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In Vitro Assessment the Antagonistic Activity of Enteric Lactobacillus Spp. against opportunistic Bacteria

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Abstract: *Currently there is interest in the development of alternative therapies in the treatment of gastrointestinal tract disorders. Attention has turned to gut microbiota as bacteria based therapies. Many enteric lactobacilliare potentially probiotics and approved as alternative therapy for curing lists of gastric diseases.The present study was aimed to seek for therapeutic efficacy of enteric Lactobacillusagainst human opportunistic bacteria(Achromobacterxylosoxidans and Klebsiellaoxytoca), that were isolated from gastric endoscopic biopsy specimens of peptic ulcerative patients on continuous medication. Thirteen Lactobacillus isolates were isolatedfrom breast – feed infant faces on De Man Rogosa and Sharpemedium (MRS) supplemented with vancomycin (1µg.ml^{-1}), mainlyheterofermentative lactobacilli were the predominant.Isolateswere identified on the basis of microscopicexamination, biochemical tests, and sugars fermentation profile.Lactobacillus isolates were screened for their (in vitro) antagonistic effects. All isolates showed antagonistic activity in respect of inhibition zones in agar well – diffusion technique, the most potent isolates wereA9 and A20,that were exhibited the highest inhibition zones 26.93 ± 1.93 and 23.20 ± 1.91 mm respectively against Achromobacterxylosoxidans and Klebsiellaoxytoca. Identification of thetwo potentantibacterial lactobacilli isolateswasconfirmed with molecular analysis by the amplification of universal bacterial 16S rRNA gene, followed by DNA sequencing of this gene and alignment of sequencing in National Center for Biotechnology Information (NCBI).*

Keywords: *Probiotics, Enteric Lactobacillus, in vitro antagonism, opportunisticbacteria,Achromobacterxyl osoxidans, Klebsiellaoxytoca*

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I. Introduction

The term "Probiotics" is derived from a Greek word 'biotikos' meaning 'for life', which was first coined by Parkers [1], and defined as life microorganisms when they were administrated in adequate amounts to confer health benefits on the host [2]. Lactic acid bacteria (LAB), especially *Lactobacillus*, are the most commonly used microorganisms as probiotics, members of lactobacilli are "Generally Recognized as Safe" (GRAS) ingredients, and are desired members of the gastrointestinal tract (GIT) microflora, contribute mainly in maintaining the GIT homeostasis [3]. *Lactobacillus* colonize the gastrointestinal tract of mammals and human immediately after birth [4], and represents the major digestive system microflora, that contribute approximately 75% in gastric functions, they known to benefit health as natural predominant microflora [5]. The beneficial biological functions of gastric *Lactobacillus* include; reduction of serum cholesterol, amelioration of diarrhea or constipation, elimination of procarcinogens, synthesis of vitamin B, activation of immune system , improve of adhesive ability, and prevent gastrointestinal infections [6, 7]. Lactobacilli therapeutic actions of gastric disorders attributed to different mechanisms, such as, competitive exclusion of enteric pathogens [8], enhancement of GIT lining epithelial barriers, and production of bioactive molecules like; organic acids particularly lactic acid, hydrogen peroxide, diacetyl, and antimicrobial substances, bacteriocins and bacteriocins – like peptides [9, 10].

Opportunistic pathogens have become increasingly relevant as the causative agents of many clinical diseases[11]. Many opportunistic bacterial strains are becoming important pathogens of human, and being implicated in the increasing morbidity amongst the patient population.*Achromobacterxylosoxidans*and *Klebsiellaoxytoca*are belongs tophylum proteobacteria[12], now are emerging as important opportunistic microorganism and frequently causes infections at nearly any body site. Infections are proceeded by gastrointestinal colonization, the pathogenic potential of these two bacteria are essentially unknown [13]. Recently they are associated to many gastric ulcerative diseases [14], and frequently isolated from many peptic ulcer cases in human [15,3]specially those patients who are on long – term antibiotics treatments. Consequently multi – drug strains of these opportunistic bacteria attack the inflamed damaged gastric lining mucosal surfaces[16]. In vivo studies in murine model support the role of proteobacterial members in the development of

gastritis from peptic ulcers to gastric neoplasia [17].Such studies demonstrated patient's that taking massive drugs for curing of gastritis infections were showed a neutralization of the gastric environment, and this closely correlated the alterations in the gastric microbiota and significantly increased colonization of proteobacterialopportunistic [18].

This study aimed to seek for in vitro antagonistic behavior of human derived *Lactobacillus* spp. against human gastric opportunistic bacteria.

II. Materials and methods

Isolation of bacteria and cultural conditions

Forty fecal samples were randomly collected from naturally delivered and fully breast fed infants around Baghdad province / Iraq. Their ages ranged from hours to five months old. The collected samples were placed in sterile plastic containers and transported to the laboratory within 8 to 12 hrs. meanwhile kept at 4°C until cultivation.One gram of each fecal sample was taken,serially 10 – fold diluted in saline, and 0.1 ml was inoculated into 10 ml de Man Rogosa - Sharpe broth (MRS, Homedia, India) supplemented with erythromycin 5µg. ml^{-1}[19]. The tubes were incubated anaerobically (anaerobic jar supplied with Gas pack Oxoid/ England) at 37°C for 48 hrs. A loop full of the cultured broths was streaked triplicates on MRS agar supplemented with erythromycin (MRS – E), plates were incubatedanaerobically. The colonies with the interesting characteristic features were streaked on MRS agar supplemented with vancomycin (1µg $.ml^{-1}$) (MRS – V), pH 5.5 [20]. The plates were incubated anaerobically at 37°C for 72 hrs. The interested colonies were repeatedly cultured on MRS agar to obtain pure colonies. The culture isolates were identified to genus level by: gram staining, colonies morphology, and biochemical tests. The isolates sugars fermentation profile was achieved and compared with sugars fermentation scheme described in Bergey's manual of systematic bacteriology [21].

Antagonistic activity screening

Lactobacillus isolates were assessed for their antagonistic activities against test bacteria *Achromobacterxylosoxidans* and *Klebsiellaoxytoca*(previously isolated from endoscopic gastric biopsy specimens of peptic ulcerative patients),by agar – well diffusion method.Briefly; Melted Brain heart infusion (BHI) agar was seeded with overnight culture of test bacteria at a final concentration 10^6CFU/ ml, poured into sterile petri dishes and allowed to solidify at room temperature, wells 5mm were hollowed out in agar using a sterile cork borer, wells were filled with 80 µl (10^8CFU/ml) of the *Lactobacillus* isolates suspensions individually, plates were incubated at 4°C for 3h to facilitate diffusion into agar, after plates were incubated at 37°C for 48h. Formed inhibition zones around the wells were measured and recorded in millimeter after subtraction 5mm,wells diameter[22].

DNA extraction and PCR identification

Genomic DNA of the bacterial isolates that exhibited potent antagonism against test bacteria was extracted directly from overnight broth culture by using genomic DNA purification kit (Intron Biotechnology, Korea), and according to manufactures instructions.Isolates were subjected to PCR analysis to detect the bacterial universal *16S rRNA* gene, using a universal *16S rRNA* primer: 20F 5'-AGTTTGATCCTGGCTC-3', 1530R 5'-AAGGAGGTGATCCAGCC-3'[23].

PCR amplification mixture which was used for the detection of the universal *16S rRNA* gene was carried out in 25 µl volume includes GoTaq® Green Master Mix, 2X (12.5 µl), 3 µl of 25 ng DNA template, 1 µl (1 Mm) of each forwarded and reversed primers and 7.5 µl of nuclease free water to complete the amplification mixture to 25µl. Amplification was performed in a thermal cycler (Eppendorf®) programmed temperatures as the following: Initial denaturation 95 °C (3 min), Denaturation 95 °C (45 sec), Annealing 62°C (45 sec), Extension 72°C (1 min), and the Final extension 72°C (10 min).

PCR product was examined on agarose gel to confirm that there is a specific product with the desired size. The product was electrophoresed on 1% agarose gel containing Ethidium bromide (0.5 mg/ml) in Tris-Acetate-EDTA buffer (TAE buffer) and photographed under UV illumination.

Sequence analysis of the*16 rRNAgene*

The sequence analysis of*16S RNA*gene was performed to confirm identification of *Lactobacillus*. It was carried out by sending the PCR products of amplified *16S rRNA* gene to Macrogen Company/ Korea to preform Sanger sequencing by using AB13730XL, automated DNA sequencer. The result analyzed by BLAST website on NCBI.

Statistical analysis

The results of antagonisms are expressed as the mean ± standard and the data subjected to analysis system – SAS program [24]. Least significant difference –LSD test was used to significant compare between means of data. The level of significance was set at $P < 0.05$.

III. Results and Discussion

A total of forty fecal sampleswere randomly collected from naturally delivered, breast fed, and healthy infants for isolation of *Lactobacillus*(coded as A1 – A40). The feces of breastfeeding is the best source for isolation many beneficial LAB, due to their gut are continuously supply with fresh viable bacteria (probiotics), and they have a more stable and uniform population of microorganisms composed mainly of *Lactobacillus* and *Bifidobacterium*[25]. In contrast formula fed infants have an increased bacterial diversity with decreased prevalence of *Lactobacillus*, and this add several additional steps to the isolation of *Lactobacillus* and leads to difficulty in diagnosis steps [26]. Besides breast milk supplies bioactive non digestible oligosaccharides, that are digested in the colon, stimulating the growth and / or activity of specific fecal bacteria (including lactobacilli) that impact heath positively in infant receiving breast milk [27].

Naturally delivered infants are preferred for proper *Lactobacillus* isolation due to the fact that full – term vaginally delivered infants are exposed to massive amount of maternal vaginal microbiome while passing throughthe birth channel, in addition the infant are inoculated continuously with maternal intestinal bacteria , that makes lactobacilli the pioneering colonizers of newborn GIT[4].

The MRS medium was used for isolation of fecal lactobacilli, known as selective medium for the isolation of LAB, combination of salts and varying antibiotics are supplemented to the base medium to improve the medium selectivity to a certain member of LAB [28]. Supplementation of erythromycin to MRS suppresses the growth of *Bifidibacterium*, which are associated to *Lactobacillus* in fecal samples [19]. Out of forty infant fecal samples were cultivated on MRS – E medium, thirteen colonies (26%) were suspected belong to *Lactobacillus* on the bases of colony morphology (whitish – creamy colored, glistening, small, round , and non – convex colonies). These colonies were picked up and cultivated on MRS – V, twelve colonies (93.3%) werepronounced clearlyon this medium. Theisolates were considered as heterofermentative species because of their resistance to vancomycin is intrinsicresistant [29]. Vancomycin usually targets and binds to the terminus D-alanine of the peptidoglycan on the cytoplasmic side of peptidoglycan of cell wall, instead, in heterofermentativelactobacilli, the D-alanine is replaced with D-lactate or D-serine and therefore preventing the binding of vancomycin [30]. This really helped in isolating pure colonies from primary isolation which helped decrease the amounts of sub culturing necessary to purify the isolates.

The identification was achieved by biochemical characteristics as it is summarize in table (1). Attempt for identification of isolates to species level was performed by sugar fermentation profile of human strain and was compared with sugar fermentation scheme [21] shown in table (2), as a classical differentiation procedure[31].

Table (1): Biochemical characteristics of *Lactobacillus* isolates.

Test	Bacterial isolate												
	A2	A4	A6	A9	A11	A20	A22	A23	A24	A26	A27	A28	A29
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatinase	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reductase	-	-	+	-	+	+	-	±	±	-	-	+	+
Arginine hydrolysis	+	±	-	-	-	-	+	-	-	+	+	+	+
Bile salt (Na-taurocholate) 2%	+	+	+	+	+	+	+	+	+	+	+	+	+
NaCl 6.5%	+	+	+	+	+	+	+	+	+	+	+	+	+
Gas	-	-	+	+	-	-	-	-	-	+	-	+	-

(+ Positive reaction, - negative reaction, ± variable reaction)

Table (2): Sugars fermentation profile of *Lactobacillus* isolates.

Sugar	Bacterial isolate												
	A2	A4	A6	A9	A11	A20	A22	A23	A24	A26	A27	A28	A29
Glucose	+	+	±	+	+	±	+	±	±	+	+	+	+
Galactose	-	-	+	-	+	+	-	+	+	+	+	+	-

Lactose	-	+	-	+	-	+	+	+	+	+	+	+	+
Arabinose	+	+	-	+	-	-	+	-	+	-	-	+	+
Mannitol	-	+	+	+	+	+	±	-	-	+	+	±	-
Maltose	+	+	±	+	+	±	+	+	±	+	+	+	+
Raffinose	±	+	+	+	+	+	+	-	+	±	±	+	+
Soribitol	-	+	+	+	+	+	-	-	+	-	-	-	-
Sucrose	±	+	+	+	+	+	+	±	±	+	+	+	+
Xylose	±	+	±	+	±	±	±	-	±	-	±	-	+
Suggested species	***L. brevis***	***L. plantarum***	***L. paracasei***	***L. plantarum***	***L. paracasei***	***L. paracasei***	***L. reuteri***	***L. acidophilus***	***L. salivarious***	***L. paracasei***	***L. paracasei***	***L. fermentum***	***L. brevis***

(+ Positive reaction, - negative reaction, ± variable reaction)

Traditionally *Lactobacillus* has been identified on the basis of cell and colony morphology, biochemical analysis, and the ability to utilize various carbohydrates substrates. The application of these approaches have proved useful tools in the classification and identification of *Lactobacillus* to species level up to 80% .In the bases of sugar fermentation profile ,seven different species were mainly detected, and these species are the most frequently identifies species in human GIT , *L. paracasei* was predominant (38.5%), followed by *L. plantarum*and *L. brevis*. This come in agreement with previous study has approved the prevalence of *L. paracasie* in breastfeeding while it was less prevalent in formula – fed infants GIT[32]. This suggests that the diet can affect the composition of infant's intestinal microbiota.The antagonistic activities of *Lactobacillus* isolates were assessed by agar-well diffusion assayagainst test bacteria, *K. oxytoca*and *A. xylosoxidans*(figure 1).

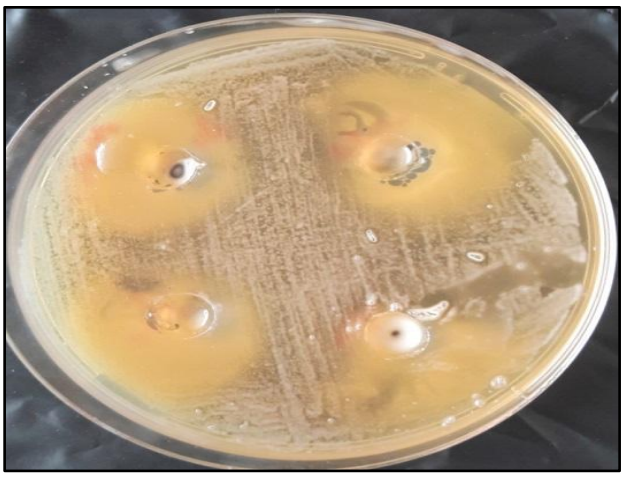

Figure (1): Antagonistic activity of*Lactobacillus* isolates (in respect of inhibition zones) against *A. xylosoxidans.*

All isolates were able to inhibit the two tested bacteria in various degrees, with average inhibition zones 26.93± 1.93 – 12.23± 0.67 mm respectively. The isolate A9 was exhibited highest inhibition against *A. xylosoxidans*, while the isolate A20 was potent against*K. oxytoca*(table 3). Both isolates assumed to be potentially probiotic isolates. To ensure their identification, subsequently subjected to genotypicidentification,the *16S rRNA* gene of the isolates was amplified and sequenced; DNA sequence was analyzed and compared with the basic Local Alignment Search Tool (BLAST), in NCBI. The alignment result of isolate A9 revealed high matching with the universal strain (LBRH025) sequence which is recorded on NCBI as *Lactobacillus plantarum*species with its accessionnumber: HM101329.1at 99% query cover of 99% identify and 0% gaps (figure 2). While The alignment for the sequence of isolate A20 was revealed high matching with the universal strain (CAU5144) sequence which is recorded on NCBI as *Lactobacillus paracasei*with its accession number: MF423812.1at 100% query cover of 99% identify and 0% gaps (figure 3).

Phenotypic methods have been most commonly used for the identification of LAB, but more recently, molecular techniques such as *16S rRNA* sequencing have been developed, enabling a more consistent and

accurate identification of individual strains. Other promising identification tools include partial *rRNA* gene sequencing for accurate identification [33].*L. plantarum* is one of the most important and versatile species has many applications in the food and pharmaceutical industries.It is well known as bacteriocinogenic species that are abundant in their productivity of plasmide – encoded plantaricin[34]. Plantaricin LpU4 was active against several pathogens with various antibiotic-resistance phenotypes including a methicillin-resistant strain [35].*L. paracasei*is natural human fecal isolate exhibits antibacterial activity against various pathogenic microorganisms (*Bacillus, Streptococcus, Staphylococcus*), including gram-negative bacteria *Salmonella* and *Pseudomonas*. In addition, *L. paracasei* was found to be able to control the overgrowth of pathogen *S. aureus*[36]. Therefore, it would be of interest to isolate thesespecies and assesse the antimicrobial activity against opportunistic strains.

Table (3): Antagonistic activity of*Lactobacillus* isolates in respect of inhibition zones (mm) against *A. xylosoxidans* and *K. oxytoca.*

***Lactobacillus* Isolate**	Mean ± SE (mm)	
	A. xylosoxidans	***K. oxytoca***
A2	21.56 ± 2.58	12.93 ± 1.24
A4	18.83 ± 3.08	13.13 ± 1.41
A6	21.73 ± 5.35	15.46 ± 1.58
A9	26.93 ± 1.93	19.90 ± 6.14
A11	16.88 ± 2.55	16.80 ± 3.42
A20	15.26 ± 1.79	23.20 ± 1.91
A22	19.56 ± 1.46	15.16 ± 1.78
A24	14.30 ± 1.28	20.10 ± 0.98
A26	12.23 ± 0.76	12.23 ± 1.32
A27	19.40 ± 0.25	13.03 ± 1.29
A28	19.70 ± 0.17	13.26 ± 1.7
A29	12.97 ± 1.44	14.73 ± 2.74
LSD value	6.791 *	7.400 *
* (P<0.05).		

Score	**Expect**	**Identities**	**Gaps**	**Strand**
1635 bits(885)	**0.0**	**895/899(99%)**	**4/899(0%)**	**Plus/Plus**

Query 1
ACGTGCTTGCACTGAATGAGATTTTAACACGAAGTGAGTGGCGGACGGGTGAGTAACACG 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 3
ACGTGCTTGCACTGAATGAGATTTTAACACGAAGTGAGTGGCGGACGGGTGAGTAACACG 62

Query 61
TGGGTAACCTGCCCAGAAGCAGGGGATAACACCTGGAAACAGATGCTAATACCGTATAAC 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 63
TGGGTAACCTGCCCAGAAGCAGGGGATAACACCTGGAAACAGATGCTAATACCGTATAAC 122

Query 121
AGAGAAAACCGCCTGGTTTTCTTTTAAAAGATGGCTCTGCTATCACTTCTGGATGGACCC 180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 123
AGAGAAAACCGCCTGGTTTTCTTTTAAAAGATGGCTCTGCTATCACTTCTGGATGGACCC 182

Query 181
GCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGATGATGCGTAGCCGACC 240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 183
GCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGATGATGCGTAGCCGACC 242

Query 241
TGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC 300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 243
TGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC 302

Query 301
AGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAA 360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 303
AGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAA 362

Query 361
GGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACGTGGGTGAGAGTAACTGTTCAC 420
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 363
GGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACGTGGGTGAGAGTAACTGTTCAC 422

Query 421
CCAGTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA 480
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 423
CCAGTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA 482

Query 481
CGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTCTTTTA 540
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 483
CGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTCTTTTA 542

Query 541
AGTCTAATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATTGGAAACTGGGAGACTTGA 600
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 543
AGTCTAATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATTGGAAACTGGGAGACTTGA 602

Query 601
GTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGA 660
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 603
GTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGA 662

Query 661 ACACCAGTGGCGAA-
GCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGG 719
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Sbjct 663
ACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGG
722

Query 720
TAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGATTACTAAGTGTTG
779
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 723
TAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGATTACTAAGTGTTG
782

Query 780
GAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGTAATCCGCCTGGGGGAGTAC
839
|||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct 783 GAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGTAATCCGCCT-
GGGGAGTAC 841

Query 840 GACCGCAAGGTTGAAACTCAAAAGA-
TTGACGGGGGGCCCGCACAAGCGGTGGAGCATG 897
|||||||||||||||||||||||| ||||| ||||||||||||||||||||||||
Sbjct 842 GACCGCAAGGTTGAAACTCAAAAGAATTGAC-
GGGGGCCCGCACAAGCGGTGGAGCATG 899

Figure (2): Alignment of isolate A9 with Lactobacillus plantarum strain LBRH025 16S ribosomal RNA gene, partial sequence. Sequence ID: HM101329.1**Query:** the target (studied) nucleotide sequence. **Subject:** nucleotide sequences within a database.

Score	Expect	Identities	Gaps	Strand
1421 bits(769)	0.0	777/780(99%)	3/780(0%)	Plus/Plus

Query 1
GACGTGCTTGCACTGAATGAGATTTTAACACGAAGTGAGTGGCGGACGGGTGAGTAACAC
60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 32
GACGTGCTTGCACTGAATGAGATTTTAACACGAAGTGAGTGGCGGACGGGTGAGTAACAC
91

Query 61
GTGGGTAACCTGCCCAGAAGCAGGGGATAACACCTGGAAACAGATGCTAATACCGTATAA
120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 92
GTGGGTAACCTGCCCAGAAGCAGGGGATAACACCTGGAAACAGATGCTAATACCGTATAA
151

Query 121
CAGAGAAAACCGCCTGGTTTTCTTTTAAAAGATGGCTCTGCTATCACTTCTGGATGGACC 180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 152
CAGAGAAAACCGCCTGGTTTTCTTTTAAAAGATGGCTCTGCTATCACTTCTGGATGGACC 211

Query 181
CGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGATGATGCGTAGCCGAC
240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 212
CGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGATGATGCGTAGCCGAC
271

Query 241
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 272
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
331

Query 301
CAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGA
360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 332
CAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGA
391

Query 361
AGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACGTGGGTGAGAGTAACTGTTCA
420
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 392
AGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACGTGGGTGAGAGTAACTGTTCA
451

Query 421
CCCAGTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
480
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 452
CCCAGTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
511

Query 481
ACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTCTTTT
540
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 512
ACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTCTTTT
571

Query 541
AAGTCTAATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATTGGAAACTGGGAGACTTG
600
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 572
AAGTCTAATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATTGGAAACTGGGAGACTTG
631

Query 601
AGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAG
660
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 632
AGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAG
691

Query 661
AACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGG 720
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct 692
AACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCAT-G 750

Query 721
GGTAGCGAACAGGATTAGATACCCTGGGTAGTCCATGCCCGTAAACGATGATTACTAAGT 780
|||||||||||||||||||||||| ||||||||| ||||||||||||||||||||
Sbjct 751 GGTAGCGAACAGGATTAGATACCCTGG-TAGTCCATG-CCGTAAACGATGATTACTAAGT 808

Figure (3): Alignment of isolate A20 with Lactobacillus paracasei strain CAU5144 16S ribosomal RNA gene, partial sequence Sequence ID: MF423812.1**Query:** the target (studied) nucleotide sequence. **Subject:** nucleotide sequences within a database.

The *Lactobacillus*isolates were isolated from infant feces, it means they are GIT associated microbiota, this site in healthy human induce the beneficial bacteria to production and secretion variety of antibacterial substances, it was reported in previous study that 99% of human associated lactobacilli make at least one of antimicrobial substance [37]. In vitro antagonistic behavior of *Lactobacillus* considered to be multifactorial, fundamentally due to accumulation of many primary and secondary metabolites like; H2O2, ethanol, organic acids (lactic and acetic acids), bacteriocins and bacteriocins – like peptide products[9, 10]. The production of organic acids in particular lactic acid, from fermentation of hexoses, decreases milieu pH, the concomitant reduction in pH of microenvironment and accumulation of lipophilic organic acids results in broad - spectrum inhibition activity against Gram- positive and Gram-negative bacteria [38]. Lipophilic acids antagonistic effects against of many potential pathogenic bacteria attributed to the penetration of microbial cellular membranes and intracellular dissociate to produce hydrogen ions, which interfere with essential metabolic functions. Such as the enzymatic activity, membrane permeability and bioavailability of some nutrients which depends on ionic balance [39]. *Lactobacillus* also is capable of producing antimicrobial compounds such as, bacteriocins and bacteriocins- like substances. These compounds are also responsible for the anti - microbial efficacy. Bacteriocins are biologically active protein moieties with bacteriocidal mode of action [40].Bacteriocins gain entry into the target cells by recognizing specific cell surface receptor then kill the cell by forming ion – permeable channel in the cytoplasmic membrane, by nonspecific degradation of cellular DNA, inhibiting the protein biosynthesis through the specific cleavage of *16S rRNA*, or by cell lysis [41]. Most of reported *Lactobacillus*bacteriocins fall into class l bacteriocins (lantobiotics). The antibacterial activity of lantobiotics based on interaction with the bacterial membrane, they binds specifically to phosphoethanolamine which results in inhibition of phospholipase A2 and various other cellular functions. Most of bacteriocins l dissipates the proton motive force (PMF) of target cells, via pore formation [42].

IV. Conclusion

Potentially probiotic *Lactobacillus*strains have emerged as great alternatives to chemicals andantibiotics in the field's therapy and have demonstrated antimicrobial activities against vast array of pathogens. In the current era of antibiotic resistance, probiotic lactobacilli and their bioactive products may be the remedy for choice to cure opportunistic strains. Therefore, more focusedresearch studies need to be conducted to include in vitro and in vivo analyses, animal model studiesand human trials, in order to validate health claims, and to ensure the safety and efficacy of *L.plantarum*and *L. paracasei.*

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