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Full Length Research Paper

Prohibitive effect of lactoperoxidase system (LPS) on some pathogen fungi and bacteria

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Lactoperoxidase is a peroxidase enzyme found in milk. This enzyme has antimicrobial and antioxidant properties. It is fairly heat resistant and was widely used as an indicator of over pasteurization of milk. In the present study, bovine lactoperoxidase (LPO) was purified from skimmed milk using amberlite-CG-50-H^+ resin, CM-sephadex-C-50 ion-exchange chromatography and sephadex-G-100 gel filtration chromatography. Enzyme activity was determined using 2, 2-azino-bis-diammonium salt as a chromogenic substrate at pH 6, and purification degree was controlled by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Antibacterial and antifungal activity of bovine lactoperoxidase was determined by disk diffusion method. Bovine LPO exhibited high antifungal and antibacterial activity in 100 mM thiocynate-100 mM hydrogen peroxide (H_2O_2) medium on some fungi (*Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis* and *Saccharomyces boulardii*) and bacteria (*Citrobacter freundii, Escherichia coli, Streptococcus pneumoniae, Staphylococcus epidermidis* and *Staphylococcus intermedius*), respectively. Antibacterial and antifungal activity of LPO system was compared to those of well known antibacterial and antifungal substances such as cefaclor, erythromycin, tetracycline and fluconazole. This study has investigated for the first time the antifungal and antibacterial effects of bovine LPO system on *C. albicans, C. glabrata, C. krusei, C. parapsilosis, S. boulardii*, *S. pneumoniae, S. epidermidis* and *S. intermedius*.

Key words: Milk, enzyme, lactoperoxidase, LPO, antibacterial, antifungal.

INTRODUCTION

The immune systems of organisms are not fully developed at the beginning of their life; therefore, organisms cannot protect themselves from pathogens. Milk contains some essential antimicrobial factors such as lactoferrin, lysozyme, immunoglobulins and lacto-peroxidase (Elagamy et al., 1992). Lactoperoxidase (LPO) (donor: hydrogen peroxide oxidoreductase E.C.1.11.1.7) is one of the prominent enzymes in milk with oxidoreductase activity. This enzyme has a crucial role in the protection of the lactating mammary gland and the intestinal tract of newborn infants against pathogenic microorganisms (Ueda et al., 1997). Several studies have reported that LPO is a glycoprotein which is present in milk, saliva and tears (Dumonte and Rousst, 1983; Morin et al., 1995; Shin et al., 2000). LPO consists of a single polypeptide chain containing 612 amino acid residues, whereas the molecular weight of bovine LPO is about 80 kDa (Paul and Ohlsson, 1985; Cals et al., 1991). It contains 15 half-cysteine residues and carbohydrate moieties that comprise about 10% of the molecular weight (De-Wit et al., 1996; Sisecioglu et al., 2009, 2010).

It was demonstrated that lactoperoxidase constitutes one of the defense systems for organisms and its components system include lactoperoxidase, thiocyanate and hydrogen peroxide. The generation of H_2O_2 by activated phagocytes is known to play an important part in the killing of several bacterial and fungal strains

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(Gülçin, 2007, 2009, 2010; Gülçin et al., 2006, 2009). Biological systems can produce hydrogen peroxide (Gülçin, 2006a, 2006b), in that the enzyme catalyzes the oxidation of endogenous thiocyanate (SCN^-) by H_2O_2, thereby producing antibacterial hypothiocyanate ($OSCN^-$) (Kumar and Bhatla, 1995). However, much of the research has focused on bacterial inhibition. LPO isolated from goat and camel milk was found to be antibacterial even in the absence of a medium containing thiocyanate-H_2O_2 (Jacob et al., 1998, 2000). Some studies have shown that lactoperoxidase has antifungal activity, for instance, goat milk lactoperoxidase inhibited different fungal species (Sisecioglu et al., 2009; Jacob et al., 1998, 2000; Lenander-Lumikari, 1992; Popper and Knorr, 1997).

In dairy cows, some bacteria (that is, *Staphylococcus spp., Streptococcus spp*.) cause mastitis diseases, which brings about an important economic problem for the dairy industry around the world. Lactoperoxidase content of bovine milk is higher than that of other mammals' milk. The LPO system destroys the cell wall of the bacterium that proliferates in milk and thus, contributes to immunological defense and protects the mammary gland (Sandholm and Korhonen, 1995; Owens et al., 1997). The present study was carried out to determine the effects of different concentrations of thiocyanate-H $_2O_2$ medium on antibacterial and antifungal properties of purified LPO. Some of the bacteria and fungi used in this study were found to cause bovine mastitis diseases.

MATERIALS AND METHODS

Materials

Raw cows' milk was obtained from the Veterinary Faculty of Ataturk University (Erzurum, Turkey). The following chemicals were used: Sephadex G-100, ammonium sulfate, sodium acetate, CM-Sephadex C-50, sodium phosphate, 2,2 -azino-bis(3-ethylbenz-thiazoline-6 sulfonic acid) diammonium salt (ABTS), 3x30 cm column, NaCl, Coomassie Brilliant Blue R-250, standard proteins (Bovine Carbonic Anhydrase, bovine albumin, rabbit phosphorylase B and rabbit muscle myosin) (Sigma Aldrich, Steinheim, Germany) and Amberlite CG-50 resin (Fluka, Buchs, Switzerland). SDS-PAGE was performed using a Mini-Protean 3 system® (Bio-Rad, München, Germany).

Purification of LPO

Bovine milk was centrifuged (Heraus Sepatech Suprafuge 22, Hanau, Germany) at 1135xg at 4°C for 15 min to remove fat. Amberlite CG 50 (NH^{4+}) Resin (equilibrated with 5 mM sodium acetate pH 6.8) was added in the proportion of 22 g/L to the fresh raw skimmed bovine milk (Cals et al., 1991; De-Wit et al., 1996; Khmelnitsky et al., 1998; Sisecioglu et al., 2009, 2010). The supernatant was decanted and the resin was washed with distilled water and 20 mM sodium acetate at pH 6.8. The bound protein was eluted with 0.5 M sodium acetate pH 6.8. To the green-colored mixture, solid ammonium sulfate was gradually added (I. precipitation; saturation 90%) over a period of 30 min, while it was being stirred magnetically and the enzyme solution was dialyzed overnight against 5 mM sodium phosphate buffer at pH 6.8. The clear greenish supernatant obtained as described above was loaded onto a column of CM Sephadex C- 50, previously equilibrated with 10 mM sodium phosphate buffer (pH 6.8). The column-bounded enzyme was washed with 100 ml of 10 mM phosphate buffer at pH 6.8 containing 100 mM NaCl. The enzyme was eluted with a linear gradient of 100 - 200 mM NaCl in 10 mM phosphate buffer at pH 6.8 and was subjected to ammonium sulfate precipitation (II, saturation 90%). Subsequently, the enzyme solution was dialyzed overnight against 5 mM sodium phosphate buffer at pH 6.8. Lactoperoxidase enzyme obtained from the CM Sephadex C-50 column was applied to a column of Sephadex G 100. The column-bound enzyme was eluted with 0.1 M phosphate buffer at pH 6.8 and was salted out with ammonium sulfate precipitation (III, 90% saturation). The enzyme solution was dialyzed overnight against 0.5 M sodium phosphate buffer at pH 6.0. Fractions were lyophilized and checked for purity by SDS-PAGE.

SDS-PAGE

SDS-PAGE was performed under denaturing conditions after LPO purification, according to the Laemmli's procedure (1970) described previously (Beydemir et al., 2003; Gülçin et al., 2004; Beydemir and Gülçin, 2004; ArasHisar et al., 2004; Aras et al., 2005) . The stacking and running gels comprised 3 (w/v) and 10% (w/v) acrylamide, respectively and 0.1% (w/v) SDS. The electrode buffer was 0.025 M Tris/0.2 M glycine (pH 8.3) . The sample buffer was prepared by mixing 0.65 mL of Tris-HCl (1 M, pH: 6.8), 3 mL of 10% (w/v) SDS, 1 mL of neat glycerol, 1 mL of 0.1% (w/v) bromphenol blue, 0.5 mL of -mercaptoethanol and 3.85 mL water. A 20 mg aliquot of enzyme (50 mL) was added into 50 mL sample buffer and the mixture was heated in a boiling water bath for 3 min and cooled (Hisar et al., 2005; Gülçin and Yıldırım, 2005; Beydemir et al., 2005).

LPO sample was loaded into each space of the stacking gel and was analyzed separately by polyacrylamide gel electrophoresis. Initially, an electric potential of 80 V was (Hoefer Scientific Instruments SE 600) applied until the bromphenol dye reached the running gel, then it was increased to 200 V for 3 - 4 h. However, gels were stained for 1.5 h in 0.1% (w/v) Coommassie Brilliant Blue R-250, 50% (v/v) methanol and 10% (v/v) acetic acid, and distained with methanol/acetic acid (Sisecioglu et al., 2009, 2010).

Determination of LPO activity

Lactoperoxidase activities were determined by the procedure of Shindler and Bardsley with a slight modification and one unit of activity is defined as the amount of enzyme catalyzing the oxidation of 1 µmol of ABTS min^{-1} at 298°K (molar absorption coefficient; 32400 M^{-1} cm^{-1}) (Ozdemir et al., 2001; U uz and Ozdemir, 2005; Sisecioglu et al., 2009, 2010). Protein concentration was determined according to the method of Lowry and co-workers (1951). UV-VIS [CHEBIOS (s.r.l.) Optimumone, Roma, Italy] spectrophotometer was used for the detection of LPO activity and the determination of protein concentration.

Kinetic studies

The kinetic parameters are used to study enzymes extensively (Coban et al., 2009; Öztürk Sarıkaya et al., 2010; Innocenti et al., 2010). To obtain K_m and V_{max} values at pH 6, the enzyme activity was measured at 20°C 412 nm for five different substrate concentrations. For this purpose, 0.2, 0.5, 0.8, 1.1 and 1.5 ml volume from stock solution of the substrates were used. A total volume of 2.8 ml was achieved with an appropriate buffer solution

of the enzyme activity and then, 0.1 ml enzyme and 0.1 ml H_2O_2 were added. To calculate K_m and V_{max} values, one unit of activity was defined as the amount of enzyme catalyzing the oxidation of 1 µmol of ABTS min^{-1} at 25°C and then, V_{max} and K_m values were calculated by the Lineweaver-Burk graph (1934) that was previously described (Gülçin et al., 2004, 2005; Çoban et al., 2007, 2008; Köksal and Gülçin, 2008; entürk et al., 2008, 2009).

Antifungal and antibacterial studies

Antibacterial and antifungal activity of bovine lactoperoxidase was determined by disk diffusion method using Mueller -Hinton Agar Medium with slight modification (Jacob et al., 2000; Chen et al., 2003; Gülçin et al., 2003, 2004, 2008) and was compared to the well-known antibacterial and antifungal substances such as cefaclor, erythromycin, tetracycline and fluconazole. For LPO (200 µL of 100 mM KSCN, 100 µL of 100 mM H_2O_2 and 200 µl of LPO dissolved in phosphate buffer pH 6.8), (200 µL of 50 mM KSCN, 100 µL of 50 mM H_2O_2 and 200 µl of LPO dissolved in phosphate buffer pH 6.8), (200 µL of 50 mM KSCN, 100 µL of 20 mM H_2O_2 and 200 µl of LPO dissolved in phosphate buffer pH 6.8) and (200 µL of 10 mM KSCN, 100 µL of 10 mM H_2O_2 and 200 µl of LPO dissolved in phosphate buffer pH 6.8) were transferred into sterile test- tubes, respectively. The mixture was allowed to react for 1 min and 30 µL of each mixture was added into sterile disks. Filter-sterilized protein was used in the reactions. Control disks were employed for denatured LPO instead of native LPO, while other conditions were maintained. Filter-sterilized protein was denatured by using a boiling water bath for 1 h.

For the antibacterial substances, BBL Sensi-Disks Antimicrobial Susceptibility test disks containing 30 mcg/disk of cefaclor, 15 mcg/disk of erythromycin, 30 mcg/disk of tetracycline and antifungal substances and 25 mcg/disk of fluconazole disk were used and incubated for 16 - 24 h at 37°C. The bacterial and fungal strains used were obtained from the Veterinary Faculty of Atatürk University. Antibacterial action of bovine LPO-thiocyanate-hydrogen peroxide system, cefaclor, erythromycin and tetracycline against *Citrobacter freundii, Streptococcus pneumoniae, Staphylococcus epidermidis* and *Staphylococcus intermedius* and antifungal action of bovine LPO-thiocyanate-hydrogen peroxide system**,** fluconazole against *Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis* and *Saccharomyces boulardii* were determined for bovine LPO. According to the Clinical and Laboratory Standards Institute, antibacterial zone interpretive criteria were: inhibition zone for cefaclor < 13 mm, which was considered to be resistant, while zone of inhibition for cefaclor between 15 - 17 mm was considered as slightly sensitive (intermediate) and > 19 was considered as sensitive (susceptible). Zone of inhibition for erythromycin < 12 mm was resistant, while erythromycin between 14 - 17 mm was intermediate and > 19 was susceptible. Inhibition zone for tetracycline < 13 mm was resistant, between 15 - 18 mm was intermediate and > 20 was susceptible. Fluconazole inhibition zone interpretive criteria were resistant for < 12 mm, intermediate for 13 - 18 and susceptible for > 20 (Meis et al., 2000).

RESULTS AND DISCUSSION

Lactoperoxidase is a natural bacterial defense system through the oxidation of thiocyanate ions (SCN^-) by H_2O_2. These are both present in biological fluids, and together with lactoperoxidase, they are termed the lactoperoxidase System (LPS). LPS has proven to be both bactericidal and bacteriostatic to a wide variety of microorganisms, while having no effect on the proteins and enzymes of the organisms producing it (Hannuksela et al., 1994) and is a naturally occurring, antimicrobial mechanism found in raw milk (Haddain et al., 1996). The mechanism of action of the lactoperoxidase system has been explained in detail by DeWit and Hooydonk (1996). LPO is more active at acidic pH levels, but is less stable under acidic conditions (Wever et al., 1982) . However, it can exert antibacterial and antifungal effect on both bacteria and fungi (Jacob et al., 2000; Lenander -Lumikari, 1992; Popper and Knorr, 1997). Its system in bovine milk has been well established and it has been reported that activation of the system depends on the concentration of thiocyanate (SCN) and hydrogen peroxide (H_2O_2) . LPO system has the ability to catalyze the oxidation of SCN by H_2O_2 with the production of antibacterial hypothiocyanate ($OSCN^-$) (Reiter, 1985; Ozdemir et al., 2002; Sisecioglu et al., 2009, 2010). Lactoperoxidase has been extensively used in biochemistry as a means to radioiodinate protein model of thyroid (U uz and Ozdemir, 2005), as well as in dairy industry to preserve raw milk during transportation and processing in chemical industry (Kumar and Bhatla, 1995). LPO enzyme has been purified and characterized from different sources and species (Morin et al., 1995; Shin et al., 2000; U uz and Ozdemir, 2005). It was reported that most pathogenic bacteria are inhibited by a medium containing thiocyanate-H_2O_2. LPO-thiocynate-H_2O_2 system is an effective agent against many of the diseases caused by organisms in plants and animals (Jacob et al., 1998, 2000).

The lactoperoxidase system is a naturally occurring, antimicrobial mechanism found in raw milk (Sisecioglu et al., 2009, 2010). LPO can exert antibacterial and anti-fungal effect on both bacteria and fungi (Lenander-Lumikari, 1992; Popper and Knorr; 1997; Jacob et al., 2000), in that its system in bovine milk has been well established and it has been reported that activation of the system depends on the concentration of thiocyanate (SCN) and hydrogen peroxide (H_2O_2) . LPO system has the ability to catalyze the oxidation of SCN by H_2O_2 with the production of antibacterial hypo-thiocyanate ($OSCN^-$). However, LPO enzyme has been purified and character-rized from different sources and species (Morin et al., 1995; Shin et al., 2000). It was reported that most pathogenic bacteria are inhibited by a medium containing thiocyanate-H_2O_2. LPO-thiocynate-H_2O_2 system is an effective agent against many of the diseases caused by organisms in plants and animals (Jacob et al., 1998, 2000).

As shown in Table 1, specific activity was calculated for crude extract and purified enzyme solution, yielding a purification of 15.8 fold and 8.4 mg (R_z: 0.8) was obtained from 1 L bovine milk. Kinetic parameters such as optimum pH, K_m and V_{max} were calculated from graphics of ABTS substrate on LPO. Also, radical form of ABTS was extensively used as a radical agent (Gulcin et al., 2006a, 2006b, 2010; Ak and Gulcin, 2008; Koksal and Gulcin, 2008; Koksal et al., 2009; Talaz et al., 2009). Optimum pH value was found to be 6 by means of activity-pH

Table 1. Purification steps of lactoperoxidase (LPO) from bovine milk.

Purification steps	Total volume (mL)	Enzyme activity (EU/mL)	Total enzyme activity (EU)	Protein (mg/mL)	Total protein (mg)	Specific activity (EU/mg)	Yield (%)	Purification fold
Homogenate	230	2.56	588.8	1.57	361.1	1.63	100	1.00
Ammonium sulfate precipitation	35	14.3	500.5	2.35	82.3	6.09	85.00	3.73
CM-Sephadex C-50 column chromatography	265	1.68	445.2	0.12	31.8	14.00	75.61	8.58
Ammonium sulfate precipitation	32	11.05	353.6	0.73	23.4	15.14	60.05	9.28
Sephadex G-100 column chromatography	180	1.58	284.4	0.088	15.8	17.95	48.30	11.01
Ammonium sulfate precipitation and dialyze	27	6.02	162.5	0.31	8.4	19.42	27.59	11.91

graphs, whereas K_m value at optimum pH was 0.25 mM and V_{max} was 7.95 µmol/mL.min. All purification steps were controlled by SDS-PAGE (Figure 1).

Bovine LPO-H_2O_2 (100 mM)-thiocyanate (100 mM) system showed much larger inhibition zones against bacteria and fungi than bovine LPO-H_2O_2 (50 mM)-thiocyanate (50 mM), LPO- H_2O_2 (50 mM)-thiocyanate (20 mM) and LPO- H_2O_2 (10 mM)-thiocyanate (10 mM) system (Tables 2 and 3).

Lactoperoxidase was purified from different milk sources such as goat, bovine, buffalo and camel and has shown antibacterial and antifungal effects with thiocyanate-H_2O_2 medium on different bacterial and fungi, for example, *Aeromonas hydrophila, Citrobacter freundi, Escherichia coli, Klebsiella pneumoniae, Proteus mirablis, Pseudomo Shigella dysenteries nas aeruginose, Salmonella enteritidis, Salmonella schotmuelleri, Salmonella typhi, Serratia marcescens, Shigella sonnei, Klebsiella oxytocica, Staphylococcus aerogenes, Streptococcus faecalis, Mycobacterium smegmatis CCM 2067*, *Shigella dysenteries, Aspergillus niger, Pencillium chrysogeum, Aspergillus flaws, Alternaria sp., Trichoderma sp., Corynespora cassiicola, Phytopthora meadii, Claviceps sp.* and *Corticium salmonicolor* (Elagamy et al., 1992; Jacob et al., 2000; Ozdemir et al., 2002; Uguz and Ozdemir, 2005). In another study, lactoperoxidase was isolated from goat milk and its antibacterial properties were investigated to explore the possibilities of using it as an agent to help fight against diseases (Jacob et al., 1998). Uguz and co-workers showed that bovine lactoperoxidase exhibited antibacterial effects on different pathogenic bacteria at different concentrations of thiocyanate medium (2005). However, there is no detailed study on pathogenic fungi with different concentrations of thiocyanate mediated bovine LPO.

Cefaclor, erythromycin, tetracycline and fluconazole are well known as antibacterial and antifungal substances. As shown in Table 3, erythromycin has inhibitory effects on *Staphylococcus epidermidis* and *Staphylococcus intermedius*, yet, inhibition zone of erythromycin is weaker

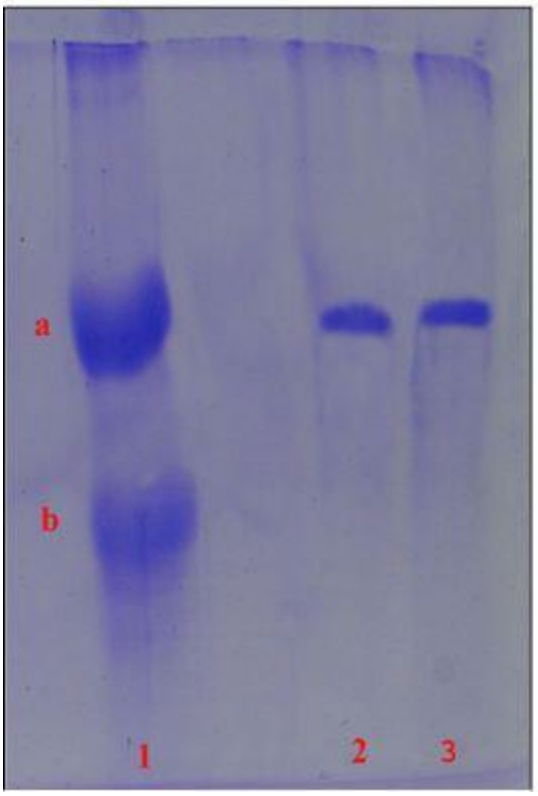

Figure 1. SDS-PAGE bands of LPO. Line 1: Standard proteins; a: Bovine albumin (66 kDa, Sigma, Lot 032K9285), b: Egg albumin c (45 kDa; Sigma, Lot 092K9284). Line 2: Purified LPO from Bovine milk (80 kDa). (SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis, LPO: Lactoperoxidase enzyme).

Table 2. Inhibition zones of LPO-thiocyanate-H_2O_2 system against some bacteria by disk diffusion method.

	Diameter of inhibition zone*							
	LPO-system					Antibacterial substances		
Microorganisms	I[a]	II[b]	III[c]	IV[d]	V[e]	CEC[,]	E[,]	TE[,§]
Citrobacter freundii	14	10	10	-	-	17	-	33
Escherichia coli	16	13	-	-	-	-	-	24
Streptococcus pneumoniae	25	21	20	20	-	32	30	34
Staphylococcus epidermidis	15	12	-	-	-	26	13	23
Staphylococcus intermedius	16	11	11	-	-	18	15	26

* Inhibition zone in diameter around the discs (mm).
: They were used as positive reference standards antibiotic discs (Oxoid).
[a]**:** 100 mM KSCN-100 mM H_2O_2,
[b]**:** 50 mM thiocyanate-50 mM H_2O_2,
[c]: 20 mM thiocyanate-50 mM H_2O_2,
[d]: 10 mM KSCN-10 mM H_2O_2,
[e]**:** Control values (Denatured LPO),
: 30 mcg/disk of cefaclor,
: 15 mcg/disk of erythromycin,
[§]**:** 30 mcg/disk of tetracycline,
: 0.9 μg/disk of LPO.

Table 3. Inhibition zones of LPO-thiocyanate-H_2O_2 system against some fungi shown by disk diffusion method.

	Diameter of inhibition zone*					
	LPO-system					Antibacterial substances
Microorganisms	I[a]	II[b]	III[c]	IV[d]	V[e]	F[,]
Candida albicans	11	-	-	-	-	14
Candida glabrata	14	11	11	10	-	17
Candida krusei	12	10	10	-	-	23
Candida parapsilosis	12	11	-	-	-	19
Saccharomyces boulardii	17	14	10	10	-	27

(I: 100 mM KSCN-100 mM H_2O_2, II: 50mM thiocyanate-50 mM H_2O_2, III: 20 mM Thiocyanate-50 mM H_2O_2, IV: 10 mM KSCN-10 mM H_2O_2, V: Control (Denatured LPO).
* Inhibition zone in diameter around the discs (mm).
: It was used as positive reference standards anticandidal disc (Oxoid).
[a]**:** 100 mM KSCN-100 mM H_2O_2,
[b]**:** 50 mM Thiocyanate-50 mM H_2O_2,
[c]: 20 mM Thiocyanate-50 mM H_2O_2,
[d]: 10 mM KSCN-10 mM H_2O_2,
[e]**:** Control values (Denatured LPO),
: 25 mcg/disk of Fluconazole,
: 0.9 μg/disk LPO.

than 100 mM bovine LPO system for *Staphylococcus epidermidis* and *Staphylococcus intermedius*. Bovine LPO system exhibits inhibition property on *Citrobacter freundii* and *Escherichia coli*; however, *erythromycin* does not have any effects on *Escherichia coli* and *Citrobacter freundii,* while cefaclor does not have any effects on *Escherichia coli.* All of the fungi are inhibited by bovine LPO system (Table 3). This result demonstrated that immune systems of organisms are resistant against bacteria and fungi. Some research has demonstrated that the LPO system purified from different sources inhibited different strains of *E. coli.* For example, goat milk showed 20 mm inhibition zone, buffalo lactoperoxidase showed 17 mm inhibition zone (Jacob et al., 2000; Ozdemir et al., 2002) and bovine LPO exhibited similar results.

Meis and co- workers reported that fluconazole has inhibition effects on *Candida strains* (Meis et al., 2000). Fluconazol inhibited 99% of *C. albicans* (in 14.368 isolates), 94% of *C. parapsilosist* (in 752 isolates), 90% of *C. tropicalis* (in 869 isolates), 67% of *C. glabrata* (in 2.073 isolates) and 26% of *C. krusei* (in 351 isolates). The present study showed that fluconazole is an efficient antibiotic and inhibited all of the fungi. The LPO system also exhibited the same property; nevertheless, the effect of the LPO system is weaker than that of fluconazole.

In this study, LPO was purified from bovine milk and its

antibacterial and antifungal properties were investigated against some bacteria and fungi. Different concentrations of bovine LPO-H_2O_2-thiyocyanate system were shown to be sensitive against certain bacteria and fungi and their sensitivity were equal or close to that of cefaclor, erythromycin, tetracycline and fluconazole substances. It was known that the action of LPO against bacteria is caused by sulfydryl (-SH) oxidation. The oxidation of -SH groups in the bacterial cytoplasmic membrane results in loss of the ability to transport glucose and also in leaking of potassium ions, amino acids and peptides.

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