Determination of Antibacterial Effectiveness of 2-Bromo-2-Nitropropane-1,3-Diol Against Mix Population of Bacteria Isolated from the Salt-Pack Cured Hides

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Abstract

The aim of this study was to research the antibacterial effectiveness of different concentrations of 2-Bromo-2-nitropropane-1,3-diol against mix population of Gram negative (*Enterobacter cloacae, Vibrio fluvialis* and *Pseudomonas luteola*), Gram positive (*Staphylococcus cohnii* and *Enterococcus faecium*) and Gram positive endospore forming bacteria (*Bacillus pumilus*). The *in vitro* antibacterial effectiveness of different dilutions of 2-Bromo-2-nitropropane-1,3-diol against mix population of *Enterobacter cloacae, Vibrio fluvialis, Pseudomonas luteola, Staphylococcus cohnii, Enterococcus faecium* and *Bacillus pumilus*, which were isolated from salt-packed cured hides and identified with API test kits, was examined by the agar disc diffusion method on Nutrient Agar according to the guidelines of the National Committee for Clinical Laboratory Standards. The discs containing 7 µL of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1% (w/v) of 2-Bromo-2-nitropropane-1,3-diol were prepared and placed on surface of Nutrient Agar inoculated with the mix population of bacteria (10^8 c.f.u./mL). Zones were detected around the discs containing all concentrations of 2-Bromo-2-nitropropane-1,3-diol. Zone diameters increased proportionally to the concentrations of the test agent. Inhibition zone diameters of ≥20 mm of were observed at 0.7, 0.8, 0.9 and 1% of 2-Bromo-2-nitropropane-1,3-diol. Although inhibition zone diameter of 15 mm was observed around the disc containing 0.1% of the antibacterial agent, inhibition zone diameter of 23 mm were seen around the disc containing 1% of the agent. As a conclusion, 2-Bromo-2-nitropropane-1,3-diol was found to be fairly effective at the dilutions tested against Gram positive, Gram negative and endospore forming bacteria.

Keywords: 2-Bromo-2-nitropropane-1,3-diol, Antibacterial activity

1.Introduction

Drying, salting, freezing, electric current, heat treatment, pasteurization, γ-irradiation, and electron beam irradiation can be used to control bacterial growth in different industries (Farkas 2001; Ricke et al. 2005; Birbir et al. 2008; Bailey et al. 2001). In addition to physical methods, salt, boric acid, enzymes, sodium citrate, nanoparticles, and quaternary ammonium compounds are used to prevent bacterial growth. In the study carried out by Cho et al. (2005), silver napoparticles were found to be effective against *E.coli* and *S.aureus.* Natural antimicrobial treatments such as bacteriocin, anthocyanins, carotenoids, and flavenoids are also used by scientists to eliminate bacterial growth (Ricke et al. 2005; Guo et al. 1997; Donovan et al. 1998; Stacewicz-Sapuntzakis et al. 2001). Bacteriocin producing *Salmonella* species were used to inhibit the growth of other *Salmonella* species which do not produce bacteriocin (Patankar and Joshi 1985). Bacteriocin is usually used in food industry as a food preservative. The effectiveness of an antibacterial agent is conditioned by pH, organic content, temperature, pathogenity of the bacteria, concentration of the agent, and exposure time. Microbial growth was inhibited by a wide variety of antimicrobial agents such as 1-2 dichlorobenzene, trichloro-S-triazinetrione, sodium sulphate, sodium bisulphite, acetic acid, alkyl phenol ethoxylates, potassium dimethyldithiocarbonate, 2-(thiocyanomethylthio)benzo - thiazole, methylene bis (thiocyanate), sodium-orthophenylphenate, ortho-benzyl-para-chlorophenol, 5-chloro-2-methyl-4-isothiazolin-3-one, potassium dimethyldithiocarbamate, sodium salt of o-phenylphenate (Birbir and Bailey 2000), quaternary ammonium compounds (Bilgi et al. 2009), plus 2-bromo-2-nitropropane-1,3-diol (Muthusubramanian and Mitra 2006), antibacterial complex of copper (II) with benzothiazole (Haibin et al. 2008).

Moreover, antimicrobial activity of nitro compounds against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa,* 2-Bromo-2-nitropropane-1,3-diol was found to be very effective against these test bacteria (Stretton and Manson 1973). It is a colourless and odourless crystalline solid (Croshaw et al. 1964). It is used as fungicidal and bactericidal agent in industrial water applications, paper mills, cosmetic and personal-care products, water-based paints, agriculture and adhesives (BIOBAN 2007; Reregistration Eligibility Decision: Bronopol 1995). The agent, a formaldehyde-releasing preservative (FRP), is environmentally friendly due to its biodegradability and does not accumulate in the food chain or groundwater (Product Safety Assessment Bronopol Antimicrobial 2010). It is

widely used at concentrations of up to 0.1% (w/v) in personal-care products (Shepherd et al. 1988; Croshaw et al. 1964; Bryce et al. 1978). The antibacterial activity of 2-Bromo-2-nitropropane-1,3-diol is explained by its chemical reaction with the significant thiols such as cysteine, glutathione and proteins found in the bacterial cell (Shepherd et al. 1988; Stretton and Manson 1973; Bryce et al. 1978). When 2-Bromo-2-nitropropane-1,3-diol oxidizes thiol-containing molecules under aerobic conditions, active oxygen products such as superoxide and peroxide, which have bactericidal activity for bacteria, are produced (Shepherd et al. 1988). In addition, the agent causes cell membrane damage (Stretton and Manson 1973).

Hence, in the present study, the antibacterial efficacy of 2-Bromo-2-nitropropane-1,3-diol was tested against the mix culture of *Enterobacter cloacae, Vibrio fluvialis, Pseudomonas luteola, Staphylococcus cohnii, Enterococcus faecium* and *Bacillus pumilus* which were isolated from salt-pack cured hides.

2. Material and Methods

2.1. Test isolates and the antibacterial agent

In the present study, we assessed the antimicrobial effectiveness of 2-Bromo-2-nitropropane-1,3-diol against six isolates of bacteria, such as *Enterobacter cloacae, Vibrio fluvialis, Pseudomonas luteola, Staphylococcus cohnii, Enterococcus faecium* and *Bacillus pumilus*. The bacteria were selected as representative potential bacterial pathogens from salt-pack cured hides. All test isolates were isolated and identified in the previous studies (Aslan and Birbir 2011a, Aslan and Birbir 2011b; Aslan and Birbir 2012). API® 20E (bioMériux, Inc, France), API® 20NE (bioMériux, Inc, France), API Staph (bioMériux, Inc, France), API 20 Strep (bioMériux, Inc, France) and API 50CH (bioMériux, Inc, France) test strips were used to identify Gram-negative (*Enterobacter cloacae, Vibrio fluvialis* and *Pseudomonas luteola*) and Gram-positive bacteria (*Staphylococcus cohnii, Enterococcus faecium* and *Bacillus pumilus*), respectively**.** The test bactericide was obtained from Buckman International, Memphis, TN. Varied concentrations of the test agent were prepared as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1% (w/v).

2.2. Determination of protease activity.

Proteolytic activity of the isolates was screened on Tryptic Soy Agar containing 4% gelatine (Table 1). These isolates were seperately inoculated onto Tryptic Soy Agar and incubated at 37°C for 48 hours. After incubation period, the plates were flooded with a saturated solution of ammonium sulfate. Clear zones around the colonies were taken as evidence of protease activity (Barnett and Venghaus 1988).

2.3. Determination of lipase activity.

Lipolytic activity of these isolates was examined on Tween 80 Agar medium (Table 1). The isolates were seperately inoculated onto Tween 80 Agar and incubated at 37°C for 24-48 hours. After incubation period, clear zones around the colonies were taken as evidence of lipase activity (Birbir et al. 2007).

2.4. Determination of antibacterial effectiveness of 2-Bromo-2-nitropropane-1,3-diol

The antibacterial effectiveness of 2-Bromo-2-nitropropane-1,3-diol was investigated by Kirby-Bauer Disk-Diffusion method in accordance with the procedures recommended by National Committee for Clinical Laboratory Standards (Madigan et al. 2012; Bilgehan 2004). During the experiments, the mix culture of *Enterobacter cloacae, Vibrio fluvialis, Pseudomonas luteola, Staphylococcus cohnii, Enterococcus faecium* and *Bacillus pumilus* were used. Initially, the test isolates were separately grown in Nutrient Broth (Merck, Darmstadt, Germany) at 37°C for 24 hours. After incubation period, each of these bacterial cultures was separately suspended in sterile physiological saline to a final cell density of 10^8 c.f.u./mL. The colony forming units were determined by plate counting. In this respect, the mixed culture of the test isolates was prepared from these physiological saline solutions. In order to determine the resistance or sensitivity of the mix culture of test bacteria against 2-Bromo-2-nitropropane-1,3-diol, the disk diffusion method was applied. Test discs (6 mm) were prepared from Whatman No.1 filter paper and sterilized at 121°C for 20 minutes. Afterwards, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 (w/v) percentages of the agent were prepared. Seven of these concentrations of the agent were impregnated into the discs. Then, 100 µL of the mix culture of the test bacteria was spread on Nutrient Agar plates. Subsequently, the discs containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1% (w/v) of the

antimicrobial agent were placed on the Nutrient Agar plates. The plates were incubated at 35°C for 24 hours. The zones of inhibition around the discs were recorded after 24 h at 35°C.

3.Results and discussion

In the present study, *Pseudomonas luteola, Enterococcus faecium, Staphylococcus cohnii* and *Bacillus pumilus* showed both proteolytic and lipolytic activities, while *Vibrio fluvialis* and *Enterobacter cloacae* were found as protease positive and lipase negative (Table 1).

In our previous studies we isolated a wide variety of Gram positive (396 isolates) and Gram negative bacteria (256 isolates) from salt-pack cured hides. *Enterobacter cloacae, Vibrio fluvialis, Pseudomonas luteola, Staphylococcus cohnii, Enterococcus faecium* and *Bacillus pumilus* were isolated from all of the salt-pack cured hides examined. Furthermore, these isolates also were found in soak liquors treated with 0.8 g/l of Didecyldimethyl ammonium chloride (Berber et al. 2010).

The most common Gram-positive genera on the salted hides were *Staphylococcus* (115 isolates), *Bacillus* (111 isolates) and *Enterococcus* (75 isolates), while the most common Gram-negative genera on the salt-pack cured hides were found to be *Enterobacter* (66), *Pseudomonas* (59) and *Vibrio* (32) (Aslan and Birbir 2011a; Aslan and Birbir 2011b; Aslan and Birbir 2012). These isolates which showed both proteolytic and lipolytic activities were in the highest numbers on the hides (Aslan and Birbir 2011a; Aslan and Birbir 2011b; Aslan and Birbir 2012).

The prevalence of bacteria in main soak liqour may result from high organic content of the soak liquors and antibacterial resistance of bacteria. High organic content of soak liqours may affect antibacterial activity adversely. Moreover, the bacteria may develop resistance to antibacterial agent and they can transfer the genes carrying antibacterial resistance among themselves. As a result, despite the presence of antibacterial agents in soak liquors, the bacteria can survive (Birbir et al. 2008).

Hence, we selected *Enterobacter cloacae, Pseudomonas luteola, Bacillus pumilus, Vibrio fluvialis, Enterococcus faecium* and *Staphylococcus cohnii* as the test isolates in the present study.

Table 1. Proteolytic and lipolytic activities of the test isolates

Isolates	Proteolytic Activity	Lipolytic Activity
Enterobacter cloacae	+	-
Vibrio fluvialis	+	-
Pseudomonas luteola	+	+
Enterococcus faecium	+	+
Staphylococcus cohnii	+	+
Bacillus pumilus	+	+

The antibacterial activity of 2-Bromo-2-nitropropane-1,3-diol against mixed bacterial population, as measured by disk diffusion method, is shown in Table 2. While the concentration of the antimicrobial agent was increased, the size of inhibition zone was also increased. Inhibition zone diameters of ≥20 mm were observed at 0.7, 0.8, 0.9 and 1% of the agent concentrations. Although an inhibition zone diameter of 15 mm was observed around the disc containing 0.1% of the agent, inhibition zone diameter of 23 mm was seen around the disc containing 1% of the agent. The inhibition zone of 20 mm is generally evaluated as effective concentration of antimicrobial agent against bacteria. The results can be interpreted as demonstrating 0.7, 0.8, 0.9 and 1% of the test agent were found to be effective against mix population of the test bacteria (Table 2).

Table 2. *In vitro* antibacterial effectiveness of 2-Bromo-2-nitropropane-1,3-diol against mixed population of Gram-negative, Gram-positive and Gram-positive endospore forming bacteria

Test bacteria*	Concentration of the test agent % (w/v)	Zone diameter range (mm)
Mixed culture of *Enterobacter cloacae, Vibrio fluvialis, Pseudomonas luteola, Staphylococcus cohnii, Enterococcus faecium* and *Bacillus pumilus*	0.1	15
	0.2	17
	0.3	18
	0.4	19
	0.5	19
	0.6	19
	0.7	20
	0.8	20
	0.9	21
	1	23

* The inoculum density of the test bacteria was 10^8 c.f.u./mL before experiment.

Similar results were obtained from the studies carried out by other researchers. Among the studies of inhibition of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* by nitro compounds, 2-Bromo-2-nitropropane-1,3-diol was found to be very effective against these bacteria (Stretton and Manson 1973). In the study of Croshaw et al. (1964), all the test bacteria such as *P.aeruginosa* were inhibited at the concentrations of 12.5-50 µg/ml of 2-Bromo-2-nitropropane-1,3-diol. Also, the minimum inhibitory concentration of 2-Bromo-2-nitropropane-1,3-diol for *E.coli* ATCC 8739 was found as 13 µg/ml (Shepherd et al. 1988).

4.Conclusion

2-Bromo-2-nitropropane-1,3-diol has been shown to have antibacterial effectiveness against the mix culture of *Enterobacter cloacae, Vibrio fluvialis, Pseudomonas luteola, Staphylococcus cohnii, Enterococcus faecium* and *Bacillus pumilus.* Concentrations of 0.7, 0.8, 0.9 and 1% of the test agent were found to be more effective against mix population of the test bacteria. Due to its high antibacterial activity, environmentally friendliness and biodegradability, it can be used in brine curing and soaking process of hides in the leather industry to reduce the total bacterial counts and cease bacterial damage. We recommend using this antibacterial agent in the leather industry.

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