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Comparison of antibiotics and bacteriocins antibacterial activity on *Xanthomonas citri subsp.citri*

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Abstract

Citrus canker is a citrus disease species created by the bacterium *Xanthomonas citri subsp.citri*. Many citrus, such as oranges, lime, and grapefruit are affected by the infectious bacteria and stems, leaves and fruits are experiencing loss. In this experiment antibacterial effects of five kinds of antibiotics including ampicillin, kanamycin, chloramphenicol, penicillin, streptomycin, Cronobacteriocin DGH2 and Enterobacteriocin DGH4 were evaluated on 107 strains of *Xanthomonascitri subsp.citri*. MIC and MBC data for antibiotics and bacteriocins against Xanthomonas strains were performed. According to this project, Xanthomonas strains were comparatively susceptible and resistant to Cronobacteriocin DGH2, Enterobacteriocin DGH4, ampicillin, kanamycin, chloramphenicol, penicillin and streptomycin. NIGEB-183 strain is the most sensitive to these antibiotics and bacteriocins. However, only the NIGEB-242R1 strain is resistant to chloramphenicol. Penicillin has minimum inhibitory effects on Xanthomonas strains. Based on this case study, chloramphenicol is the most antibacterial activity among antibacterial agents and this compound is a good candidate for inhibitory activity. Cronobacteriocin DGH2 has a moderate antibacterial activity against Xanthomonas strains.

Keywords: Antibiotics, Bacteriocins, Citrus canker, MIC and MBC data, *Xanthomonas citri subsp.citri*

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Introduction

Citrus canker is a pandemic disease that led to a severe economic downturn in the citrus industry (Gottwald *et al.,* 2009; Behlau *et al.,* 2010; Chen *et al.,* 2012). Disease symptoms consist of water soggy ulcers that develop into band scorch, then pimples and finally, canker. Citrus canker can lead to defoliation and immature fruit fall, in severe cases (Brunings and Gabriel, 2003). The method of choice to control the disease is sup plantation of putrefied plants where it is not yet endemic. In cases where the disease is endemic, control is attempted by seeding trees that are not patient, limiting the extension between orchards, and using preventive copper sprays (Graham *et al*., 2004). However, none of these actions control citrus canker efficiently (Leandro *et al*., 2010). Antibiotics are the most successful family of medicines to protect human health and plants (Stepanauskas, 2006). Because of the emergence and dissemination of antibiotic resistance and their association with continuous use of antibiotics, therapies based on antimicrobial peptides are attractive candidates as valid alternatives to antibiotic treatments (Chen *et al*., 2012). Bacteriocins are bacterial ribosomally synthesized and extracellular peptides or proteins with an antibacterial activity usually against bacteria intimately related to the producer (Morisset and Frère, 2002; Opsata *et al*., 2010; Castro *et al*., 2011; Tashakor *et al.,* 2017). These antimicrobial therapies offer additional advantages over drug therapies currently used, because bacteriocins are considered as natural bioactive compounds. Multiple aspects discern bacteriocins from antibiotics; (i) Bacteriocins are produced on the surface of ribosomes in microbial cells, while antibiotics are primarily secondary metabolites of the cell; (ii) Bacteriocin producers are insusceptible to the bactericidal agents, unlike producers of antibiotics; (iii) Bacteriocins can attach to the target cell wall anyplace on the surface, as no specific receptors on the target cell wall apparently exist; (iv) The mechanism of bacteriocin on target cells is diverse and is associated with the method of pore formation in the outer cell membrane. Bacteriocins bind to cell walls of sensitive microbes, motive ionic imbalances, and produce pores (Morisset and Frère, 2002). Inorganic ions leak the target cells through the created pores and thereby killing them. Antibiotics, on the other hand, can inhibit synthesis of the subcellular processes (cell wall synthesis, intracellular protein production, and DNA and RNA replication) (Svetoch *et al*., 2011). Their bactericidal and bacteriostatic mechanisms are diverse and may comprise pore formation, degradation of cellular DNA, disruption via specific cleavage of 16S rRNA, and blockage of peptidoglycan synthesis (Todorov *et al*., 2011a). In this study, the inhibitory effect of both Cronobacteriocin DGH2 and

Enterobacteriocin DGH4 also antibiotics including ampicillin, kanamycin, chloramphenicol, penicillin, and streptomycin over 107 strains of Xanthomonas were examined and the inhibitory power of antibacterial agents were compared with each other on Xanthomonas. Diffusion assays used to determine the sensitivity of organisms isolated from clinical samples have their limitations; when two-sided outcomes are obtained, the quantitation of antibiotic activity needs to be more precise versus the pathogen. As well as the designation 'Resistant' and 'Susceptible' may have a realist explanation. So until in doubt, detailed assessment method is to determine the MIC of the antibiotic to the organisms. In addition, the identical tubes which can be used to test the MBC as well have the added value benefit (Adel *et al.,* 2015).

Materials and methods

Bacterial strains and media

Two strains of *Cronobacter sp.* DGH1 and *Enterobacter sp.* DGH3 bacterium were isolated from citrus orchards in Sistan and Baluchestan region and was screened for bacteriocin production against indicator strains *Xanthomonas citri subsp.citri* following previously described method (Gholami *et al.*, 2014; Gholami et al., 2014b; Gholami *et al.*, 2015). All the producer and indicator strains were cultured in Nutrient Broth (Maryam *et al.*, 2011; Pragalaki *et al.*, 2013) and Nutrient Agar (Foltz and Martin, 1938) medium. The bacteria against which the antimicrobial spectrum of Cronobacteriocin DGH2 and Enterobacteriocin DGH4 was studied are enlisted in Table 1. All the bacteria were procured from bacterial collection of NIGEB, Iran.

Bacteriocin production

Nutrient broth was inoculated with an 18h culture (2%, v/v) of bacterial strains and moved to shaker incubator with 180 rpm at 37ºC (Svetoslav *et al.*, 2010). After cultivation of bacteria, cells were removed by centrifugation (10,000×g for 30min). The supernatant was filtered through a center glass filter with 0.2µm, pore size (Minisart, 17597, Sartorius, Germany). The bacteriocin present in the cell free supernatant fraction by Amicon® ultra-15 PL-5 was concentrated.

Bacteriocin enzymatic digestion test

Culture supernatant was incubated at 37ºC for 1h in the presence of various enzymes (proteinase K, trypsin, papain, pepsin) at a final concentration of 1 mg mL^{-1} and 10 mL of each supernatant was spotted onto soft agar inoculated with 250 µL of 0.5 Mac Farland pre-cultivated target strains. The plates transferred at 32ºC and inhibition zones were recorded.

Table 1: Antimicrobial[1] agents effect on *Xanthomonas citri subsp.citri strains*.

Sample	AMP	KAN	CHL	PEN	STR	CRO	ENT
NIGEB-26	11[2]	12	15	8	10	5	7
NIGEB-88	9	12	18	9	10	5	3
NIGEB-201	8	10	13	8	7	2	2
NIGEB-88R	6	10	12	10	6	5	1
NIGEB-197	4	10	8	6	10	4	3
NIGEB-112	2	11	13	7	4	2	2
NIGEB-3369	4	11	10	6	10	7	8
NIGEB-204	9	10	13	8	9	2	2
NIGEB-K32	4	10	6	6.5	8	11	7
NIGEB-196	2	4	6	3.5	3	-	-
NIGEB-126	5	7	9	5	8	3	1
NIGEB-170	6	-	5	7	5	-	-
NIGEB-142	-	-	5	-	2.5	1	-
NIGEB-242R1	2.5	5	-	2.5	3.5	2	-
NIGEB-87	5	5.5	8	7	6	4	1
NIGEB-224	5	6	7	5	7	-	-
NIGEB-52	6	5	8	7	6	2	-
NIGEB-162	4	3.5	6	4	5	-	-
NIGEB-262sc1	5	3.5	7	4.5	5	1	4
NIGEB-260R2	5	5	7	5	4	4	2
NIGEB-157	4	5	8	7	5	-	-
NIGEB-286R1-2	4	2.5	6	5	4	-	-
NIGEB-243R1	4	5	8	5	5.5	-	1
NIGEB-244R1	5	3	7	3.5	3	-	-
NIGEB-147-1	5	4	6	3	3.5	2	-
NIGEB-153	4	5	9	6	5	-	3
NIGEB-249R2	3	4	7	6	7	4	2
NIGEB-136	2	2.5	5	-	3	-	-
NIGEB-213R2	6	5	8	8	6	3	1
NIGEB-54	6	5	11	10	8	-	-
NIGEB-7	5	6	10	8	7	2	-
NIGEB-211R1-1	5	-	6	9	5	4	-
NIGEB-247R1	5	6	6	6	4	2	-
NIGEB-10	6	6	9	8	7	-	-
NIGEB-53	6	8	9	8	9	2	2
NIGEB-313R1	7	6	10	8	8	-	-
NIGEB-232	7	8	10	8	9	-	-
NIGEB-96	2.5	3.5	7	-	4	3	2
NIGEB-246	8	7	9	8	10	2	1
NIGEB-13	5	12	18	9	12	-	1
NIGEB-180	7	5	16	-	10	2	3
NIGEB-186	9	6	17	7	6	2	3
NIGEB-259R1	9	11	15	9	8	-	2
NIGEB-75	8	6	14	10	8	3	-
NIGEB-79	8	16	15	5	11	-	-
NIGEB-22	12	8	18	10	8	4	-
NIGEB-264R1	6	7	16	3	8	3	3
NIGEB-94	11	10	20	10	4	2	1
NIGEB-112	4	4	6	4	-	-	-
NIGEB-81	5	7	11	4	3	-	-
NIGEB-287R2	8	5	10	11	5	6	5
NIGEB-138	3	4	11	6	6	3	3
NIGEB-227	9	5	12	11	5	1	-
NIGEB-36	6	7	11	5	6	2	3
NIGEB-16	10	11	17	8	13	5	3
NIGEB-168	10	15	20	14	14	4	-

Sample	AMP	KAN	CHL	PEN	STR	CRO	ENT
NIGEB-169	6	12	18	12	15	2	2
NIGEB-65	8	11	16	-	10	4	1
NIGEB-173	17	7	13	20	4	-	-
NIGEB-98	2	4	13	3	11	4	5
NIGEB-127	11	10	18	10	2	2	1
NIGEB-184-1	15	7	10	20	10	2	3
NIGEB-184-2	-	6	7	-	-	-	-
NIGEB-122	7	2	13	4	10	-	-
NIGEB-134	5	9	12	5	10	1	2
NIGEB-125	7	2	12	8	13	7	4
NIGEB-71	10	9	15	13	8	-	-
NIGEB-116	2	-	6	-	-	-	-
NIGEB-263R1	5	8	11	-	9	-	-
NIGEB-195	3	10	11	4	10	-	3
NIGEB-90	-	3	10	-	5	4	2
NIGEB-44	3	9	13	-	14	3	2
NIGEB-113	-	9	10	5	11	-	2
NIGEB-78	7	8	15	-	2	-	-
NIGEB-74	5	7	15	6	7	5	-
NIGEB-187	6	7	15	4	-	-	-
NIGEB-183	10	9	27	8	10	1	1
NIGEB-97	11	6	13	10	5	8	-
NIGEB-135	10	11	7	6	4	2	-
NIGEB-101	6	9	18	-	10	-	-
NIGEB-107	10	10	20	10	7	-	4
NIGEB-84	4	7	16	4	13	4	4
NIGEB-119	10	6	15	4	8	4	-
NIGEB-131	4	8	12	5	5	-	-
NIGEB-104	11	7	20	15	13	4	3
NIGEB-9171	-	2	11	-	4	-	2
NIGEB-9179	3	5	12	3	4	2	3
NIGEB-2525	2	5	14	-	5	4	2
NIGEB-31	4	5	14	3	10	6	-
NIGEB-56	8	9	10	3	9	-	-
NIGEB-66	3	5	14	3	5	4	2
NIGEB-45	5	6	12	5	10	2	2
NIGEB-K56	7	5	14	5	12	4	2
NIGEB-261R2	9	5	10	9	5	2	-
NIGEB-288	9	5	10	9	5	4	-
NIGEB-92	8	9	16	10	14	3	1
NIGEB-151	12	7	15	11	10	4	2
NIGEB-233R2	7	6	13	5	7	4	4
NIGEB-155	9	14	20	12	16	9	10
NIGEB-242R2	22	10	5	5	6	2	4
NIGEB-38	3	11	12	5	9	-	5
NIGEB-41	3	4	11	4	10	2	4
NIGEB-761	-	5	12	3	9	4	-
NIGEB-108	3	7	14	6	10	-	-
NIGEB-58	3	8	10	3	10	-	6
NIGEB-26	5	4	14	3	6	-	-
NIGEB-265	7	5	10	11	4	8	3

[1)] Abbreviation of mentioned antimicrobial agents are AMP, ampicillin; KAN, kanamycin; CHL, chloramphenicol; PEN, penicillin; STR, streptomycin; CRO, Cronobacteriocin DGH2 ; ENT, Enterobacteriocin DGH4.

[2)] Halo diameter in scale of mm.

Bacteriocin temperature resistance test

Considering that protein activity is reduced in the high-temperature; thermal diagnostic test for bacterial supernatant in the range of different temperatures was performed. Thus, after the bacterial supernatant obtained the amount of 50µL into single vials; each vial was placed in a hotplate with temperatures of 35, 45, 55, 75 and 100ºC for 30 minutes. 10 µL of each vial was transferred on antibiogram disks and disks placed on the plates that were inoculated with pathogenic bacteria and was incubated at 32ºC, then formation of halo was investigated after 24 hours. All samples were readjusted to pH 6.0 with sterile 1.0 M HCl or 1.0 M NaOH. In this experiment, unheated supernatant served as the control (Mathieu *et al*., 1994; Gao *et al.*, 2010).

Bacteriocins and antibiotics sensitivity determination

Bacterial strains of Xanthomonas were tested for sensitivity to the Cronobacteriocin DGH2 and Enterobacteriocin DGH4, and to some antibiotics such as; ampicillin, kanamycin, chloramphenicol, penicillin, and streptomycin. Initial testing was performed by the DD - AWDA[1] method as described by Gholami *et al.* (2013). For Xanthomonas strains, minimal inhibitory concentrations (MICs) were determined by the broth micro-dilution method in 96-well microtiter plates (Becton Dickinson Labware, Franklin Lakes, NJ) as described by the Clinical and Laboratory Standards Institute (CLSI) 2009. Mueller-Hinton Broth from Germany (Merck Company), ampicillin, kanamycin, chloramphenicol, penicillin, and streptomycin from USA (Sigma Company) were used in this experiment.

Preparation of stock dilution and broth dilution

The stock solutions are created in higher concentrations to preserve their qualities and afterward stored in aliquots at -20°C. Dilution range of some antibacterial that is needed for this research is shown in Table 2. Stock dilutions were prepared of the antibiotic in 1000 and 100 µg L^{-1} concentrations as required from primary stock solution (10,000 mg L^{-1}). A minimum of 1 mL of each dilution per vial is needed for the experiment. The antibacterial agent (antibiotic or bacteriocin) is diluted in sterile broth followed by mixed with broth inoculated with bacterial strains.

Preparation of broth microdilution

100 µL of antibacterial agent dilution are distributed into the microwell plates then inoculated with 5 µL of overnight broth of the test organism which were diluted to 1 in 1000 in an appropriate broth culture. The amount of the inoculums affected the outcome of the test significantly.

[1] Disc Diffusion – Agar Well Diffusion Assay

Table 2: Suggested antibiotic microdilution ranges for MIC and MBC Testing.

Antimicrobial agent	Concentration range (ug mL^{-1})
Penicillin G*	0.015 – 32
Ampicillin*	0.15 – 32
Chloramphenicol*	0.06 – 256
Tetracycline*	0.06 – 256
Streptomycin*	16 – 256
Sakacin DGH6	14 – 2500
Ciprofloxacin	0.15 – 32

*Source- (Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy, 1991).

Microwell plates were incubated for 18 hours at 37ºC and 500 µL of broth culture inoculated with the organism and kept at +4°C in a cold room overnight to be used as standard.

Determination of MIC by the broth micro dilution method

The Broth micro dilution procedure is one of the easiest and most convenient methods for assessment of a small number of isolates. (Domig *et al.*, 2007; Kushiro *et al.*, 2009). This method employs double-strength Mueller-Hinton broth and 4X strength antibiotic solutions prepared as serial two-fold dilutions. Also the test organism is at a concentration of 0.5 McFarland. A mixture of double-strength Mueller-Hinton broth (100 mL), the antibiotic dilutions (50 mL) and the target organism suspension were incubated for 18-24 h at 35ºC. The MIC of the antibacterial agents will be considered the lowest concentration showing inhibition of growth (Pfaller *et al.*, 2010).

Determination of MBC by the broth microdilution method

The 'Broth microdilution' method which was used in MIC determination can willingly be converted to determine the MBC as well (Bär *et al.*, 2009; Mandal *et al.*, 2010).

Reading of results

MIC is explicit as the minimum concentration of antibacterial agent inhibited growth arbitrage by lack of turbidity in the test tube. Standard strain of recognized MIC is applied to check the antibacterial activity and statements as the control.

Assay of activity and determination of antimicrobial spectrum

Antimicrobial substance (bacteriocin) produced by the strain was detected by DD - AWDA method. For DD - AWDA method, Nutrient agar plates (1.5% agar) were overlaid with 5 ml of soft agar (0.75% agar) inoculated with 100µL of an overnight culture of the indicator microorganism. The concentration of bacteria in the suspension was adjusted to 1.5×10^8 CFU/mL using Mc Farland's scale (0.5 McFarland) (Patel *et al.*, 2011; Nazemi

et al., 2017) and confirmed by a serial dilution plating method (Blodgett, 2005). 15µL of bacteriocin was placed in each disc and disc was moved on the top agar layer that had hardened. The plates were incubated for 24 h at 35°C and afterwards, zones of inhibition were measured.

Antibacterial activity of bacteriocins and antibiotics

Antibacterial effects of five types of antibiotics including ampicillin, kanamycin, chloramphenicol, penicillin, streptomycin, and Cronobacteriocin DGH2 and Enterobacteriocin DGH4 were evaluated on 107 strains of Xanthomonas (Jayapradha *et al.*, 2009) (Table 1).

Results

Bacteriocin enzymatic digestion identification test

As shown in Table 3, after treatment by proteolytic enzymes such as proteinase-K, trypsin and pepsin the antibacterial activity of secreted components was decreased by bacterial strains, but the inhibitory activity of cell free supernatant was not significantly diminished when treated with papainase. Catalase had no effect on the antimicrobial activity indicating that the inhibitory activity is not due to hydrogen peroxide production (results not shown). This result showed that these components are proteinaceous molecules, and probably bacteriocins (Todorov *et al.*, 2011a; Jiang *et al.*, 2012).

Bacteriocin temperature resistance identification test

Halo formation of the bacterial supernatant in the temperature range of 25, 45, 65, and 100°C was investigated. The zone formation at 25 and 45°C did not change but reduced at 65°C and there wasn't any zone at 120°C (Table 3). It can be deduced from this experiment that the antibacterial activity is factor that has a protein structure (Garver and Muriana, 1993; Todorov *et al.*, 2011b).

Bacterial susceptibility

Table 2 shows MIC data for antibiotics and bacteriocins against Xanthomonas strains. Based on this analysis, Xanthomonas strains were comparatively susceptible and resistant to Cronobacteriocin DGH2, Enterobacteriocin DGH4, ampicillin, kanamycin, chloramphenicol, penicillin and streptomycin. MBC data for these antimicrobial agents also was shown in Table 4.

Table 3: Stability of bacteriocins produced by *Cronobacter* sp. DGH1 and *Enterobacter* sp. DGH3 after treatment with enzymes, detergents, urea, heat, NaCl and pH.

Effector	Concentration	Enterobacteriocin DGH4*	Cronobacteriocin DGH2*
Proteinase K	1mg mL^{-1}	-	_
Pepsin	1mg mL^{-1}	-	_
Trypsin	1mg mL^{-1}	-	_
pH 3-6	3 h	+	++
pH 6-8	3 h	++	++
pH 8-10	3 h	+	+
Temperature 4, 55, 60, 85, 100 ºC	1 h	+	+
Temperature 25, 35,45 ºC	1 h	+	++
Temperature 121 ºC	20 min	-	_
Triton X-100, Tween 20, Tween 80	1% (v/v)	+	++
SDS, urea, NaCl, EDTA,	1% (w/v)	+	++
manganese sulphate	1% (w/v)	++	++

*Activity of bacteriocin was expressed in + = presence of inhibition zone (1-5 mm); ++ = presence of inhibition zone (5-16mm); − = no inhibition.

Table 4: Information about antimicrobial agents, break point, MIC and MBC range and antimicrobial resistance percentage for 107 samples of *Xanthomonas citri subsp.citri*.

Antimicrobial agent[1]	Breakpoint for resistance (µg mL^{-1})[2]	MIC range in isolates (µg mL^{-1})	MBC range in isolates (µg mL^{-1})	No. of isolates resistant to antimicrobial agents (%)
				Xanthomonas strains (n=107)
AMP	≥32	<184 - >390	<368 - >780	5.6
KAN	≥16	<115 - >340	<225 - >899	3.7
CHL	≥32	<90 - >257	<115 - >350	0.9
PEN	≥4	<315- >610	<315- >610	13.1
STR	≥64	<210 - >512	<504 - >1536	3.7
CRO	≥4	<400- >2048	<550- >3000	35.5
ENT	≥4/76	<182 - >390	<370 - >782	41.1
No. of multi-drug resistant (MDR) isolates [3]				2.8

1) Abbreviation of mentioned antibiotics are AMP, ampicillin; KAN, kanamycin; CHL, chloramphenicol; PEN, penicillin; STR, streptomycin; CRO, Cronobacteriocin DGH2; ENT, Enterobacteriocin DGH4

2) Breakpoints were adopted from CLSI (Clinical and Laboratory Standards Institute)

3) In this study isolates which were resistant to at least 4 groups of antimicrobial agents considered as MDR *Xanthomonas citri subsp.citri* strains

Bacterial resistance

Among the antimicrobials, most of Xanthomonas strains are resistant to Enterobacteriocin DGH4 (41.1%); While the lowest of strains are resistant to chloramphenicol (0.9%). Since the bacteriocin functional range is much smaller than antibiotics. Such results were quite predictable. Among types of antibiotics the most common bacterial strains resistant to the penicillin were shown. Penicillin eliminates gram-positive bacteria and Xanthomonas is the gram-negative bacterium, therefore most bacterial strains studied were resistant to penicillin. The inhibitory effect of this antibiotic is lower than other antibiotics (Table 4). MIC range of the isolates indicated that strains show the greatest sensitivity to

chloramphenicol and have the least sensitivity to the Enterobacteriocin DGH4, on the other hand; MBC range of this strain also gives similar results (Table 4). As can be seen in Table 2, number of multi drug resistance (MDR) strains (Kazem *et al.*, 2012) are included in 2.8% of the total monocytogenes.

Comparison of sensitivity and resistance to antibiotics

As can be seen in Table 1, chloramphenicol has the most potent inhibitory effect among antibacterial agents. NIGEB-183 strain is the most sensitive to these antibiotics by formed a halo with 27 mm diameter. Only NIGEB-242R1strain is resistant to chloramphenicol. Penicillin also has minimum inhibitory effects on Xanthomonas strains, and strains of NIGEB-142, NIGEB-136, NIGEB-96, NIGEB-180, NIGEB-65, NIGEB-184-2, NIGEB-116, NIGEB-263R1, NIGEB-90, NIGEB-44, NIGEB-78, NIGEB-101, NIGEB-9171 and NIGEB-2525 are resistant to this antibiotic, but strain of NIGEB-104 is the most sensitive to this antibiotic with a 27 mm zone diameter. Strains of NIGEB-142, NIGEB-761, NIGEB-90, NIGEB-761, NIGEB-90, NIGEB-113, NIGEB-9171 and NIGEB-184-2 are resistant to ampicillin while the strain of NIGEB-242R2 is the most sensitive to this antibiotic with a 22 mm zone diameter. Furthermore, strains of NIGEB-170, NIGEB-142, NIGEB-211R1-1 and NIGEB-116 are resistant to kanamycin while strain of NIGEB-79 is most sensitive to this antibiotic with a 16 mm zone diameter. Strains of NIGEB-187, NIGEB-184-2, NIGEB-116 and NIGEB-112 are resistant to streptomycin whereas NIGEB-155 strain is most sensitive to streptomycin with a 16 mm zone diameter. One example of antibiotic antibacterial activity was shown in Fig. 1.

Comparison of sensitivity and resistance of Xanthomonas strains to bacteriocins

Thirty eight strains are resistant to *Cronobacteriocin* sp. DGH2; NIGEB-K32 strain is the most sensitive to this bacteriocin with an 11 mm zone diameter. Forty seven strains are resistant to Enterobacteriocin DGH4 whereas NIGEB-155 strain is the most sensitive to this bacteriocin with a 10 mm zone diameter (Table 1). One example of bacteriocin antibacterial activity was shown in Fig. 1.

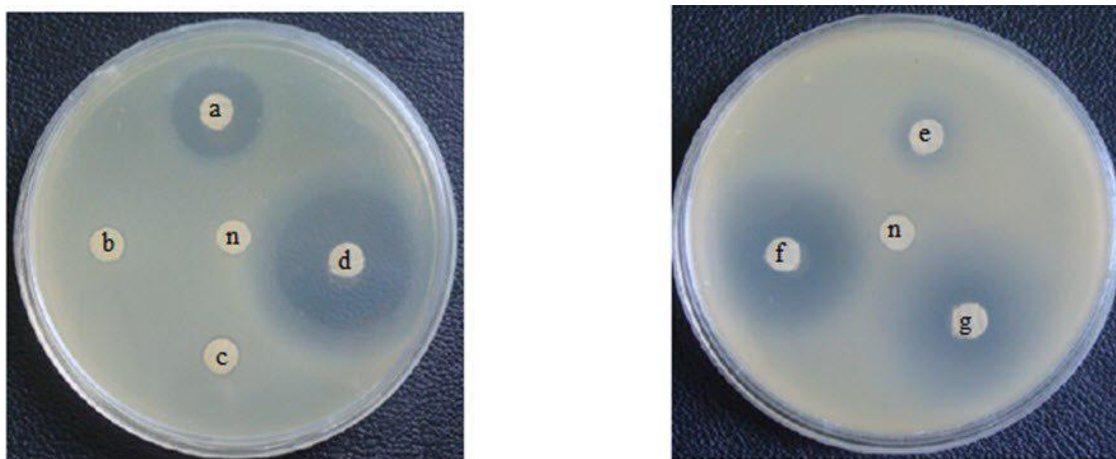

Figure 1: Antibacterial activity of antimicrobial agents that were examined in this study. a: ampicillin, b: penicillin, c: Enterobacteriocin DGH4, d: chloramphenicol, e: Cronobacteriocin DGH2, f: kanamycin, g: streptomycin, n: negative control. Plate surface inoculated with pathogenic bacteria (NIGEB-65 strain).

Discussion

Citrus is one of the most universal fruit crops with high economic and health value and is important in daily life (Pergola *et al.*, 2013). Citrus fruit is produced all over the tropic and subtropical areas of the world, where there is sufficient water, rich soil and adequate moisture for tree growth and fruit production. These natural products in addition to their use as a food source are also an important component with many pharmaceutical and healthcare applications (Gmitter and Hu, 1990).

The pathogen *Xanthomonas citri subsp. Citri* is a Gram-negative bacterium which is the main factor of citrus canker, a threatening disorder that affected citrus crops all around the world (Brunings and Gabriel, 2003; Paola *et al.*, 2011). Antibiotics were very important discovery in the healthy life of plants and animals, since the recognition of penicillin in 1928 as the first antibiotic, and come behined by it full-scale output (Bennett, 2001). Babes and Cornil found an antagonism in Staphylococcus genus in 1885 (Cornil *et al.*, 1885). Gratia first found bacteriocins in 1925 (Gratia, 1925). All these discoveries are confined to a few years and it is exposition the importance of bacteriocins. As the functional limited area of antibiotics is more than bacteriocins (Kheadr *et al.*, 2004; Pogány *et al*., 2010; Naghmouchi *et al.*, 2012), in this study, we attained a resembling conclusion and inhibitory effects of antibiotics on Xanthomonas strains is a wider span of bacteriocins, whereas activity of Xanthomonas strains was controlled by each bacteriocin was less than types of antibiotics. In this project the range of concentration of each antibiotic is from 256 µg mL^{-1} to 0.015 µg mL^{-1}. This range for bacteriocins is 14 to 2500 µg mL^{-1}. Studies have shown that the antibacterial effects of antibiotics on pathogenic bacteria have begun three

years ago and have contributed in the inhibition of microbial diseases and infections (Hayes G Fau-Moens *et al.*, 2013). Comparison of antibacterial effects and the range of antibiotics with bacteriocins has not been studied well (Kheadr *et al.*, 2004). On the other hand, comparison of antibacterial effects and the range of antibiotics with bacteriocins inhibiting the bacterial citrus canker disease has been examined for the first time in this project and no previous studies have been done in this context and on Xanthomonas pathogenic bacterium strains, so far. Considering that the best way to control citrus canker disease is the use of antibiotics, and pathogenic strains are sensitive to these antibiotics, in order to better control the disease by antibiotics, examination of the rate of bacterial resistance to these antimicrobial agents is useful. Knowing that these strains were inhibited by either antibiotic or how much antibiotics is needed to control bacteria is cost-effective economically, and saves antibiotics application. As mentioned above, most of the inhibitory effect among antibacterial agents is chloramphenicol, NIGEB-183 strain is the most sensitive to these antibiotics, and only NIGEB-242R1strain is resistant to chloramphenicol. Because Penicillin is an antibiotic inhibitor of Gram-positive bacteria, it was expected that it has a small anti-bacterial effect on the Xanthomonas strains as a Gram-negative bacteria. Bacteriocins have a narrow antibacterial spectrum to antibiotics; for this reason, strains that were resistant to bacteriocins were more. The study shows that the best antibiotics to inhibit strains of the pathogenic bacteria, is chloramphenicol. The required amount of this antibiotic to inhibit bacteria is less than other antibacterial agents. Moreover, the range of its antibacterial activity is greater than other antimicrobial agents.

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