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RESEARCH ARTICLE

Molecular Detection of Lysinibacillus Fusiformis Isolated from Milk Samples of Cow in Iraq

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Abstract

Raw milk infected by bacteria can start from exceptional resources: air, milking devices, feed, soil, dangs and hays.The strategies of nourishing and lodging cows may impact the germs pleasant of milk. Bacterial contaminants can cause infection, or deterioration of drain and its auxiliary items and the destructive impacts on the grade and security of dairy items as a result of oxygen consuming spore-forming bacteria got from crude drain were characterized by isolation of Lysinibacillus fusiformis from 90 milk samples of cow at 21.6l%, the bacteria were identified by routein bacteriological methods and Molecular distinguishing proof of the confines was carried out by 16S rRNA sequencing and the bacterial confines were taxonomically classified as Lysinibacillus fusiformis. The groupings were stored in NCBI GenBank with the accession number KY038703, KF916675. and KF916675.1 with identity of 100%, 96 and 99% respectively.

Keywords: *Raw milk, Lysinibacillus fusiformis, 16S rRNA PCR sequencing.*

Introduction

Milk can be considered as a huge supply of microorganisms because milk contains high level of supplements makes it a particularly reasonable development medium for different microscopic organisms. In truth, these microorganisms can accomplish more populace densities taking after contamination amid drain preparing in dairy ranches and in the dairy industry [1].

In truth, it is exceptionally critical to preserve them in reasonable conditions (heat degree and wetness), that maintain a strategic distance from the expansion of the microflora display on their surfaces [2]. Indeed, in commercially sterilized drain, the decay caused by Bacillus species has been detailed, in spite of the fact that usually generally caused by thermostable proteolytic and lipolytic proteins or by re-contamination of the sterilized drain amid filling [3].

In arranging to test the microbial groups colonizing books and documented facts, various strategies are handy. In such methodologies can be isolated essentially to two assorted procedures: the culture-dependent strategies (based on the development of microorganisms on particular microbiological media) and the culture independent methods (based on the extraction of nucleic acids and their examination). Lysinibacillus fusiformis is Gram-positive, rod-shaped microbes non motile having a place to the family Bacillaceae [4,5]. The bacteria have a type of peptidoglycan in their cell wall and many researchers' studies its pathogenicity [6].

Lysinibacillus fusiformis separated from other near of Bacillacea since of the presence of lysine and aspartate in thepeptidoglycan of the cell wall [7]. The bacteria resist many metals like inmercury-contaminated sites and boron [8,9].

Lysinibacillus fusiformis is pathogenic to human [10], other researcher studies its biosurfactant activity against biofilm formation of Escherichia coli and Streptococcus mutans [11] , else it increased the biofilm of Bacillus subtilis by the overabundance of hypoxanthine inside B. subtilis cells, which may prompt cell stress and death [12]. This study aimed was isolating of Lysinibacillus fusiformis from raw milk of cow and detect it by 16 serene.

Material and Methods

Sampling

Crude drain tests (90) were aseptically collected from dairy ranches at geologically distinctive areas within the locale of Baghdad from a late summer/autumn and a winter.

Bacterial Strain Isolation and Identification

Milk samples were collected in sterile tubes, 0.1ml of every raw milk sample turned into streaked out on blood agar plates (Oxoid) and incubated at 37C with 5% CO2 for 24 h. Spore-forming bacteria were isolated from mdia. each colonies were purified by sub-culturing. Isolates were preserved in 20% glycerol at -80 ^{0}C.

Biochemical and Molecular Characterization of Isolate

Based on the isolated colony and its morphological and biochemical characteristics were explored. The biochemical characterization of this strain, referred to as Lysinibacillus fusiformis.

DNA Extracted

DNA extract was done as described by [13].

PCR and DNA Sequencing

The 16S rRNA may be a well-conserved, all inclusive bacterial genewidely utilized to distinguish contrasts among bacteria. The PCR amplification was performed using universal primers f FμM (5'-AGAGTTTGATCCTGGCTCAG- 3')and RμM (5'- GGTTACCTTGTTACGACTT) – the procedure done as (Coorevits et al 2008) . All isolatewere sended to (NCBI) and BioEdit program.

Results

Bacterial Isolation

Three isolated bacteria classified as Lysinibacillus fusiformis out of 90 raw milk samples in 21.6l% .

Characterization of Bacterial Strains

We screened a collection of 90 raw milk samples obtained from different sampling sites in Baghdad in order to distinguish microbes that are found in raw milk. The isolated bacteria from raw milk were distinguished based on biochemical test as Lysinibacillus fusiformis as shown in Table 1

Table 1: Some Biochemical test on *Lysinibacillus fusiformis*

Biochemical test and gram stain	*Lysinibacillus fusiformis*
Gram stain	Negative
Motility	+
Oxidase	+
catalase	+
Citrate utilization	v
Indole	-
Methyl red	-
Urea	+
Hydrolysis of esculin	-
Voges Proskauer's	-
Phenyl alaninedeamination	+
Glucose	-
Lactose	-

+indicates positive result,- indicates a negative result,V indicates variable

Evaluation of Bacterial Detection and Identification by 16SrRNA and Sequencing

Lysinibacillus fusiformis nucleotide sequence was submitted to GenBank in NCBI with the accession number the PCR-sequenced done with the Maxime PCR PreMix kit (i-Taq) 20μlrxn with 100%,96% and 99% identitied as shown in Table 2. The 16s rRNA was amplified (Fig. 1) and subjected to purification and sequencing.

Table 2: Identified bacteria based on sequencing and their similarity with GenBank

Bacteria	Sequence ID	Gen Bank accession no.	% similarity/GenBank closest relative
Lysinibacillus fusiformis	KY038703.1	JN1425	100%
	KF916675.1	JN1435	96%
	KM280032.1	JN915	99%

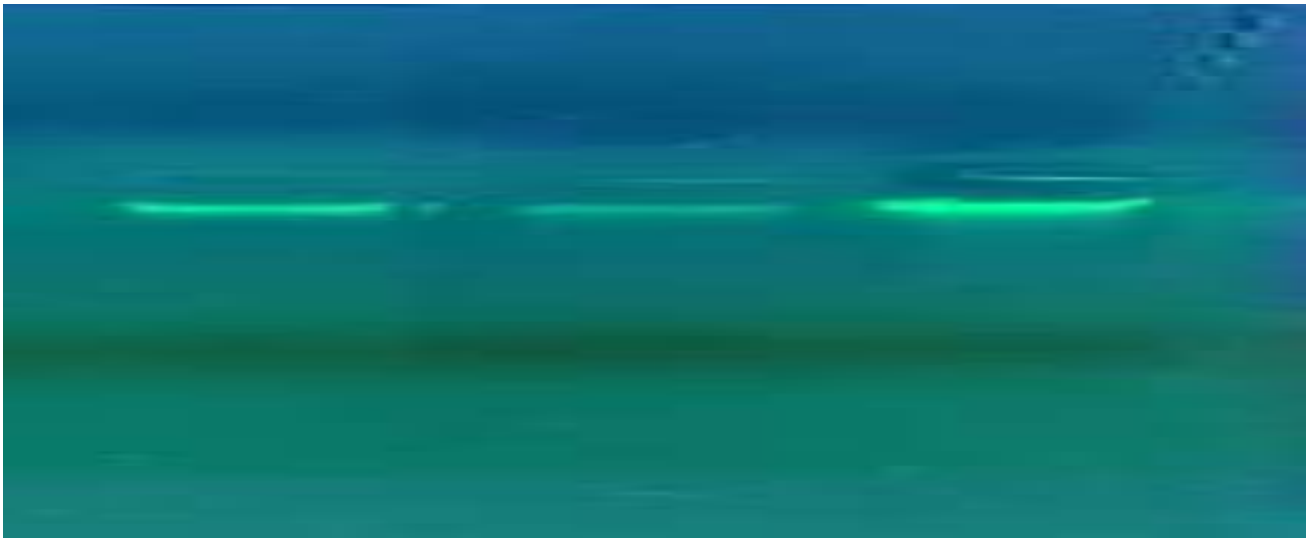

Figure |1: Gel electrophoresis of genomic DNA extraction from bacteria, 1% agarose gel at 5 vol /cm for 1:15 houre

The homology to *Lysinibacillus fusiformis* DNA sequencing demonstrate 100%,96% and 99% of isolates as shown (Fig.3,4 and 5 respectively). The sequence (approximately 1620, 1817and 1315 bp, respectively) (Fig. 2).

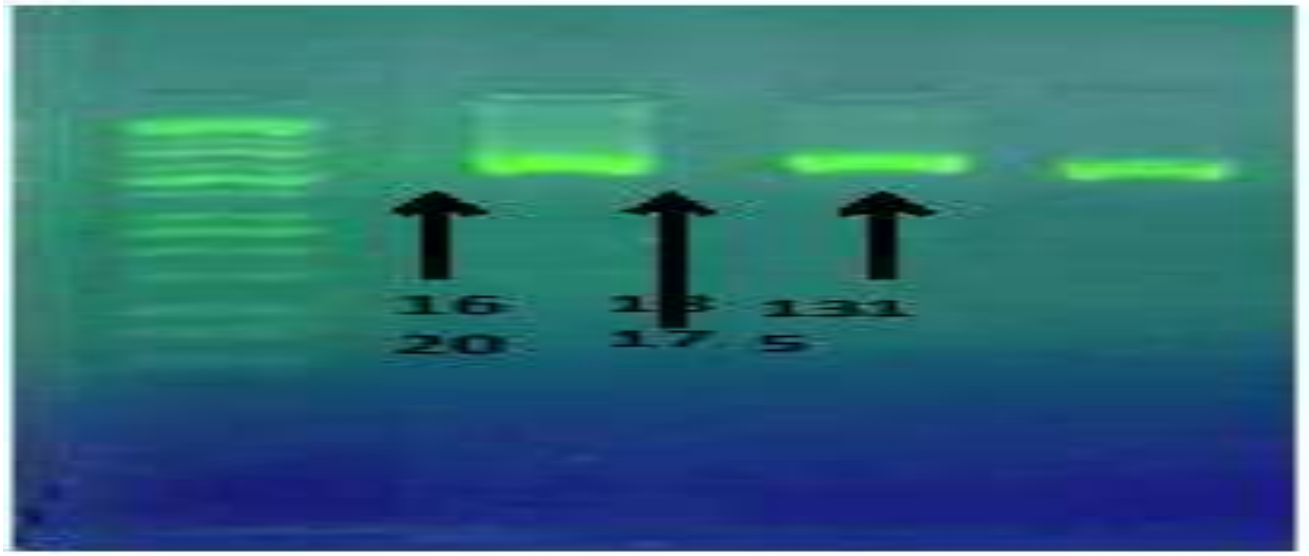

Figure 2: PCR product the band size 1600 bp. The product was electrophoresis on 2% agarose at 5 volt/cm². 1x TBE buffer for 1:30 hours. N: DNA ladder (100)

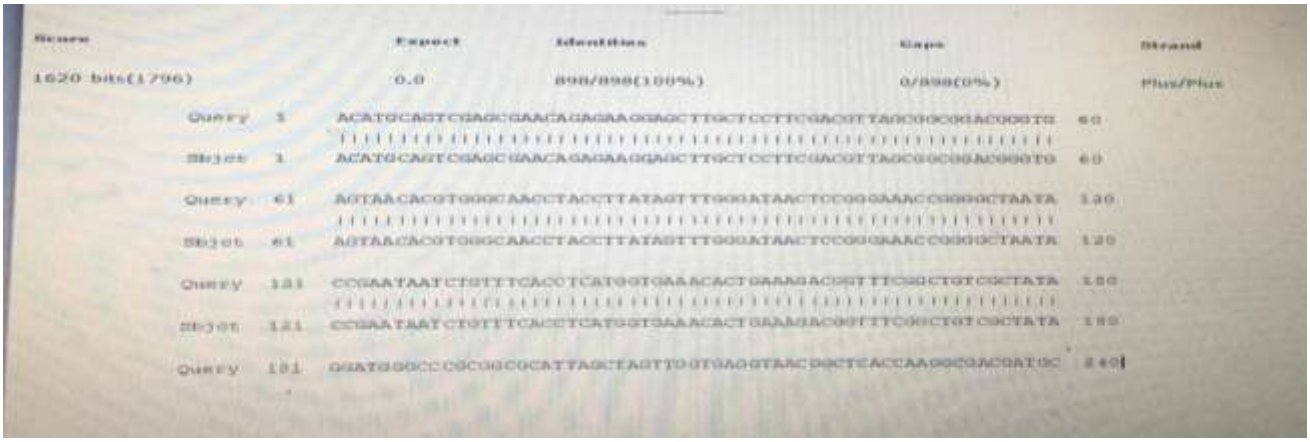

Figure 3: The sequence of *Lysinibacillus fusiformis* that explain similarity 100% of the first isolate as shown in table 2

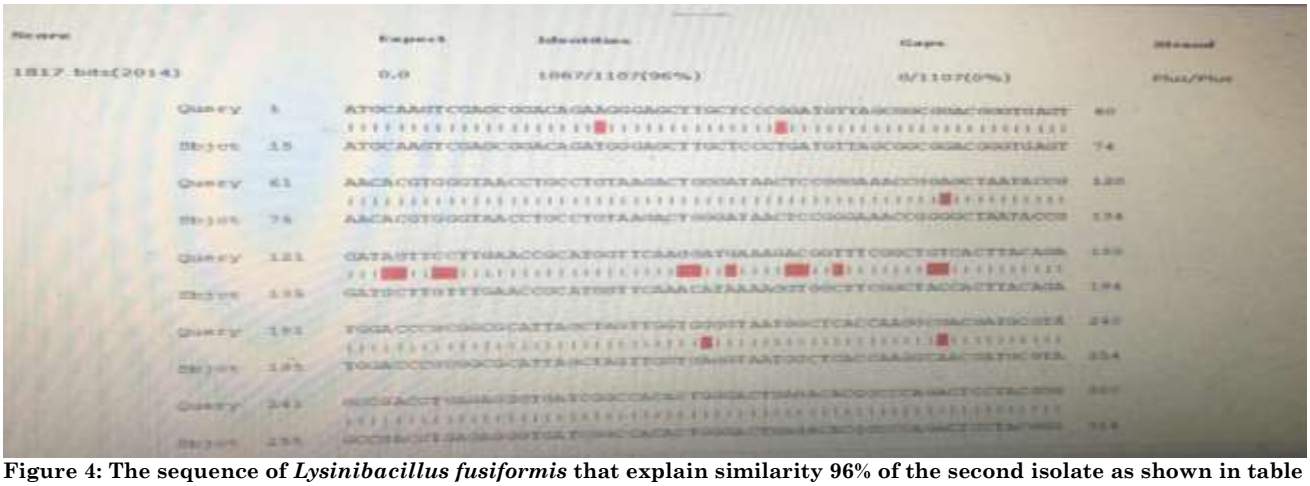

Figure 4: The sequence of *Lysinibacillus fusiformis* that explain similarity 96% of the second isolate as shown in table 2

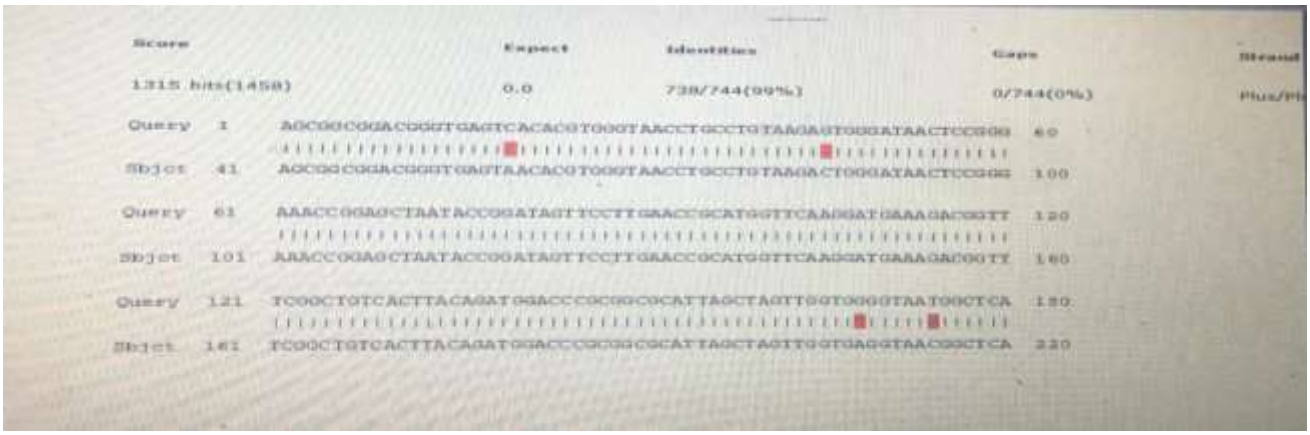

Figure 5: The sequence of *Lysinibacillus fusiformis* that explain similarity 99% of the third isolate as shown in table 2

Discussion

The genus Lysinibacillus contain more species and the bacteria L. fusiformis is one of the most recognized species in this genera [14]. (Priest et al 1988).The bacterial strain Lysinibacillus fusiformis was first isolated in 1901 from crop, Beta vulgaris cited by [15].

The bacteria confined from crude drain test among several bacterial colonies and appeared capacity to spoilage the raw milk as well as isolated from different environmental sources like exfoliating cream , tamarind seed (Tamarindus indica,cow dung,raw milk and dairy farms, waste material and industrial effluent etc. [15,16,17,18,3,19,8]. The bacteria resist many metal and antibiotic and it cause a disease to human such as tropical ulcers and dermal respiratory infections [8,10].

The aim of this research was to evaluated the spore forming bacteria in untreated milk and the influence of season on bacterial isolate, the prevalence of Lysinibacillus fusiformis isolation was 21.61% this percent indicate that the raw milk contaminated with bacteria Besides, as changes in cultivate operational management will influence the high-impact spore-forming microbiota in drain, conceivably indeed select for as yet unknown species, uncommon sharpness on microbial milk quality is required [3]. Morever Pasteurization and refrigeration of milk very necessary to decreased contamination which lead to milking spoilage that's agree with [20], all isolated obtained during spring and early summer that indicate the bacteria prefer moderate tempreture for its multiplication .

The bacteria was isolated and identified by culturing characteristic, biochemical test and confirm diagnosis via 16S rRNA gene sequence Besides, 16S rRNA gene sequencing has been connected to affirm the bacterial species distinguishing proof data[21,22] .

The 16sRNA gene sequencing of Lysinibacillus fusiformis was compared with sequences in the NCBI GenBank database with pairwise similarity values of 100%, 96% and 99 %, respectively in the present study one Lysinibacillus fusiformis obtaine 96% similarity which needs to be further characterized.

To the authors' knowledge, this is the first documentation of Lysinibacillus fusiformis bacteria, isolated from raw milk in Baghdad,Iraq.

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