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Cairo University
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Department of Microbiology

Molecular characterization and identification of bacilli isolated from broiler gastrointestinal tract

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For The degree of

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ABSTRACT

The importance of *Bacillus* bacteria is increasing day after day. They are considered potential alternatives for antibiotics due to their antimicrobial activity against most pathogenic bacteria. The goal of the present study was to isolate and identify *Bacillus* species with antimicrobial activity. A total of 110 intestinal samples taken from broiler chickens of different ages (3- 5 weeks) from different retail markets in Cairo were examined bacteriologically to detect bacilli. *Escherichia coli, Proteus* species and *Bacillus* species were isolated. The *Bacillus* isolates were confirmed by VITEK, PCR, sequencing of the 16S rRNA gene and phylogeny. Thirty two bacilli species (17 *Bacillus amyloliquefaciens,* 7 *Lysinibacillus sphaericus,* 3 *Bacillus cereus,* 2 *Bacillus licheniformis* and 2 *Lysinibacillus macrolides* and one *Bacillus altitudinis*) were detected. The isolates were screened for their antimicrobial activity against indicator Gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Salmonella* Enteritidis), Gram positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), and Yeast (*Candida albicans*) using cross streak, disc diffusion and well diffusion agar methods. The best inhibition results were achieved by cross streak method and disc diffusion method. *S. aureus, L. monocytogenes* and *Candida albicans* were the most sensitive indicator bacteria followed by *Salmonella* Enteritidis then *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli*. Interestingly, using the cross streak method, and disk diffusion method of the whole bacterial culture, showed that nearly all the tested bacilli could possess their antimicrobial activity against the indicator bacteria. On the other hand, by using well diffusion method of the culture filtrate, all the tested bacilli couldn't inhibit the growth of the indicator bacteria except *Bacillus amyloliquefaciens* that showed inhibition activity against *S. aureus,* and *Escherichia coli*. These findings demonstrate that the local spore forming isolates have probiotic properties which can be further developed as poultry feed additive or used in further industrial uses. In addition, the cross streak method and the disk diffusion method were suitable for a preliminary assessment of the antagonistic effects of the isolates.

Keywords: Antimicrobial activity, *Bacillus* species, Broilers, *E. coli*, Intestine, *Lysinibacillus sphaericus*, Phylogeny, *Proteus* species.

Dedication

I would like to express my appreciation and gratitude to my family for their patience, help and encouragement, especially my parents, who supported me too much during this work. May Allah bless them more, grant them with the best life and accept their good deeds.

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List of Contents

List of Tables

List of Figures

List of abbreviations

16S rRNA	16 S ribosomal Ribonucleic acid
BLIS	Bacteriocin like inhibitory substance
BLSs	bacteriocin-like substances
CFS	Cell free supernatant
CLPs	Cyclic lipopeptides
gDNA	Genomic Deoxyribonucleic acid
GIT	gastrointestinal tract
IFC	Imaging flow cytometry
LPs	lipopeptides
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Multiple sequence alignment
NCBI	National Centre for Biotechnology Information
NRPSs	Non-ribosomal peptide synthetases
OTC	Oxytetracycline
PBS	Phosphate Buffered saline
PCR	polymerase chain reaction
PPA	Phenyl pyruvic acid
SC-CSPB	Spore coat and canoe-shaped parasporal body
SNE	Subclinical necrotic enteritis
VP	Voges-Proskauer
WGS	Whole-genome sequencing

1- Introduction

Introduction

The gastrointestinal tract (GIT) of poultry is densely populated with microorganisms which closely and intensively interact with the host and ingested feed (**Desouky *et al*., 2021)**. The gut microbiome benefits the host by providing nutrients from otherwise poorly utilized dietary substrates and modulating the development and function of the digestive and immune system (**Pan and Yu, 2014**).

Elimination of the antibiotics from feed can make poultry more vulnerable to infection by certain pathogens (e.g. *Escherichia coli* and *Salmonella* species) resulting in poor performance and serious damage to poultry industry. Considering these issues, probiotics, live microbial feed useful for animal health have substituted some growth promoting and prophylactic products. Several studies indicated that broilers fed with probiotics showed greater resistance to pathogen infection as well as better performance (**Teo and Tan, 2006**). In addition, the multidrug resistant bacteria cause serious problems for the treatment of bacterial diseases. Antimicrobial peptides such as bacteriocins or bacteriocins-like molecules could be a comfortable solution to remedy the resurgence of antibiotics (**Najafi *et al*., 2005)**.

Genus *Bacillus* was established in 1872 by Cohn and encompasses over 200 described species and subspecies belonging to the *Firmicutes* phylum based on the morphological characteristics **(Logan and De Vos, 2009**).

Members of the genus *Bacillus* are Gram-positive, aerobic and endospore forming bacteria characterized by rod-shaped cell morphology, catalase production and their ubiquitous distribution. They are found in diverse environments such as soil, clay, rocks, dust, aquatic environments, vegetation, food and the gastrointestinal tracts of various insects and animals **(Nicholson, 2002)**.

Most species of *Bacillus* and *Brevibacillus* are difficult to distinguish from each other based on their phenotypic characteristics (**De Vos, 2009**).

Bacillus species are among the most investigated biocontrol agents i.e., bio pesticides which contribute to suppression of plant pathogens by antagonism and/or competition **(Mnif and Ghribi, 2015**). Inhibition of pathogen growth by *Bacillus* species entails the involvement of mechanisms such as competition for