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Evaluation of resistance to conventional antibiotics in medicine by 5 strains of lactic acid bacteria isolated from the stomach of honey bee

Sepideh Gharehyakheh[1], Amir HosseinElhamirad*, Leila Nateghi[2], Kambiz Varmira[3]

1 Department of Food Sciences and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran
2 Department of Food Science and Technology, Faculty of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
3 Kambiz Varmira, Research Center of Oils and Fats, Food and Drug Administration, Kermanshah University of Medical Sciences, Kermanshah, Iran

E-mail : ah.elhami@gmail.com   Contact No. : +9....................................

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Abstract

This study describes the sensitivity of bacteria isolated from the stomach of the honey bee to conventional antibiotics in medicine. *The bacteria include Lactobacillus pentosus* (HM027640)*, Lactobacillus* sp. Taj Naser-1(GQ451611)*, Lactobacillus fermentum* (HM027642)*, Lactobacillus kunkeei* (GQ451631)*, and Lactobacillus* sp. Makhdzir Naser-1(GQ451633)isolated from the honey stomach of the honey bee. The disk diffusion method was used in this study. *Lactobacillus pentosus* (HM027640)exhibited the highest sensitivity to amoxicillin (30 µg), kanamycin (30µg), and streptomycin(10µg)*; Lactobacillus kunkeei* (GQ451631)to ampicillin (10 µg); *Lactobacillus* sp. Makhdzir Naser-1(GQ451633) to chloramphenicol (30 µg) and penicillin (10µg) and*Lactobacillus* sp. Taj Naser-1 *(GQ451611)* toerythromycin (15 µg).The highest resistance toamoxicillin (30µg) was exhibited by*Lactobacillus fermentum*(HM027642)*; against* kanamycin (30 µg) and ampicillin (10µg)by *Lactobacillus* sp. Taj Naser-1(GQ451611)*,* against chloramphenicol (30 µg), and streptomycin(10µg) *Lactobacillus kunkeei* (GQ451631), and against erythromycin (15 µg) and penicillin (10 µg) by*Lactobacillus pentosus* (HM027640). The impact of antibiotic type on the resistance of lactic acid bacteria was quite effective. In some bacteria, resistance to several antibiotics was observed;however, this was not problematic, because the diameter of the inhibitory zone was much larger than standard.

Key words : antibiotic, lactic acid bacteria, honey stomach, disk diffusion, the diameter of inhibitory zone, resistance.

INTRODUCTION

A wide range of lactic acid bacteria (LAB, *Lactobacillus species*) have been isolated from kefir and were named in 1881 by the Russian researcher Kern. In 1995, the Australian researcher Moro isolated optional anaerobic, rod-shaped bacteria from the stool of breastfed babies, and named them *Bacillus acidophilus*, which is now considered to be a general name for intestinal lactobacilli. Lactic acid bacteria, an important group of gram-positive bacteria, are widely distributed in the environment and are found in fermented foods, vegetables, and intestine of man and animals. As a component of the human genital tract and digestive system flora, LAB is considered to be one of the important components of the natural microflora of the human and animal body [1-3].

The dispersal of bacteria depends on environmental factors, such as access to oxygen, the number of raw materials required, and the presence of secretory and bacterial activity [3]. Lactic acid bacteria are rarely associated with the gastrointestinal system or other body organs, and bacterial strains used throughout the environment are considered to be safe [1]. Additionally, it has been reported that lactic acid bacteria have the ability to remove heavy metals [4], cyanotoxins[5] and mycotoxins[6] from aqueous solutions in the laboratory environment. This group of bacteria is chemoorganotrophic and requires complex and nutrient-rich growth media; its metabolism is fermentative and saccharolytic. Lactobacilli are long and uniform gram-positive bacteria that are 10 microns in length. These bacteria are free of spores and are mainly non-motile. They are polymorphic and are anaerobes or facultative anaerobes. Some species are mandatory anaerobes and carry out fermentative metabolism that produces energy through fermentation of sugars, of which lactic acid forms at least half the product. The optimal temperature and pH for growth of lactobacillus ranges from 30°C to 40°Cand from 5 to 6.5, respectively, and a 5% concentration of carbon dioxide has a significant effect on their growth. *Lactobacillus* grows in anaerobic conditions but can tolerate oxygen [7].

According to previous studies, honey produced by honeybees contains bacteria that originate from the honey stomach of the honeybee [8, 9]. Large amounts of LAB in honey originate in the honey stomach of the honeybee and protects bees from pathogens [8]. Fifty years ago, antibiotics were introduced for the treatment of diseases. Currently, the biggest threat to the use of these materials is related to bacterial resistance [10]The emergence of antimicrobial resistance has been observed shortly after introduction of any new antimicrobial compound[11]. One of the most important mechanisms for resistance is the transfer of resistance genes from existing bacteria to food [12]. In 1998, a research project was carried out entitled "Reservation of Antibiotic Resistance" in order to prevent the release of antibiotic-resistant bacteria [13]. Since LAB are a component of microflora in human and animal digestive systems, the risk of transferring a resistance gene from the fermented product to these bacteria in the digestive system, especially because of their position within the intestinal microflora, led to the introduction of probiotics, which are susceptible to antibiotics. Many studies have been conducted on the distribution and source of LAB antibiotic resistance due to biological concerns regarding the use of starter and probiotic microorganisms. In Europe, the nature of antibiotic resistance, prior to the use of microorganisms, is determined according to safety prerequisites established by the European Food Safety Authority (EFSA). In general, excellent safety has been reported for probiotics belonging to the EFSA qualified presumption of safety list [14]. Although many of the LAB are not pathogenic initially, bacteria adapt to the environment over time in order to survive [15, 16]. A gene may become resistant to antibiotics and a horizontal gene transfer to another strain may occur. If this strain becomes pathogenic, it may become associated with many problems [13, 16, 17]. Horizontal gene transfer is one of the most important antibiotic resistance factors in bacteria. Horizontal gene transfer often occurs through transduction [18].

Today, the emergence of resistance to antibiotics has had a significant effect on clinical applications. In general, resistance to aminoglycosides occurs by three methods: 1. Change in drug targeting, 2. Change in drug transfer, and 3. Enzymatic inactivation by producing aminoglycoside-modifying enzymes (AMEs) [13].

Antibiotic resistance phenomena are so important that in 2011, the World Health Organization (WHO) named World Health Day as a Day against Drug Abuse. If pathogens are resistant to at least three groups of antibiotics, they are called multi-drug-resistant (MDR) pathogens [13]. Because pesticides are widely used on farms, it seems likely that bacteria become resistant to antibiotics over time, so antibiotic susceptibility testing is performed [19].

Because honey is a common food in the marketplace and because of a high probability of bacteria entering from the honey stomach to the honey, this study was carried out to evaluation of resistance to conventional antibiotics in medicine by 5 strains of lactic acid bacteria isolated from the stomach of the honey bee**.**

MATERIALS AND METHODS

To carry out this study, the following bacteria were collected from the microbial collections of the Animal Science Research Institute of Iran (ASRI), Karaj: *Lactobacillus pentosus* (HM027640), *Lactobacillus* sp. Taj Naser-1(GQ451611), *Lactobacillus fermentum* (HM027642), *Lactobacillus kunkeei* (GQ451631)and *Lactobacillus* sp. Makhdzir Naser-1(GQ451633).

Bacteria for activation (with a minimum of three replicates) were incubated in 10 ml of 2% MRS broth growth medium (volumetric/volumetric) and placed in an oven at 37 ^{o}Cfor 24 hours under aerobic conditions. After activation, the bacteria were incubated in Falcon tubes containing 30 ml of 2% MRS agar growth medium (volumetric/volumetric) and placed in an oven at 37 ^{o}Cfor 16-20 hours under aerobic conditions. Agar medium from each bacterial suspension (0.5 MacFarland standard) was densely cultured using sterilized swabs. After complete absorption of the aqueous bacterial suspension, disks containing antibiotics were placed on the growth medium using sterile forceps while maintaining appropriate spacing of each disk between its neighbors and the edge of the plate. Plates were placed in the oven for one hour and the diameter of the inhibitory zone was then measured. This experiment was repeated three times [20]

RESULTS

Five lactic acid bacteria isolated from the honey stomach of the honeybee [21] were tested in terms of their susceptibility to conventional antibiotics by the disk diffusion method and its results are shown in Table 1.

The results indicated that *Lactobacillus fermentum* (HM027642) and *Lactobacillus* sp. Taj Naser-1(GQ451611) exhibited the highest susceptibility to chloramphenicol (30 µg) and the least susceptibility to kanamycin (30 µg) ($p \geq 0.05$).

Lactobacillus pentosus (HM027640)exhibited the highest susceptibility to amoxicillin (30 µg) and the least susceptibility to penicillin (10 µg) ($p \geq 0.05$).

Lactobacillus sp.MakhdzirNaser-1(GQ451633) and *Lactobacillus kunkeei* (GQ451631)exhibited the highest susceptibility to chloramphenicol (30 µg) and exhibited the lowest susceptibility to streptomycin (10 µg) ($p \geq 0.05$).

Table 1: The susceptibility of bacteria to antibiotics by measuring the diameter (mm) of the inhibitory zone of selected strains of lactobacilli isolated from the stomach of the honey bee

Sample	*Lactobacillus fermentum* (HM027642)	*Lactobacillus* spTaj Naser-1 (GQ451611)	*Lactobacillus kunkeei* (GQ451631)	*Lactobacillus* sp. MakhdzirNaser -1 (GQ451633)	*Lactobacillus pentosus* (HM027640)
Amoxicillin 30 µg	9.500 ±0.424^{dC}	18.050 ±0.212^{cB}	18.250 ±0.693^{aB}	16.975 ±0.460^{bB}	21.400 ± 0.608^{aA}
Kanamycin 30 µg	8.250 ±0.509^{dC}	7.680 ±0.382^{fC}	8.680 ±0.537^{cdC}	9.680 ±0.297^{dB}	11.330±0.438^{deA}
Ampicillin 10 µg	13.280 ±0.071^{cA}	12.750 ±0.198^{dA}	14.650 ±0.622^{bA}	14.455 ±0.573^{cA}	13.500 ±0.552^{cA}
Chloramphenicol 30 µg	21.100 ±0.269^{aA}	21.860 ±0.297^{aA}	18.850 ±0.283^{aB}	22.100 ±0.212^{aA}	21.300 ±0.226^{aA}
Erythromycin 15 µg	18.280 ±0.354^{bB}	19.600 ±0.410^{bA}	18.800 ±0.339^{aB}	18.230 ±0.240^{bB}	17.470 ±0.467^{bB}
Penicillin 10 µg	13.250 ±0.523^{cA}	10.125 ±0.460^{eB}	10.275 ±0.247^{cB}	13.250 ±0.396^{cA}	9.615 ±0.389^{eB}
Streptomycin 10 µg	9.100 ±0.566^{dB}	8.110 ±0.580^{fB}	7.640 ±0.608^{dB}	7.650 ±0.438^{eB}	12.250 ±0.665^{cdA}

The results are given as mean ± SD.
Different letters indicate a significant difference in each column
Different letters indicate a significant difference in each row

DISCUSSION

In general, all bacteria were susceptible to antibiotics due to the diameter of the inhibitory zone and did not exhibit multiple resistance. Among the bacteria studied, *Lactobacillus pentosus* (HM027640)exhibited the highest susceptibility to amoxicillin (30µg), whereas the highest resistance occurred for *Lactobacillus kunkeei* (GQ451631) against streptomycin (10µg). This was the first study carried out to investigate antibiotic susceptibility of bacteria extracted from the honey stomach of the honey bee.

Resistance to erythromycin in LAB has been reported for foods from various sources, such as Indonesian fermented foods [22, 23]. Among the strains studied, only *Lactobacillus* sp. Taj Naser-1(GQ451611) is susceptible to erythromycin, whereas the other strains are resistant and no significant (p<0.05) differences were observed. Resistance genes underlie this resistance. One possible mechanism is related to rRNA methylase, which can produce a single mutation in 23srRNA [14].

According to the results, resistance to penicillin was observed equally in *Lactobacillus pentosus* (HM027640), *Lactobacillus* sp. Taj Naser-1 (GQ451611) and *Lactobacillus kunkeei* (GQ451631). In a report of 55 European probiotic products, 23% were resistant to penicillin [13]. This results from resistance against cell wall synthesis inhibitors that are hydrolyzed by penicillinase antibiotics, which lead to impermeability of the cell wall, a process that has also been reported to provide resistance against ampicillin [16, 24].

Lactobacillus fermentum (HM027642)exhibited the highest resistance to amoxicillin (30 µg). Resistance can be attributed to the presence of β-lactamase, which prevents the effect of amoxicillin on trans peptidases that lead to production of cell wall peptidoglycans [25]. *Lactobacillus kunkeei* (GQ451631), *Lactobacillus fermentum* (HM027642) *and Lactobacillus* sp.Taj Naser-1(GQ451611) exhibited the highest resistance to kanamycin (30 µg), with no significant difference (p<0.05) observed between them. The most sensitive bacteria to this antibiotic was *Lactobacillus pentosus* (HM027640). The reason for this sensitivity can be explained by the fact that kanamycin and other drugs in this group are aminoglycoside antibiotics, which pass through the bacterial membrane and inhibit protein biosynthesis by ribosomes.Streptomycin is also considered to be an aminoglycoside antibiotic. *Lactobacillus pentosus* (HM027640) exhibited the highest susceptibility to streptomycin compared to the other bacteria.All aminoglycosides bind to the ribosome and inhibit bacterial protein synthesis, thereby causing bactericidal effects. Penetration of these drugs into the bacterial cell coating depends on oxygen, therefore they have no significant effect on absolute anaerobes[25].Chloramphenicol eliminates a wide range of microorganisms, and the results of recent research also indicate a high sensitivity by the tested bacteria; however, resistance to this antibiotic was observed in previous studies. In *Lactobacillus acidophilus* and *Lactobacillus delberocytes*, the chloramphenicol resistance gene bulgaricus subspecies produces acetyltransferase chloramphenicol, which inhibits binding of antibiotics to the 50S ribosomal subunit [26]

Today, the annual mortality rate due to antibiotic resistance in humans is higher than the annual mortality rate due to AIDS. Hospital costs have risen to at least $ 4.5 billion per year due to antibiotic resistance [13]. According to studies, an average of 70% of antibiotics consumed are disposed of and discharged into the environment. With entry of antibiotics into the environment, the pressure on microbial groups will increase, and they will become resistant. This process disrupts the ecosystem balance[19].

CONCLUSION

Antibiotics play an important and key role in the treatment of diseases in humans and animals. Antibiotics also have an important role in improving growth and controlling pests in the environment. The cell wall, bacterial membranes, nucleic acids, and bacterial proteins are considered to be the main targets of all antibiotic groups. In spite of the benefits of antibiotics, their excessive consumption has created serious problems in the global arena. The WHO, in a published statement, argued against inclusion of LAB in food due to the probability of increasing antibiotic resistance.

Probiotics should be used only after more careful examination, due to an increasing interest in using genetically modified probiotics for various purposes. Although certain probiotics are used in combination with antibiotics and exhibit multiple resistance, generally the biological safety of LAB should not be ignored due to increasing resistance. For this reason, before promoting probiotics consumption by humans, standards and guidelines, as well as standardized marketing, biological testing and market surveillance should be evaluated with predetermined criteria.

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