor oluşturmuştur. Altı gıda tuzundaki tüm bakteriler hem kuru ısı sterilizasyonu (175ºC'de 2 saat) hem de yüksek basınçlı buhar sterilizasyonu (121ºC'de 30 dakika) kullanılarak öldürülmüştür. Sonuç olarak, gıda bozulmalarını ve insanlarda gıda kaynaklı hastalıkları önlemek için gıda tuzlarında endotoksin içeren Gram-negatif tuza toleranslı bakterileri ve endosporlu Gram-pozitif tuza toleranslı bakterileri öldürmek için kuru ısı sterilizasyonu veya yüksek basınçlı buhar sterilizasyonu kullanılmasını öneriyoruz.
Anahtar kelimeler: Halotolerant bakteriler, gıda tuzları, biyokimyasal testler, enzimatik aktiviteler, fenotipik karakteristikler, gıda tuzlarının sterilizasyonu

I. INTRODUCTION

Foods such as fish, tomato paste, grape leaves, olives, cheese, butter, sausage, bacon, vegetables, and sauerkraut are preserved with salt to prevent food spoilage. This traditional preservation method has been applied as dry

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salting and brine salting. In these methods, it is assumed that NaCl inhibits bacterial growth by reducing moisture content and water activity in food and slowing down the cellular metabolism of microorganisms [1]. In addition to its preservative properties, salt provides flavor to food products, especially processed foods [2].

The fermentation process, which is an easy and cheap method, is applied since ancient times to preserve foods [3, 4]. While vine leaf, fish, and daikon radish are preserved with the dry salting method, brining method is used to preserve cabbage, carrot, and cucumbers [4]. After the salting process, the products are fermented for approximately 14-days at room temperature. During the fermentation process, essential amino acids and various vitamins are produced by beneficial microorganisms such as lactic acid bacteria and yeasts [4, 5]. These microorganisms may produce compounds that give taste, aroma, and flavor to food [6].

Although salt is used as a preservation agent, preservation salt may contain some pathogenic and spoilage bacteria which can survive in the presence of salt. Bacteria that can be grown in both the absence and presence of salt are called halotolerant bacteria [7]. Halotolerant bacteria are able to grow at 0-25% NaCl, pH 5-10, and 4-50ºC [7, 8]. Halotolerant bacteria produce different compatible solutes which help to balance the osmotic level inside the cell with the outer environment [9]. When salt is used as a preservation agent, halotolerant bacteria may contaminate the dry-salted and brine-salted food products. In previous studies, halotolerant bacteria were isolated from salted fish [10], fish sauce [11], cheese brine [12], fermented food [13], salt crystal [14], sugarcane press mud [15], grasshopper sub shrimp paste [16], cheese rinds [17], and fermented *Solanum macrocarpon* food [18].

Halotolerant microorganisms may produce amylase, protease, lipase, and cellulase enzymes [19]. Protease and lipase enzymes produced by halotolerant microorganisms have been reported as salt-tolerant enzymes [13, 20]. These enzymes are also tolerant to high pH, temperature, and organic solvents [21, 22]. Though enzymes of halotolerant bacteria have industrial importance, these enzymes may cause food spoilage. Due to the fact that the dry-salted or brine-salted dairy, meat and vegetable products have an important commercial market worldwide, the microbial contamination of these products must be prevented to extend their shelf-life [4, 23].

When salt is used as a preservation agent, the halotolerant bacteria found in salt and their hydrolytic activities should be checked before the preservation process [24]. When the preservation salt contaminated with halotolerant bacteria is used in food preservation, it may cause degradation of salted foods [25]. Researchers reported that dried salted codfish cured with Mediterranean Sea salt contained extremely halotolerant bacteria in high numbers [26]. In another study, codfish samples were separately cured with three different salt samples such as only NaCl, commercial sea salt obtained from southern Europe, and natural salt collected from northern Europe [27]. Codfish cured with commercial sea salt contained $1.3x10^4$ CFU/g halotolerant bacteria and $1.25x10^3$ CFU/g coliform bacteria [27]. Researchers stated that extremely halotolerant microorganisms such as staphylococci were detected at salted cod. Investigators emphasized that sea salt contained ten times more microorganisms compare to the microorganisms of mine salt [28]. Moreover, total counts of halotolerant bacteria in 30 curing salt used in the leather industry were detected between 10^4 and 10^6 CFU/g [24]. These studies showed that halotolerant microorganisms are common inhabitants in food salt and salt used in the leather industry.

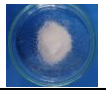
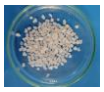
Bacterial contamination of food products, derived from salt, may cause food spoilage, reduce the shelf life of the product, reduce of nutritional quality of food and cause foodborne diseases in humans [29]. Hence, the goals of this study were to examine the moisture contents, ash contents, and pH values of the food salt samples, determine the total numbers of viable bacteria and total numbers of viable halotolerant bacteria and isolate halotolerant strains from these salt samples. Moreover, the growth and optimum ranges of halotolerant isolates at different salt concentrations, different pH, and different temperature values were investigated. Cell morphology, motility, Gram reaction, colony characteristic, and biochemical activities of each halotolerant isolate were also examined in detail. The efficacy of dry-heat sterilization and high-pressure steam sterilization for the inactivation of bacterial populations including halotolerant bacteria in six food salt samples was also investigated.

II. MATERIAL AND METHOD

2.1. Salt Samples

In this study, 25 salt samples belonging to Cyprus black lava sea salt (one sample), Himalaya salt (five samples), Rock salt (seven samples), Table salt (eight samples), Nepal salt (one sample), Sea salt (three samples) were used. The salt samples coded as 1FSS (Cyprus black lava sea salt), 2FSS, 3FSS, 7FSS, 13FSS, 15FSS (Himalaya salt), 4FSS, 10FSS, 16FSS, 19FSS, 20FSS, 23FSS, 25FSS (Rock salt), 5FSS, 11FSS, 12FSS, 14FSS, 17FSS, 18FSS, 22FSS, 24FSS (Table salt), 6FSS (Nepal salt), 8FSS, 9FSS, 21FSS (Sea salt) were collected from different countries (Table 1).

Table 1. Countries where salt samples were collected, types, colors, codes of salt samples

Countries	Types	Colors	Codes
France	Cyprus black lava sea salt 	Black	1FSS[a]
France	Himalaya salt 	Red	2FSS
France	Himalaya salt 	White	3FSS
France	Rock salt 	White	4FSS
France	Table salt 	White	5FSS
France	Nepal salt 	Pink	6FSS
Türkiye	Himalaya salt 	White	7FSS
Türkiye	Sea salt 	White	8FSS
Türkiye	Sea salt 	White	9FSS
Türkiye	Rock salt 	White	10FSS
Türkiye	Table salt 	White	11FSS
Türkiye	Table salt 	White	12FSS
Türkiye	Himalaya salt 	Pink	13FSS
Türkiye	Table salt 	White	14FSS
Pakistan	Himalaya salt 	Pink	15FSS
Pakistan	Rock salt 	White	16FSS
Pakistan	Table salt 	White	17FSS
Pakistan	Table salt 	White	18FSS
Germany	Rock salt 	White	19FSS
Germany	Rock salt 	White	20FSS
Germany	Sea salt 	White	21FSS
England	Table salt 	White	22FSS
England	Rock salt 	White	23FSS
Switzerland	Table salt 	White	24FSS
Austria	Rock salt 	White	25FSS

[a]1FSS: 1 Food Salt Sample.

2.2. Detection of Moisture Contents of Food Salt Samples

Five grams of each sample were weighed and the samples were separately put into a small cylindrical capped glass bottle. These bottles were placed in a drying oven at 102°C for 6 hours. The dried samples were then placed into a desiccator for 30 minutes. After 30 minutes, the salt samples were weighed and placed in a drying oven for 1 hour. After the cooling process, the samples were weighed again. The drying process was repeated until the first dry weight was closely equal to the second dry weight. Finally, the moisture contents of the salt samples were calculated [18].

2.3. Detection of Ash Contents of Food Salt Samples

Five grams of each salt sample were put into ceramic crucibles. Then the samples were ashed in a muffle furnace at 600ºC for 8 h. After the cooling process, the samples were weighed for the determination of ash content [30, 31].

2.4. Detection of pH values of Food Salt Samples

Five grams of each salt sample were put into a flask with 100 mL of distilled water. The flasks were shaken for 1 hour at 150 rpm. The pH values of the salt sample solutions were measured with a pH meter (Sartorius Professional Meter PT-10P, Goettingen, Germany) [30].

2.5. Determination of Total Bacterial Numbers and Total Halotolerant Bacterial Numbers of Salt Samples

To determine the total numbers of bacteria and total numbers of halotolerant bacteria in these salt samples, one gram of each salt sample was weighed and placed in 9 mL of sterile physiological saline solution containing 0.85% NaCl. Then, the salt solution was shaken in a shaking incubator at 100 rpm for 30 minutes at 24°C. Each of these solutions was passed through a sterile cellulose nitrate membrane (0.2 µm pore size) and the membrane was placed onto the surface of a Petri plate containing Modified Nutrient Agar (MNA) medium (peptone, 5 g; glucose, 10 g; beef extract, 3 g; agar, 20 g; distilled water, 1000 mL; pH 7) without salt. The plates were incubated at 37°C for seven days. After incubation, the bacterial colonies on the cellulose nitrate membrane placed on the MNA medium were counted to determine colony-forming units (CFU/g) of the total number of bacteria. All colonies grown on the cellulose nitrate membrane placed on MNA were restreaked several times to obtain pure cultures. The growth of pure bacterial culture was examined on both MNA medium without salt and MNA media with 10% NaCl and 20% NaCl. According to the test results, the total numbers of halotolerant bacteria in the food salt samples were determined.

2.6. Examination of Morphology, Motility and Gram Reaction of Halotolerant Bacterial Isolates

Each of the pure halotolerant bacterial isolates was grown on a Modified Nutrient Broth (MNB) medium at 37°C for 24 h for the determination of cellular morphology, and motility. Then, the morphology and motility of each halotolerant isolate were examined on wet mounts under a light microscope [32]. The Gram staining procedure of each pure halotolerant bacterial isolate grown on MNA medium at 37ºC for 24 h was performed according to the previously described method [32].

2.7. Examination of Growth Range of Halotolerant Bacterial Isolates at Different NaCl Concentrations, Different pH and Temperature Values

For detection range and optimal concentrations of NaCl required for the growth of each halotolerant isolate, each isolate was grown at both MNA medium without NaCl and MNA media supplemented with 10% and 20% NaCl (w/v). To determine pH range and optimal pH values for the growth of the isolates, each halotolerant isolate was grown on salt-free MNA media adjusted with 5, 7, and 9 pH values. The range and optimal growth temperatures of each isolate were detected on the MNA medium without NaCl with the optimal pH 7, at temperatures of 4°C, 24°C, and 37°C [33, 34].

2.8. Examination of Colony Properties of Pure Halotolerant Bacterial Isolates

Each of the pure halotolerant bacterial isolates was grown on MNA media without salt at 37°C for 24 h for the detection of colony form, elevation, margin, pigmentation, texture, appearance, and optical property [32-34].

2.9. Examination of Endospore Structures of Halotolerant Bacterial Isolates

To examine the endospore structure of each halotolerant bacterial isolate, the isolate was streaked out over MNA medium and the medium was incubated at 37°C for 72 h. Then, the heat-fixed smear of each halotolerant bacterial isolate was prepared and endospore staining of the isolate was performed according to the previously described method [32]. Malachite green solution (5%) was added to the slide covered with paper toweling, and the slide was placed on a hot plate for 5 min. Then, the paper towel was removed and the cooled slide was rinsed with water for 30 sec. Finally, the slide was covered with safranin for 60 sec and rinsed with water for 30 sec. The slides were examined under oil immersion [32].

2.10. Biochemical Tests (Catalase, Oxidase, Protease, Lipase, Amylase, Caseinase, Ammonia Production from Peptone) of Halotolerant Test Isolates

Catalase, oxidase, protease, lipase, amylase, and caseinase activities, and ammonia production of each halotolerant isolate were investigated. Catalase test was carried out by adding three drops of 3% H_2O_2 onto the

colonies of the isolates grown on the MNA medium without salt at 37°C for 24 h [32]. The formation of oxygen bubbles indicated positive catalase activity. For oxidase test, two drops of oxidase test agent (1% tetramethyl-p-phenylenediamine dihydrochloride) were dropped onto filter paper, and then the bacterial colony of each isolate was transferred to filter paper. Detection of purple color onto filter paper was regarded as a positive result in 30 sec [32]. Gelatine Agar medium (tryptone, 15 g; soytone, 5 g; gelatin, 20 g; agar, 20 g; distilled water, 1000 mL; pH 7) without salt was used to determine protease activity. After incubation at 37°C for 7 days, a saturated ammonium sulphate solution was added to the medium. A clear zone around the colony was accepted as a positive protease activity [35, 36]. Olive oil-containing Lipase Agar medium with rhodamine-B (trypticase peptone, 4 g; yeast extract, 4 g; olive oil, 30 mL; rhodamine-B, 0.2 g; Tween 80, 0.33 mL; agar, 20 g; distilled water, 1000 mL; pH 7) was used for lipase activity. After incubation at 37°C for 5 days, the Petri plate was checked under ultraviolet light. Fluorescent orange halos in the medium were accepted as positive lipase activity [37]. Starch medium (starch, 10 g; beef extract, 3 g; agar, 20 g; distilled water, 1000 mL; pH 7) was used for amylase test. After incubation at 37°C for 48 hours, Gram's Lugol solution was added to the medium. A clear zone around the colony was accepted as a positive amylase activity [32]. Plate Count Agar medium (tryptone, 5 g; yeast extract, 2.5 g; glucose, 1 g; skim milk, 20 mL; agar, 20 g; distilled water, 1000 mL; pH 7) containing skim milk was used for caseinase test. After incubation at 37°C for 48 h, a clear zone around the colony was accepted as a positive caseinase activity [32]. Peptone Broth medium (peptone, 10 g; sodium chloride, 5 g; distilled water, 1000 mL; pH 7) was used for detection of ammonia production from peptone. After incubation at 37°C for 24 h, 1-2 drops of Nessler reagent were dropped into tubes. Brown precipitate in the test tube was an indicator of ammonia formation of peptone [38].

2.11. Determination of Efficacy of Dry-Heat Sterilization and High-Pressure Steam Sterilization for Inactivation of Bacteria in Food Salt Samples

In this experiment, the efficacy of both dry-heat sterilization and high-pressure steam sterilization for the inactivation of bacteria including halotolerant bacteria found in six food salt samples were separately examined. Six food salt samples coded as FSS1, FSS9, FSS15, FSS18, FSS22, and FSS24 containing viable bacteria and halotolerant bacteria in high numbers were selected for sterilization experiments. Salt samples of FSS1 (Cyprus black lava sea salt obtained from France), FSS9 (sea salt, Türkiye), FSS15 (Himalaya salt, Pakistan), FSS18 (table salt, Pakistan), FSS22 (table salt, England), FSS24 (table salt, Switzerland) were used in the experiments. All experiments were done twice. Therefore, four groups of food salt samples coded as FSS1, FSS9, FSS15, FSS18, FSS22, and FSS24 were prepared. One gram of salt samples belonging to FSS1, FSS9, FSS15, FSS18, FSS22, and FSS24 was weighed. Then, each salt sample was separately placed on sterile glass Petri plates. The plates belonging to FSS1, FSS9, FSS15, FSS18, FSS22, and FSS24 were placed in a Pasteur oven (FN500, NUVE, Türkiye) and sterilized at dry-heat at 175°C for 2 h. The other plates that belonged to FSS1, FSS9, FSS15, FSS18, FSS22, and FSS24 were placed inside the autoclave (HMC, HIRAYAMA, Japan) and sterilized at high-pressure steam at 121ºC for 30 min. After sterilization processes, six food salt samples were separately added to 10 mL of sterile physiological saline solution (0.85%), and the salt solutions were mixed in a shaking incubator at 24°C for 30 min at 100 rpm. The saline solutions were passed through a sterile cellulose nitrate membrane (0.2 µm pore size), and each membrane was placed onto the MNA medium surface in the Petri plates. The plates containing membranes were incubated at 37°C for seven days. After incubation, the Petri plates were examined in terms of the presence or absence of the bacterial colonies. The absence of bacterial colonies on the membranes showed the high inactivation effect of dry-heat sterilization and high-pressure steam sterilization against bacteria found in food salt samples.

III. FINDINGS AND DISCUSSION

In the present study, although bacteria were observed in 20 salt samples, halotolerant bacteria were observed in 17 salt samples. While bacteria were not detected in five salt samples (4FSS and 5FSS collected from France; 8FSS and 12FSS Türkiye; 17FSS Pakistan), halotolerant bacteria were not found in eight salt samples (3FSS, 4FSS and 5FSS France; 8FSS and 12FSS Türkiye; 17FSS Pakistan; 20FSS Germany; 23FSS England). The moisture contents, ash contents, and pH values of salt samples changed between 2.3-3.7%, 95-97%, and 6.37-9.94, respectively. While most of the food salt samples had neutral pH values, only two salt samples coded as 5FSS (9.94) and 20FSS (8.55) had alkaline pH values (Table 2). The moisture contents of the salt samples were found to be very low. The organic content of the salt samples was calculated from the ash content. Organic contents of the salt samples were also found as fairly low. Low moisture content and low organic content may adversely affect the growth of bacteria in food salt samples. Due to the low moisture contents and low organic substances of the salt samples, 80% of salt samples contained bacteria (1-58 CFU/g), and 68% of salt samples had halotolerant bacteria (1-40 CFU/g) in low number. In a previous study, moisture contents of 40 salt samples, which were used in hide and skin preservation, ranged from 0.90% to 4.97% [38]. In another research, moisture contents and pH values of the salt samples used in the leather industry had been found between 0.90% and 5.02%, 6.23 and 7.22, respectively [24].

Table 2. The salt codes, countries, moisture contents, ash contents, pH values, total numbers of bacteria, total numbers of halotolerant bacteria of salt samples

Salt Codes	Countries	Moisture (%)	Ash (%)	pH	Total Numbers of Bacteria (CFU/g)	Total Numbers of Halotolerant Bacteria (CFU/g)
1FSS	France	3.3	96.0	7.38	35	34
2FSS	France	3.3	96.6	7.50	22	22
3FSS	France	3.5	95.2	7.21	2	0
4FSS	France	3.3	96.6	7.33	0	0
5FSS	France	3.4	95.0	9.94	0	0
6FSS	France	3.0	97.0	8.00	10	10
7FSS	Türkiye	3.3	97.0	6.54	17	14
8FSS	Türkiye	3.0	97.0	7.04	0	0
9FSS	Türkiye	3.0	96.6	6.95	40	40
10FSS	Türkiye	3.2	96.0	6.83	10	8
11FSS	Türkiye	3.3	95.0	7.43	12	11
12FSS	Türkiye	3.3	95.2	6.37	0	0
13FSS	Türkiye	3.7	96.0	7.40	30	12
14FSS	Türkiye	3.0	95.7	6.57	1	1
15FSS	Pakistan	2.5	96.3	6.82	30	30
16FSS	Pakistan	3.3	95.5	6.51	9	9
17FSS	Pakistan	3.0	97.0	6.59	0	0
18FSS	Pakistan	3.0	96.6	6.60	58	16
19FSS	Germany	3.4	95.0	6.96	1	1
20FSS	Germany	2.8	96.0	8.55	8	0
21FSS	Germany	3.0	97.0	7.10	12	10
22FSS	England	2.3	95.5	7.15	21	20
23FSS	England	2.5	96.0	7.90	2	0
24FSS	Switzerland	3.0	96.3	7.04	40	40
25FSS	Austria	3.0	97.0	7.70	9	7

A total of 138 pure halotolerant bacterial isolates were obtained from 17 salt samples. Growth ranges of the halotolerant isolates at different salt concentrations, different pH, and different temperature values are shown in Table 3. All isolates grew at the absence of salt and presence of 10% NaCl in the medium. However, only 22 isolates (6FSS5, 6FSS8, 9FSS7, 9FSS15, 9FSS16, 9FSS24, 9FSS31, 11FSS3, 11FSS7, 11FSS9, 13FSS12, 14FSS1, 18FSS7, 18FSS35, 18FSS50, 21FSS5, 21FSS9, 22FSS2, 24FSS2, 24FSS3, 25FSS1, 25FSS10) were able to grow at 20% NaCl. These results showed that the isolates were halotolerant bacteria. All halotolerant isolates showed growth at pH 5, 7, and 9. While eight isolates (13FSS22, 13FSS30, 18FSS5, 18FSS6, 18FSS7, 18FSS9, 21FSS1, 25FSS8) grew at 4°C, all isolates grew at 24°C and 37°C.

When the pH values of the food salt samples containing halotolerant bacteria were compared with the pH values at which the halotolerant isolate was grown, a close relationship was observed between the salt samples and the halotolerant isolates in terms of pH values. The pH values of 17 food salt samples (in which halotolerant bacteria were isolated) were between 6.51 and 8.00 (Table 2). All halotolerant bacteria were grown on MNA agar media with pH values of 5, 7, and 9. The optimum growth of the isolates was observed at pH 7. These results showed that the pH values of food salt samples containing halotolerant bacteria were suitable to support the growth of halotolerant isolates.

In another study, the optimal temperature ranges for halotolerant bacteria were found as 30°C-40°C [39]. In a different study, the researchers reported the growth of these microorganisms at pH 7.0-8.5 and 0%-12% NaCl concentrations [40]. The researchers stated that the optimal temperature and pH for the growth of halotolerant isolates were 37°C and 7, respectively [24]. Our test results were found to be consistent with the results of the previous studies. All halotolerant isolates showed optimal growth at MNA medium without salt, pH 7 and 37°C (Table 3).

All isolates of halotolerant bacteria were rod-shaped. Although 131 halotolerant isolates were motile, seven isolates were non-motile. While 118 halotolerant isolates (86%) were found as Gram-positive bacteria, 20 halotolerant isolates (14%) were Gram-negative bacteria. Seventy-eight isolates were endospore-forming bacteria (Table 4). The cell wall of Gram-negative bacteria contains the lipopolysaccharide layer (LPS) that is toxic to animals and humans. The toxicity of LPS is due to Lipid A called endotoxin. Some endotoxins may cause fever, gastrointestinal symptoms such as gas, diarrhea, and vomiting in humans [41]. Food salts containing Gram-negative and Gram-positive halotolerant bacteria may contaminate uncooked foods such as salads. The bacterial growth in the foods stored at room temperature may result in food spoilage and cause foodborne disease in humans.

The common presence of halotolerant Gram-positive bacteria in salt samples was also detected in the salt samples collected from the leather industry. In that study, the researchers isolated 83 halotolerant bacteria from 30 hide and skin preservation salt samples. All isolates were found as motile, rod-shaped, and endospore-forming Gram-positive bacteria in that study [24].

Forms of the colonies of halotolerant bacteria were detected as circular (91 isolates), punctiform (33 isolates), irregular (8 isolates), rhizoid (5 isolates) or filamentous (1 isolate). Elevations of the colonies were flat (76 isolates), convex (7 isolates) or raised (55 isolates). Margins of the colonies were entire (97 isolates), erose (17 isolates), filamentous (15 isolates) or undulate (9 isolates). Pigmentations of the colonies were orange (2 isolates), cream (62 isolates), dark cream (7 isolates), white (47 isolates), yellow (14 isolates), dark yellow (1 isolate) or non-pigmented (5 isolates). Textures of the colonies were smooth (73 isolates) or rough (65 isolates). Appearances of the colonies were dull (66 isolates) or shiny (72 isolates). Optical properties of the colonies were opaque (63 isolates), translucent (49 isolates) or transparent (26 isolates) (Table 4).

Catalase and oxidase reactions, protease, lipase, amylase, and caseinase activities, ammonia production from peptone of the test isolates are shown in Table 5. All isolates were catalase positive. Catalase test reactions of the halotolerant bacteria showed that these microorganisms are aerobic or facultative aerobic. Among 138 isolates, 83, 84, 48, 92, 100 isolates were oxidase, protease, lipase, amylase, and caseinase positive, respectively. Sixty isolates produced ammonia from peptone. Food salt samples coded as 1FSS, 2FSS, 6FSS, 7FSS, 9FSS, 10FSS, 11FSS, 13FSS, 14FSS, 15FSS, 16FSS, 18FSS, 19FSS, 21FSS, 22FSS, 24FSS, 25FSS had halotolerant bacteria with hydrolytic activities (Table 5). In a study carried out with 30 salt samples collected from different leather factories in Corlu and Tuzla (Türkiye), catalase and protease activities of all isolates (83 strains) in the salt samples were found as positive and 55 isolates showed positive oxidase activities [24].

Table 3. Growth ranges of halotolerant isolates at different NaCl concentrations, different pH and different temperature values

		NaCl Concentrations			pH Values			Temperature Values		
	Isolate Codes	**0%**	**10%**	**20%**	**pH 5**	**pH 7**	**pH 9**	**4°C**	**24°C**	**37°C**
1	1FSS1	++	+	-	+	++	+	-	+	++
2	1FSS2	++	+	-	+	++	+	-	+	++
3	2FSS1	++	+	-	+	++	+	-	+	++
4	2FSS2	++	+	-	+	++	+	-	+	++
5	6FSS1	++	+	-	+	++	+	-	+	++
6	6FSS2	++	+	-	+	++	+	-	+	++
7	6FSS3	++	+	-	+	++	+	-	+	++
8	6FSS4	++	+	-	+	++	+	-	+	++
9	6FSS5	++	+	+	+	++	+	-	+	++
10	6FSS6	++	+	-	+	++	+	-	+	++
11	6FSS7	++	+	-	+	++	+	-	+	++
12	6FSS8	++	+	+	+	++	+	-	+	++
13	6FSS9	++	+	-	+	++	+	-	+	++
14	6FSS10	++	+	-	+	++	+	-	+	++
15	7FSS1	++	+	-	+	++	+	-	+	++
16	9FSS1	++	+	-	+	++	+	-	+	++
17	9FSS2	++	+	-	+	++	+	-	+	++
18	9FSS3	++	+	-	+	++	+	-	+	++
19	9FSS4	++	+	-	+	++	+	-	+	++
20	9FSS5	++	+	-	+	++	+	-	+	++
21	9FSS6	++	+	-	+	++	+	-	+	++
22	9FSS7	++	+	+	+	++	+	-	+	++
23	9FSS8	++	+	-	+	++	+	-	+	++
24	9FSS9	++	+	-	+	++	+	-	+	++
25	9FSS13	++	+	-	+	++	+	-	+	++
26	9FSS14	++	+	-	+	++	+	-	+	++
27	9FSS15	++	+	+	+	++	+	-	+	++
28	9FSS16	++	+	+	+	++	+	-	+	++
29	9FSS17	++	+	-	+	++	+	-	+	++

30	9FSS18	++	+	-	+	++	+	-	+	++
31	9FSS19	++	+	-	+	++	+	-	+	++
32	9FSS20	++	+	-	+	++	+	-	+	++
33	9FSS21	++	+	-	+	++	+	-	+	++
34	9FSS22	++	+	-	+	++	+	-	+	++
35	9FSS23	++	+	-	+	++	+	-	+	++
36	9FSS24	++	+	+	+	++	+	-	+	++
37	9FSS26	++	+	-	+	++	+	-	+	++
38	9FSS27	++	+	-	+	++	+	-	+	++
39	9FSS28	++	+	-	+	++	+	-	+	++
40	9FSS29	++	+	-	+	++	+	-	+	++

Table 3. Continued

		NaCl Concentrations			pH Values			Temperature Values		
	Isolate Codes	**0%**	**10%**	**20%**	**pH 5**	**pH 7**	**pH 9**	**4°C**	**24°C**	**37°C**
41	9FSS30	++	+	-	+	++	+	-	+	++
42	9FSS31	++	+	+	+	++	+	-	+	++
43	9FSS32	++	+	-	+	++	+	-	+	++
44	9FSS33	++	+	-	+	++	+	-	+	++
45	9FSS34	++	+	-	+	++	+	-	+	++
46	9FSS35	++	+	-	+	++	+	-	+	++
47	9FSS36	++	+	-	+	++	+	-	+	++
48	9FSS37	++	+	-	+	++	+	-	+	++
49	9FSS38	++	+	-	+	++	+	-	+	++
50	9FSS39	++	+	-	+	++	+	-	+	++
51	9FSS40	++	+	-	+	++	+	-	+	++
52	10FSS1	++	+	-	+	++	+	-	+	++
53	10FSS2	++	+	-	+	++	+	-	+	++
54	10FSS3	++	+	-	+	++	+	-	+	++
55	10FSS4	++	+	-	+	++	+	-	+	++
56	10FSS5	++	+	-	+	++	+	-	+	++
57	10FSS6	++	+	-	+	++	+	-	+	++
58	10FSS7	++	+	-	+	++	+	-	+	++
59	10FSS8	++	+	-	+	++	+	-	+	++
60	11FSS1	++	+	-	+	++	+	-	+	++
61	11FSS2	++	+	-	+	++	+	-	+	++
62	11FSS3	++	+	+	+	++	+	-	+	++
63	11FSS4	++	+	-	+	++	+	-	+	++
64	11FSS5	++	+	-	+	++	+	-	+	++
65	11FSS6	++	+	-	+	++	+	-	+	++
66	11FSS7	++	+	+	+	++	+	-	+	++
67	11FSS8	++	+	-	+	++	+	-	+	++
68	11FSS9	++	+	+	+	++	+	-	+	++
69	11FSS10	++	+	-	+	++	+	-	+	++
70	11FSS11	++	+	-	+	++	+	-	+	++
71	13FSS3	++	+	-	+	++	+	-	+	++
72	13FSS11	++	+	-	+	++	+	-	+	++
73	13FSS12	++	+	+	+	++	+	-	+	++
74	13FSS15	++	+	-	+	++	+	-	+	++
75	13FSS19	++	+	-	+	++	+	-	+	++
76	13FSS20	++	+	-	+	++	+	-	+	++
77	13FSS21	++	+	-	+	++	+	-	+	++
78	13FSS22	++	+	-	+	++	+	+	+	++
79	13FSS26	++	+	-	+	++	+	-	+	++
80	13FSS28	++	+	-	+	++	+	-	+	++
81	13FSS29	++	+	-	+	++	+	-	+	++
82	13FSS30	++	+	-	+	++	+	+	+	++

83	14FSS1	++	+	+	+	++	+	-	+	++
84	15FSS1	++	+	-	+	++	+	-	+	++
85	15FSS2	++	+	-	+	++	+	-	+	++
86	16FSS1	++	+	-	+	++	+	-	+	++
87	16FSS2	++	+	-	+	++	+	-	+	++
88	16FSS3	++	+	-	+	++	+	-	+	++
89	16FSS4	++	+	-	+	++	+	-	+	++
90	16FSS5	++	+	-	+	++	+	-	+	++
91	16FSS6	++	+	-	+	++	+	-	+	++
92	16FSS7	++	+	-	+	++	+	-	+	++

Table 3. Continued

		NaCl Concentrations			**pH Values**			**Temperature Values**		
	Isolate Codes	**0%**	**10%**	**20%**	**pH 5**	**pH 7**	**pH 9**	**4°C**	**24°C**	**37°C**
93	16FSS8	++	+	-	+	++	+	-	+	++
94	16FSS9	++	+	-	+	++	+	-	+	++
95	18FSS1	++	+	-	+	++	+	-	+	++
96	18FSS5	++	+	-	+	++	+	+	+	++
97	18FSS6	++	+	-	+	++	+	+	+	++
98	18FSS7	++	+	+	+	++	+	+	+	++
99	18FSS9	++	+	-	+	++	+	+	+	++
100	18FSS10	++	+	-	+	++	+	-	+	++
101	18FSS11	++	+	-	+	++	+	-	+	++
102	18FSS35	++	+	+	+	++	+	-	+	++
103	18FSS40	++	+	-	+	++	+	-	+	++
104	18FSS41	++	+	-	+	++	+	-	+	++
105	18FSS45	++	+	-	+	++	+	-	+	++
106	18FSS46	++	+	-	+	++	+	-	+	++
107	18FSS50	++	+	+	+	++	+	-	+	++
108	18FSS52	++	+	-	+	++	+	-	+	++
109	18FSS53	++	+	-	+	++	+	-	+	++
110	18FSS55	++	+	-	+	++	+	-	+	++
111	19FSS1	++	+	-	+	++	+	-	+	++
112	21FSS1	++	+	-	+	++	+	+	+	++
113	21FSS2	++	+	-	+	++	+	-	+	++
114	21FSS3	++	+	-	+	++	+	-	+	++
115	21FSS4	++	+	-	+	++	+	-	+	++
116	21FSS5	++	+	+	+	++	+	-	+	++
117	21FSS6	++	+	-	+	++	+	-	+	++
118	21FSS7	++	+	-	+	++	+	-	+	++
119	21FSS8	++	+	-	+	++	+	-	+	++
120	21FSS9	++	+	+	+	++	+	-	+	++
121	21FSS10	++	+	-	+	++	+	-	+	++
122	22FSS1	++	+	-	+	++	+	-	+	++
123	22FSS2	++	+	+	+	++	+	-	+	++
124	22FSS3	++	+	-	+	++	+	-	+	++
125	22FSS4	++	+	-	+	++	+	-	+	++
126	22FSS5	++	+	-	+	++	+	-	+	++
127	22FSS6	++	+	-	+	++	+	-	+	++
128	22FSS7	++	+	-	+	++	+	-	+	++
129	24FSS2	++	+	+	+	++	+	-	+	++
130	24FSS3	++	+	+	+	++	+	-	+	++
131	25FSS1	++	+	+	+	++	+	-	+	++
132	25FSS4	++	+	-	+	++	+	-	+	++
133	25FSS5	++	+	-	+	++	+	-	+	++
134	25FSS6	++	+	-	+	++	+	-	+	++
135	25FSS7	++	+	-	+	++	+	-	+	++
136	25FSS8	++	+	-	+	++	+	+	+	++
137	25FSS9	++	+	-	+	++	+	-	+	++
138	25FSS10	++	+	+	+	++	+	-	+	++

++:shows optimal growth values

Table 4. Phenotypic properties of pure halotolerant bacterial colonies

	Isolate Codes	Cell Morphology	Motility	Gram Reaction	Endospore	Colony Characteristics						
						Form	Elevation	Margin	Pigmentation	Texture	Appearance	Optical Property
1	1FSS1	Rod	+	-	-	Circular	Raised	Entire	Orange	Smooth	Shiny	Opaque
2	1FSS2	Rod	+	+	+	Punctiform	Flat	Entire	Non-pigmented	Smooth	Shiny	Transparent
3	2FSS1	Rod	+	+	-	Punctiform	Flat	Entire	White	Smooth	Shiny	Transparent
4	2FSS2	Rod	+	+	+	Punctiform	Flat	Entire	Non-pigmented	Rough	Shiny	Transparent
5	6FSS1	Rod	+	+	-	Circular	Flat	Entire	Cream	Smooth	Dull	Opaque
6	6FSS2	Rod	+	-	-	Circular	Flat	Filamentous	Cream	Rough	Dull	Opaque
7	6FSS3	Rod	+	+	+	Circular	Flat	Filamentous	White	Smooth	Dull	Opaque
8	6FSS4	Rod	+	+	+	Circular	Raised	Filamentous	White	Rough	Dull	Opaque
9	6FSS5	Rod	+	+	+	Circular	Raised	Filamentous	White	Smooth	Dull	Opaque
10	6FSS6	Rod	-	+	+	Circular	Flat	Filamentous	Cream	Rough	Dull	Opaque
11	6FSS7	Rod	+	-	-	Circular	Raised	Entire	Cream	Smooth	Dull	Opaque
12	6FSS8	Rod	+	+	+	Circular	Raised	Entire	Cream	Smooth	Dull	Opaque
13	6FSS9	Rod	+	+	+	Circular	Raised	Entire	White	Rough	Dull	Opaque
14	6FSS10	Rod	+	+	+	Circular	Raised	Entire	Yellow	Smooth	Dull	Opaque
15	7FSS1	Rod	+	+	+	Circular	Convex	Entire	Orange	Smooth	Shiny	Opaque
16	9FSS1	Rod	+	+	-	Circular	Raised	Entire	Cream	Rough	Dull	Opaque
17	9FSS2	Rod	+	+	-	Circular	Raised	Entire	Cream	Rough	Shiny	Transcluent
18	9FSS3	Rod	-	+	+	Circular	Convex	Entire	Cream	Smooth	Shiny	Opaque
19	9FSS4	Rod	+	+	-	Punctiform	Flat	Entire	Cream	Rough	Shiny	Transcluent
20	9FSS5	Rod	+	+	-	Punctiform	Flat	Entire	Cream	Smooth	Shiny	Transcluent
21	9FSS6	Rod	+	+	-	Punctiform	Flat	Entire	White	Rough	Dull	Transcluent
22	9FSS7	Rod	+	+	+	Punctiform	Flat	Entire	Yellow	Smooth	Shiny	Transcluent
23	9FSS8	Rod	+	+	-	Circular	Flat	Entire	Yellow	Smooth	Shiny	Transcluent
24	9FSS9	Rod	+	+	-	Circular	Raised	Entire	Cream	Smooth	Dull	Transcluent
25	9FSS13	Rod	-	+	-	Circular	Flat	Erose	Cream	Rough	Dull	Opaque
26	9FSS14	Rod	+	+	+	Punctiform	Raised	Entire	Cream	Smooth	Shiny	Transcluent
27	9FSS15	Rod	+	+	-	Circular	Flat	Entire	Cream	Rough	Dull	Opaque
28	9FSS16	Rod	+	+	+	Circular	Raised	Entire	Cream	Smooth	Shiny	Opaque
29	9FSS17	Rod	+	+	-	Punctiform	Flat	Entire	White	Smooth	Dull	Transcluent
30	9FSS18	Rod	+	+	+	Circular	Flat	Erose	White	Smooth	Shiny	Transcluent
31	9FSS19	Rod	-	+	-	Circular	Flat	Entire	Cream	Smooth	Shiny	Transcluent
32	9FSS20	Rod	+	+	+	Punctiform	Raised	Entire	Cream	Smooth	Dull	Transcluent
33	9FSS21	Rod	+	-	-	Circular	Raised	Entire	Yellow	Smooth	Shiny	Transparent
34	9FSS22	Rod	+	+	-	Punctiform	Flat	Entire	Yellow	Rough	Shiny	Transparent
35	9FSS23	Rod	+	+	-	Punctiform	Flat	Entire	Yellow	Smooth	Shiny	Transparent
36	9FSS24	Rod	+	+	+	Circular	Raised	Undulate	Dark cream	Smooth	Shiny	Transparent
37	9FSS26	Rod	+	-	-	Punctiform	Flat	Entire	Cream	Smooth	Shiny	Transcluent
38	9FSS27	Rod	+	+	+	Punctiform	Flat	Entire	Cream	Smooth	Shiny	Transcluent
39	9FSS28	Rod	+	+	-	Circular	Raised	Entire	Cream	Rough	Dull	Opaque
40	9FSS29	Rod	+	+	-	Punctiform	Flat	Entire	Cream	Smooth	Dull	Opaque
41	9FSS30	Rod	+	+	-	Circular	Raised	Entire	White	Smooth	Shiny	Transcluent
42	9FSS31	Rod	+	+	+	Circular	Raised	Undulate	White	Smooth	Dull	Transcluent
43	9FSS32	Rod	+	+	-	Circular	Flat	Entire	Cream	Smooth	Shiny	Transcluent
44	9FSS33	Rod	+	+	-	Circular	Raised	Undulate	Cream	Rough	Dull	Opaque
45	9FSS34	Rod	-	+	+	Circular	Flat	Entire	Cream	Smooth	Dull	Opaque
46	9FSS35	Rod	+	+	-	Punctiform	Flat	Entire	Cream	Smooth	Shiny	Transparent
47	9FSS36	Rod	+	+	-	Circular	Raised	Erose	Dark cream	Smooth	Shiny	Transcluent
48	9FSS37	Rod	+	+	-	Punctiform	Raised	Entire	Yellow	Smooth	Shiny	Opaque
49	9FSS38	Rod	+	+	+	Circular	Flat	Entire	Cream	Rough	Dull	Transcluent
50	9FSS39	Rod	+	+	+	Circular	Raised	Erose	Cream	Smooth	Shiny	Transcluent

Table 4. Continued

	Isolate Codes	Cell Morphology	Motility	Gram Reaction	Endospore	Colony Characteristics						
						Form	Elevation	Margin	Pigmentation	Texture	Appearance	Optical Property
51	9FSS40	Rod	+	+	+	Punctiform	Flat	Entire	Cream	Smooth	Shiny	Transcluent
52	10FSS1	Rod	+	+	-	Circular	Raised	Entire	White	Rough	Dull	Transcluent
53	10FSS2	Rod	+	+	+	Circular	Raised	Entire	White	Rough	Dull	Opaque
54	10FSS3	Rod	+	+	+	Circular	Raised	Entire	Cream	Rough	Dull	Opaque
55	10FSS4	Rod	+	+	-	Circular	Raised	Entire	White	Rough	Dull	Opaque
57	10FSS6	Rod	-	+	-	Punctiform	Flat	Entire	Cream	Rough	Shiny	Transcluent
58	10FSS7	Rod	+	+	-	Circular	Flat	Entire	Cream	Smooth	Shiny	Transparent
59	10FSS8	Rod	+	+	-	Punctiform	Flat	Entire	White	Smooth	Shiny	Transparent
60	11FSS1	Rod	+	+	+	Circular	Raised	Entire	Cream	Smooth	Dull	Opaque
61	11FSS2	Rod	+	+	+	Circular	Flat	Erose	Cream	Rough	Dull	Transcluent
62	11FSS3	Rod	+	+	+	Circular	Raised	Entire	White	Smooth	Dull	Transparent
63	11FSS4	Rod	+	+	+	Circular	Raised	Entire	Cream	Smooth	Dull	Opaque
64	11FSS5	Rod	+	+	+	Circular	Flat	Entire	White	Smooth	Dull	Opaque
65	11FSS6	Rod	+	+	+	Circular	Raised	Entire	White	Rough	Dull	Opaque
66	11FSS7	Rod	+	+	+	Circular	Raised	Entire	Cream	Smooth	Shiny	Opaque
67	11FSS8	Rod	+	+	+	Circular	Flat	Entire	Cream	Smooth	Shiny	Transcluent
68	11FSS9	Rod	+	+	+	Circular	Raised	Entire	White	Rough	Dull	Opaque
69	11FSS10	Rod	+	+	+	Circular	Flat	Entire	White	Rough	Dull	Opaque
70	11FSS11	Rod	+	+	+	Circular	Raised	Entire	Cream	Rough	Dull	Opaque
71	13FSS3	Rod	+	+	+	Irregular	Convex	Entire	Cream	Rough	Shiny	Opaque
72	13FSS11	Rod	+	+	+	Punctiform	Flat	Entire	Non-pigmented	Smooth	Shiny	Transparent
73	13FSS12	Rod	+	+	+	Irregular	Convex	Entire	Cream	Rough	Shiny	Opaque
74	13FSS15	Rod	+	+	+	Circular	Convex	Entire	White	Smooth	Dull	Transcluent
75	13FSS19	Rod	+	+	+	Irregular	Flat	Entire	Cream	Rough	Shiny	Opaque
76	13FSS20	Rod	+	+	+	Rhizoid	Flat	Filamentous	Cream	Smooth	Dull	Transcluent
77	13FSS21	Rod	+	+	+	Circular	Flat	Entire	White	Rough	Shiny	Opaque
78	13FSS22	Rod	+	+	+	Circular	Flat	Entire	White	Smooth	Shiny	Transparent
79	13FSS26	Rod	+	+	+	Rhizoid	Flat	Filamentous	White	Rough	Shiny	Transcluent
80	13FSS28	Rod	+	+	+	Rhizoid	Raised	Filamentous	White	Rough	Shiny	Transparent
81	13FSS29	Rod	+	+	+	Rhizoid	Flat	Filamentous	White	Rough	Dull	Transparent
82	13FSS30	Rod	+	+	+	Rhizoid	Flat	Filamentous	White	Rough	Shiny	Transcluent
83	14FSS1	Rod	+	-	-	Circular	Flat	Entire	Dark cream	Smooth	Shiny	Transparent
84	15FSS1	Rod	+	+	+	Circular	Raised	Entire	Yellow	Smooth	Shiny	Transcluent
85	15FSS2	Rod	+	-	-	Circular	Flat	Entire	Yellow	Smooth	Shiny	Transcluent
86	16FSS1	Rod	+	+	+	Punctiform	Raised	Entire	Cream	Rough	Dull	Opaque
87	16FSS2	Rod	-	-	-	Punctiform	Flat	Entire	Dark yellow	Smooth	Shiny	Transparent
88	16FSS3	Rod	+	-	-	Circular	Flat	Entire	Yellow	Smooth	Dull	Transcluent
89	16FSS4	Rod	+	+	+	Circular	Raised	Entire	Cream	Smooth	Dull	Opaque
90	16FSS5	Rod	+	+	+	Circular	Raised	Entire	White	Smooth	Dull	Opaque
91	16FSS6	Rod	+	+	+	Circular	Düz	Entire	White	Smooth	Dull	Opaque
92	16FSS7	Rod	+	+	+	Punctiform	Flat	Undulate	Cream	Rough	Dull	Transparent
93	16FSS8	Rod	+	-	-	Punctiform	Flat	Entire	White	Rough	Shiny	Transcluent
94	16FSS9	Rod	+	+	+	Circular	Flat	Entire	Cream	Rough	Dull	Transcluent
95	18FSS1	Rod	+	+	+	Punctiform	Flat	Entire	Cream	Rough	Shiny	Opaque
96	18FSS5	Rod	+	+	+	Circular	Raised	Erose	White	Smooth	Dull	Opaque
97	18FSS6	Rod	+	+	-	Circular	Flat	Erose	Dark cream	Smooth	Dull	Opaque
98	18FSS7	Rod	+	+	-	Circular	Flat	Entire	White	Smooth	Dull	Opaque
99	18FSS9	Rod	+	+	-	Circular	Convex	Entire	White	Rough	Dull	Opaque
100	18FSS10	Rod	+	+	+	Circular	Raised	Entire	White	Rough	Dull	Transcluent
101	18FSS11	Rod	+	+	+	Circular	Flat	Entire	Cream	Rough	Shiny	Transcluent
102	18FSS35	Rod	+	+	+	Circular	Flat	Erose	Cream	Rough	Dull	Transcluent
103	18FSS40	Rod	+	+	+	Circular	Flat	Entire	White	Rough	Shiny	Opaque
104	18FSS41	Rod	+	+	-	Punctiform	Flat	Entire	White	Rough	Dull	Transcluent
105	18FSS45	Rod	+	+	-	Circular	Flat	Erose	Dark cream	Smooth	Shiny	Transcluent

Table 4. Continued

	Isolate Codes	Cell Morphology	Motility	Gram Reaction	Endospore	Colony Characteristics						
						Form	Elevation	Margin	Pigmentation	Texture	Appearance	Optical Property
106	18FSS46	Rod	+	+	+	Punctiform	Convex	Entire	Dark cream	Smooth	Shiny	Transparent
107	18FSS50	Rod	+	+	+	Circular	Raised	Entire	Cream	Smooth	Dull	Opaque
108	18FSS52	Rod	+	+	+	Circular	Raised	Entire	White	Rough	Dull	Opaque
109	18FSS53	Rod	+	+	-	Circular	Flat	Entire	Dark cream	Rough	Dull	Transparent
110	18FSS55	Rod	+	+	+	Circular	Raised	Undulate	White	Rough	Shiny	Opaque
111	19FSS1	Rod	+	-	-	Punctiform	Flat	Entire	Non-pigmented	Rough	Shiny	Transparent
112	21FSS1	Rod	+	+	+	Circular	Flat	Erose	Cream	Rough	Dull	Opaque
113	21FSS2	Rod	+	-	-	Circular	Flat	Erose	Cream	Smooth	Shiny	Opaque
114	21FSS3	Rod	+	-	-	Circular	Flat	Erose	Cream	Rough	Dull	Opaque
115	21FSS4	Rod	+	-	-	Circular	Flat	Erose	Cream	Rough	Dull	Opaque
116	21FSS5	Rod	+	+	+	Filamentous	Raised	Filamentous	Cream	Smooth	Shiny	Transcluent
117	21FSS6	Rod	+	+	+	Circular	Flat	Entire	White	Rough	Dull	Transcluent
118	21FSS7	Rod	+	+	+	Circular	Flat	Erose	Yellow	Rough	Dull	Opaque
119	21FSS8	Rod	+	+	+	Circular	Raised	Undulate	Cream	Rough	Dull	Transcluent
120	21FSS9	Rod	+	+	+	Circular	Raised	Undulate	Cream	Rough	Shiny	Transcluent
121	21FSS10	Rod	+	+	+	Circular	Flat	Erose	Cream	Smooth	Dull	Opaque
122	22FSS1	Rod	+	-	-	Circular	Raised	Erose	White	Smooth	Shiny	Opaque
123	22FSS2	Rod	+	+	-	Punctiform	Flat	Entire	White	Smooth	Shiny	Transcluent
124	22FSS3	Rod	+	+	+	Punctiform	Flat	Entire	White	Rough	Dull	Transparent
125	22FSS4	Rod	+	-	-	Circular	Flat	Undulate	White	Rough	Shiny	Transcluent
126	22FSS5	Rod	+	-	-	Circular	Raised	Entire	Yellow	Smooth	Shiny	Opaque
127	22FSS6	Rod	+	-	-	Circular	Flat	Undulate	White	Rough	Dull	Transparent
128	22FSS7	Rod	+	+	-	Punctiform	Flat	Entire	White	Smooth	Shiny	Transparent
129	24FSS2	Rod	+	+	+	Circular	Flat	Entire	Yellow	Rough	Shiny	Opaque
130	24FSS3	Rod	+	+	-	Circular	Raised	Entire	White	Rough	Shiny	Transcluent
131	25FSS1	Rod	+	+	+	Irregular	Raised	Filamentous	White	Rough	Shiny	Opaque
132	25FSS4	Rod	+	-	-	Circular	Flat	Erose	Cream	Smooth	Dull	Opaque
133	25FSS5	Rod	+	+	-	Irregular	Raised	Entire	Cream	Rough	Shiny	Opaque
134	25FSS6	Rod	+	+	+	Circular	Flat	Entire	Non-pigmented	Smooth	Shiny	Transparent
135	25FSS7	Rod	+	+	+	Irregular	Raised	Filamentous	Cream	Rough	Shiny	Transcluent
136	25FSS8	Rod	+	+	+	Irregular	Raised	Filamentous	Cream	Rough	Shiny	Transcluent
137	25FSS9	Rod	+	+	-	Irregular	Raised	Filamentous	Cream	Rough	Shiny	Transcluent
138	25FSS10	Rod	+	-	-	Circular	Raised	Entire	Yellow	Smooth	Shiny	Opaque

Table 5. Biochemical test results of halotolerant test isolates

	Isolate Codes	Catalase	Oxidase	Protease	Lipase	Amylase	Caseinase	Ammonia from peptone
1	1FSS1	+	-	-	+	-	-	+
2	1FSS2	+	+	+	-	+	+	+
3	2FSS1	+	+	-	-	-	-	-
4	2FSS2	+	-	-	+	+	-	+
5	6FSS1	+	-	-	+	+	+	+
6	6FSS2	+	-	-	+	+	+	-
7	6FSS3	+	-	-	+	+	+	+
8	6FSS4	+	-	+	+	+	+	+
9	6FSS5	+	+	-	+	+	+	-
10	6FSS6	+	+	+	+	-	+	-
11	6FSS7	+	-	+	-	+	+	+
12	6FSS8	+	-	-	-	+	+	-
13	6FSS9	+	+	+	-	+	+	+
14	6FSS10	+	+	-	-	+	+	-
15	7FSS1	+	-	+	+	-	+	-
16	9FSS1	+	+	-	-	+	+	+
17	9FSS2	+	+	+	-	+	+	+
18	9FSS3	+	+	+	-	+	+	+
19	9FSS4	+	+	-	-	-	+	-
20	9FSS5	+	+	+	+	-	+	-
21	9FSS6	+	+	+	-	+	-	-
22	9FSS7	+	+	-	-	+	+	+
23	9FSS8	+	+	+	-	+	+	-
24	9FSS9	+	+	+	-	+	-	-
25	9FSS13	+	-	-	-	+	+	+
26	9FSS14	+	+	-	-	+	+	-
27	9FSS15	+	-	+	+	-	+	-
28	9FSS16	+	+	+	+	+	+	-
29	9FSS17	+	+	+	+	-	+	-
30	9FSS18	+	+	-	-	-	+	-
31	9FSS19	+	-	+	-	-	+	-
32	9FSS20	+	+	+	-	+	+	-
33	9FSS21	+	-	+	-	-	-	-
34	9FSS22	+	-	+	-	+	-	-
35	9FSS23	+	+	+	-	+	+	-
36	9FSS24	+	+	+	+	-	-	-
37	9FSS26	+	+	+	-	-	-	+
38	9FSS27	+	+	-	-	-	+	-
39	9FSS28	+	+	+	-	-	+	-
40	9FSS29	+	+	+	-	-	+	-
41	9FSS30	+	+	+	-	+	+	-
42	9FSS31	+	+	-	-	+	+	-
43	9FSS32	+	-	+	+	+	+	+
44	9FSS33	+	+	+	+	-	-	-
45	9FSS34	+	+	+	-	-	+	-
46	9FSS35	+	+	+	+	-	+	-
47	9FSS36	+	-	+	-	-	+	-
48	9FSS37	+	-	+	-	-	+	-
49	9FSS38	+	-	-	-	-	-	+
50	9FSS39	+	+	+	-	-	-	-
51	9FSS40	+	-	+	-	+	+	+
52	10FSS1	+	-	+	-	-	-	+
53	10FSS2	+	+	+	-	-	-	-
54	10FSS3	+	-	+	-	+	+	+
55	10FSS4	+	+	-	+	+	+	-
56	10FSS5	+	-	+	-	+	+	-
57	10FSS6	+	-	+	-	+	-	-
58	10FSS7	+	-	+	-	+	-	+
59	10FSS8	+	+	+	-	-	+	-
60	11FSS1	+	+	+	+	+	+	-
61	11FSS2	+	+	+	+	-	+	+
62	11FSS3	+	+	+	+	+	+	-
63	11FSS4	+	-	+	-	-	-	+

Table 5. Continued

	Isolate Codes	Catalase	Oxidase	Protease	Lipase	Amylase	Caseinase	Ammonia from peptone
64	11FSS5	+	+	+	+	+	+	-
65	11FSS6	+	+	+	+	-	+	+
66	11FSS7	+	+	+	+	+	+	-
67	11FSS8	+	-	-	+	+	+	-
68	11FSS9	+	+	+	-	+	+	+
69	11FSS10	+	+	+	-	-	+	-
70	11FSS11	+	-	-	+	+	+	-
71	13FSS3	+	-	-	-	+	+	-
72	13FSS11	+	-	-	-	+	+	-
73	13FSS12	+	-	-	-	+	+	+
74	13FSS15	+	-	-	-	+	+	-
75	13FSS19	+	-	-	-	+	+	-
76	13FSS20	+	+	+	-	+	-	+
77	13FSS21	+	+	-	-	-	-	-
78	13FSS22	+	+	-	-	+	+	+
79	13FSS26	+	+	+	-	+	+	-
80	13FSS28	+	-	-	-	-	+	+
81	13FSS29	+	+	-	-	+	-	+
82	13FSS30	+	+	+	-	+	+	+
83	14FSS1	+	+	-	-	-	+	+
84	15FSS1	+	-	-	-	-	-	-
85	15FSS2	+	+	-	-	+	-	-
86	16FSS1	+	-	-	-	+	+	-
87	16FSS2	+	-	-	+	+	+	+
88	16FSS3	+	+	+	-	-	-	-
89	16FSS4	+	-	+	+	+	+	+
90	16FSS5	+	-	+	+	+	+	-
91	16FSS6	+	+	+	-	+	-	+
92	16FSS7	+	-	+	+	+	+	-
93	16FSS8	+	-	+	-	-	+	+
94	16FSS9	+	+	-	+	+	-	-
95	18FSS1	+	+	-	+	+	+	-
96	18FSS5	+	+	+	-	-	-	-
97	18FSS6	+	+	+	+	+	+	-
98	18FSS7	+	+	+	+	+	+	-
99	18FSS9	+	+	+	-	+	-	-
100	18FSS10	+	+	+	+	+	+	+
101	18FSS11	+	+	-	+	+	-	-
102	18FSS35	+	+	+	+	-	-	+
103	18FSS40	+	+	+	-	-	+	+
104	18FSS41	+	+	+	-	+	+	+
105	18FSS45	+	+	+	-	+	-	-
106	18FSS46	+	+	+	+	+	+	-
107	18FSS50	+	+	+	-	+	+	-
108	18FSS52	+	+	+	+	+	-	+
109	18FSS53	+	+	+	-	+	+	-
110	18FSS55	+	+	+	-	+	+	+
111	19FSS1	+	-	+	+	-	-	+
112	21FSS1	+	-	-	+	+	+	+
113	21FSS2	+	+	-	-	+	+	-
114	21FSS3	+	-	+	+	+	+	+
115	21FSS4	+	-	+	-	+	+	+
116	21FSS5	+	-	-	-	+	+	+
117	21FSS6	+	-	-	-	+	+	-
118	21FSS7	+	+	+	-	+	+	+
119	21FSS8	+	-	-	-	+	+	+
120	21FSS9	+	-	-	-	+	+	+
121	21FSS10	+	+	+	-	+	+	+
122	22FSS1	+	+	+	+	-	+	+
123	22FSS2	+	-	-	+	-	-	-
124	22FSS3	+	-	-	-	-	-	-
125	22FSS4	+	-	-	+	+	+	+
126	22FSS5	+	-	+	-	-	+	-
127	22FSS6	+	+	+	+	+	+	+
128	22FSS7	+	+	+	+	+	+	-

Table 5. Continued

	Isolate Codes	Catalase	Oxidase	Protease	Lipase	Amylase	Caseinase	Ammonia from peptone
129	24FSS2	+	+	-	-	+	-	-
130	24FSS3	+	+	-	-	+	-	+
131	25FSS1	+	+	+	-	-	-	+
132	25FSS4	+	-	+	-	+	+	+
133	25FSS5	+	+	+	-	+	+	+
134	25FSS6	+	-	-	-	+	+	+
135	25FSS7	+	+	-	-	+	+	+
136	25FSS8	+	+	-	-	+	+	+
137	25FSS9	+	+	-	-	+	+	+
138	25FSS10	+	-	-	+	-	-	-

In the present study, the sterilization methods of food salt samples were also examined. Six food salt samples containing bacteria and halotolerant bacteria in high numbers were selected for sterilization experiments. Food salt samples of FSS1 (Cyprus black lava sea salt, France), FSS9 (sea salt, Türkiye), FSS15 (Himalaya salt, Pakistan), FSS18 (table salt, Pakistan), FSS22 (table salt, England), FSS24 (table salt, Switzerland) were used in the sterilization experiments. The numbers of total bacteria and halotolerant bacteria in the FSS1, FSS9, FSS15, FSS18, FSS22, FSS24 were respectively found as 35 and 34 CFU/g; 40 and 40 CFU/g; 30 and 30 CFU/g; 58 and 16 CFU/g; 21and 20 CFU/g; 40 and 40 CFU/g (Table 2). After sterilization processes of food salt samples of FSS1, FSS9, FSS15, FSS18, FSS22, and FSS24 using dry heat (175^{o}C for 2 hours) and high-pressure moist heat (121^{o}C for 30 minutes), the bacterial colony was not detected on the membranes of the MNA agar media incubated at 37^{o}C for seven days. Therefore, the dry-heat sterilization process of six food salt samples at 175^{o}C for 2 h and the high-pressure moist heat sterilization process of six food salt samples at 121^{o}C for 30 min were found highly effective sterilization methods to kill all bacterial populations including Gram-positive halotolerant bacteria containing endospore and Gram-negative halotolerant bacteria having endotoxin. After both sterilization methods, the color and appearance of the salt samples did not change.

IV. CONCLUSIONS AND EVALUATIONS

This research is the first study that examines the food salt samples collected from France, Germany, England, Pakistan, Türkiye, Switzerland, and Austria for halotolerant bacteria. A total of 138 halotolerant bacteria were isolated from the food salt samples coded as 1FSS (2 isolates), 2FSS (2 isolates), 6FSS (10 isolates), 7FSS (1 isolate), 9FSS (36 isolates), 10FSS (8 isolates), 11FSS (11 isolates), 13FSS (12 isolates), 14FSS (1 isolate), 15FSS (2 isolates), 16FSS (9 isolates), 18FSS (16 isolates), 19FSS (1 isolate), 21FSS (10 isolates), 22FSS (7 isolates), 24FSS (2 isolates), and 25FSS (8 isolates). Among the food salt samples, the most number of halotolerant isolates was recovered from 9FSS. The food salt samples contained orange, cream, dark cream, white, yellow, dark yellow, and non-pigmented colonies with the appearance of dull and shiny. The examination colors of preservation salt samples before using them in food preservation and salted foods may offer us an idea about the bacterial contamination. All halotolerant isolates grew in both absence of salt and the presence of 10% NaCl. Some of the isolates grew by 20% NaCl. Hence, those isolates were accepted as halotolerant bacteria. While a few halotolerant isolates grew at refrigerator temperature, all halotolerant isolates grew at room temperature and 37^{o}C. The halotolerant isolates recovered from food salt samples grew on acidic, neutral, and alkaline media. According to the most important results obtained from the present study, 80% of food salt samples contained bacteria, and 68% of the food salt samples had halotolerant bacteria. Moreover, Gram-positive halotolerant bacteria containing endospore structure were found in 15 salt samples, and Gram-negative halotolerant bacteria having endotoxin were detected in 10 salt samples. Most of these halotolerant isolates had hydrolytic activities such as protease, amylase, lipase, or caseinase. Based on the results of this study, Gram-positive and Gram-negative halotolerant bacteria producing hydrolytic enzymes may grow in acidic, neutral, and alkaline foods stored at room temperature, 37^{o}C, and even some of them at 4^{o}C. Some of these microorganisms may cause food spoilage and foodborne diseases. The sterilization of six food salt samples was accomplished using both dry-heat and high-pressure moist heat treatments in this study. Therefore, we suggest using sterilized salt in uncooked foods to prevent bacterial growth, food spoilage, and foodborne diseases.

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