RESEARCH ARTICLE

Molecular Identification and Pathogenic Potential of Clinical Bacillus Sp.20

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ABSTRACT

Antibiotic resistance bacteria has become a worldwide problem. The shortage of new antibiotics has prompted research into chemicals that could act as adjuvants and enhance the efficacy of available antibiotics. Bacterial susceptibility to antibiotics was determined using the e-test method to evaluate the effect of commonly used antibiotics on the selected isolate (S20). The isolate displayed resistance to cefoxitin, benzylpenicillin, oxacillin, erythromycin, ciprofloxacin, and azithromycin. Whereas it was susceptible to the rest antibiotics. In this study, we reported the draft genome sequence of S20, isolated from a patient. The genome consists of 4,557,070 bp, with a GC of 46.4%. It has 228 RNA reads that protein-coding genes encoding multidrug resistance transporters, virulence factors. The draft genome sequences project was deposited in GenBank under accession no.. PRJNA480592. The version described in this paper is the first version. To estimate the phylotypes in the selected genomes, the 16S rRNA gene sequences were retrieved from the RAST annotation and used as a query against the SILVA reference database with the threshold set to above 97%. Bacillus mycoides was the closest genus to our isolate (100%).

Keywords: Antibiotic resistance, Bacillus sp. SILVA, Database, Whole genome sequences

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INTRODUCTION

There are many soil-related bacterial pathogens can cause serious human disease. Bacillus species are gram-positive, rod-shaped and spore-forming bacteria. The growth on solid media has a particular rhizoid shape. Earlier studies showed the genome size was ranged from 5.1–6.25 megabases.[1,2]

There are many related species with *B. cereus,* such as *B. anthracis, B.mycoides, B. thuringiensis, B. pseudomycoides, B. weihenstephanensis,* and some new identified related species.[2] So far, the most clinically significant isolates were *B. anthrax, B.cereus, B. megaterium, B. polymyxa, B. pumilus, B. subtilis, and B. amyloliquifaciens* that cause of local infections.[1] The pathogenicity of Bacillus species (non-anthrax) has been investigated poorly, and several virulence factors such as motility, biofilm producing , swarming, capsule-forming can increase their potential pathogenicity.[3] However, other *Bacillus species e.g., B. mycoides, Paenibacillus* and *B. velezensis* received less attention compared to other members.[1] The different *Bacillus species* produce various extracellular products, including enzymes such as hemolysin, phospholipase, urease, protease, pigments, and antimicrobial substances.[4]

A wide range of human infections are linked with this bacteria, such as skin infections, osteomyelitis, fulminant eye infections, endophthalmitis, and sever systematic diseases.[2] Many studies demonstrated that Bacillus species are susceptible to penicillin and cephalosporin with the exception of B. cereus.[3,5] Whereas the most effective bactericidal synergy drugs were clindamycin-gentamicin combination against *B.cereus* infections. The presence of antibiotics resistance genes in these bacteria could be impacted by environmental stress, that potentially transferable genes in the environment.[6]

As far from being sterile environments, metal polluted soils often harbor unique and phylogenetically diverse bacterial communities such as actinomycetes and firmicutes, and these microorganisms could be the key elements for bioremediations systems. Next generation technique has a major role to study the whole genome of novel bacterial isolates and open new perspective in ecological studies. In general, this study focuses on the bacterial genes *Bacillus sp.* gram-positive, spore-forming, non-motile, filamentous microbes that produce wide variety of bioactive natural products, such as polyketides and non-ribosomal peptides.[7]

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MATERIAL AND METHODS

Bacterial Strain

Isolate S20 was isolated from the blood samples from patients who were hospitalized at Al-Zahraa hospital, Diyala, Iraq. Isolate was grown on Blood and LB agar (BioMerieux, France). Blood base medium was prepared as manufacturer recommendation (Difco, Detroit, Mich).

Susceptibility Test

The minimal inhibitory concentration (MIC) of 15 antimicrobial was used against the selected isolate (S20) was determined using E-test strips (Himedia laboratories Pvt Limited, India). Each isolate was inoculated into 5 mL of Muller Hinton Broth (MHB) and incubated for 18 hours at 37°C at 200 rpm. The overnight culture was adjusted to 0.5 McFarland standards. A volume of 100 µL of the standardized bacterial inoculum suspension was spread on MHA plates. A relevant antibiotic E-test strip was then placed at the center of each plate and incubated at 37°C for 18 hours.[8] The MIC was then determined by measuring the inhibition ellipse according to the manufacturer's guidelines. The MIC breakpoint for susceptibility was defined as the lowest drug concentration that exhibited complete inhibition of microbial growth. The experiments were done in triplicate and repeated twice.

DNA Extraction

Whole genome DNA sequencing and genome assembly were outsourced to the Genome Sequencing facility upon provision of genomic DNA samples. The whole genome from isolates of S20 were aligned using Rapid Annotation using Subsystems Technology (RAST).

Taxonomic Affiliation

The assembled genomes in multi-contig format. To estimate the phylotypes of the selected genomes, the 16S rRNA gene was used as query against the SILVA reference database with the threshold set to above 98%.

Resistance Gene Prediction

Isolate 20 draft genomes were analyzed using the Comprehensive Antibiotic Resistance Database (CARD; version 1.2.0) to estimate the total number of antibiotic resistance genes ARGs (https://card.mcmaster.ca/analyze/rgi).[9]

RESULTS AND DISCUSSION

Morphological and Antimicrobial Susceptibility Test

The isolate was raised rhizoid colonies with a counter-clockwise filamentous swirling pattern and was positive to gram stain. The isolate exhibited a hemolytic pattern of hemolysin BL. Isolate 20 were subjected to antibiotic susceptibility tests, which exhibited different degrees of resistance to the selected antibiotics. The isolate was resistant to cefoxitin, benzylpenicillin, oxacillin, erythromycin, ciprofloxacin, and azithromycin. Whereas it was susceptible to the rest antibiotics Table 1. Identification of potentially spore-forming microbial species in the clinical samples could explain the pathogenic tolerance to high temperature, antibiotics, metals, and toxic chemicals.[10] Early identification of these pathogenic might help to treat and reduce bacterial infections. However, many resistance genes of Bacillus mycoides sp. S20 might not be expressed under the idea that many mutations do not lead to resistant phenotype. For this reason, this research used antimicrobial databases, such as CARD and RAST tools to detect the resistance genes.

Table 1: Antibiotic susceptibly of Bacillus sp. 20

Antibiotic	*Susceptibility*
Cefoxitin	R
Benzylpenicillin	R
Oxacillin	R
Azithromycin	R
Ciprofloxacin	R
Erythromycin	R
Clindamycin	S
Daptomycin	S
Vancomycin	S
Tetracycline	S
Ampicillin	S
Ampiclox	S
Neomycin	S
Kanamycin	S
Meropenem	S

S–Susceptible; I–Intermediate; R–Resistant (CLSI guidelines, Cockerill et al., 2012).

Nucleotide Sequence Accession Number

Sequencing and assembly statistics of eight isolates which passed stringent quality criteria are shown in Table 2. The sequenced genome generated 30 contigs spanning 4.557.070 bp, with GC content 46.4%, and 228 RNAs. And the draft genome was deposited in GenBank under accesses no. PRJNA480592. The version described in this paper is the first version.

Taxonomic Identification

Taxonomic classification based on 16S rRNA gene sequence analysis using the SILVA database as reference revealed that the sequenced isolates belonged to the genus, Bacillus mycoides sp. with 99% similarity. The WGS of these isolates were selected for further comparative genomic analyses to aid the identification of genetic elements associated with differential resistance and tolerance phenotypes.

Genes Encoding Virulence Factors

Analysis of the genome revealed a large number of genes encoding virulence factors that may contribute to the pathogenicity. Most are degradative enzymes and adhesins that may facilitate infection, identification and characterization of putative antibiotic resistance genes (ARGs). The number of predicted antibiotics and metal resistance genes was obtained by browsing the annotated genomes in SEED viewer based on homology to genes in the RAST database(Figure 2), whereas

Table 2: Draft genome assembly matrix for genome sequences.

Isolate Name	*Total number of reads*	*Genome size (mb)*	*GC (%)*	*N 50*	*Number of contigs (>= 1000 bp)*	*Largest contig*	*Number of RNAs*
20	3822	4,557,007	46.4	117	30	1956809	228

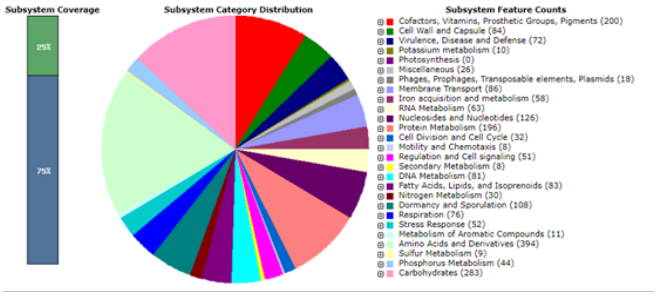

Figure 1: An overview of the subsystem categories of antibiotic resistance genes using RAST server. Number of the genes with associated functional categories of de novo assembled Bacillus sp.20 genome.

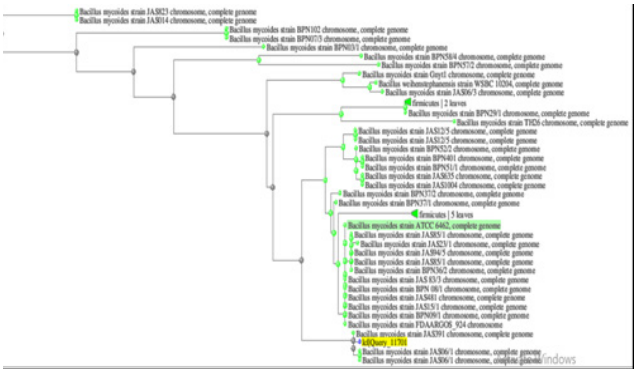

Figure 2: Taxonomic classification of the 16S rRNA genes at similarity threshold of 97 %

Table 3: The total coverage of similarity for Bacillus sp. 20 genome using reference amino acid sequences.

Gene type	Resistance profile
mprF	Antibiotic target modifying enzyme; peptide antibiotic resistance gene
bcrA	Efflux pump conferring antibiotic resistance; peptide antibiotic resistance gene
ileS	Mupirocin resistance gene
blt, emeA	Efflux pump conferring antibiotic resistance; fluoroquinolone resistance gene
EF-Tu	Antibiotic resistant gene variant or mutant; elfamycin resistance gene
lmrD	Efflux pump conferring antibiotic resistance; lincosamide resistance gene
mecC, mecB	Antibiotic resistance gene cluster, cassette, or operon; beta-lactam resistance gene; antibiotic inactivation enzyme; antibiotic target replacement protein
mecC, mecB	Antibiotic resistance gene cluster, cassette, or operon; beta-lactam resistance gene; antibiotic inactivation enzyme; antibiotic target replacement protein
PBP1b	Antibiotic inactivation enzyme; beta-lactam resistance gene; antibiotic target replacement protein
sav1866	Efflux pump conferring antibiotic resistance
rpoB	Rifampin resistance gene; antibiotic resistant gene variant or mutant
TetB	Antibiotic target protection protein; tetracycline resistance gene

the predicted genes under functional Sub-categories/virulence, disease and defense window (Figure 1).

The evolution of antibiotic resistance genes in bacteria can become a major course of concern in different environments, and bacterial resistance could be influenced by interactions with antibiotic-producing competitors.[3] However, many bacterial strains have 'intrinsic' activity of broad-spectrum efflux pumps systems which are mediated by several genes.[2]

Identification and Characterization of Putative Antibiotic Resistance Genes

(ARGs) with RAST/SEED and CARD Tool

Furthermore, CARD tool was used to explore the diversity and abundance of Antibiotics Resistance Genes (ARGs). The Resistance Gene Identifier (RGI) hits 15 predicted resistance proteins that have the same distribution among all draft genomes. Most putative resistance proteins belonged to the efflux pump system, rifampin resistance genes, putative resistance to mupirocin, lincosamide, elfamycin, fluoroquinolone, and finally Glycopeptide resistance genes (Table 3).

Whole genomes comparison was performed using CARD and RAST tools to scan the functional profiling and evaluate the genetic variations of resistance determinants in the draft genome. An earlier study demonstrated that the microorganisms isolated from coal combustion ash settling basins were resistant to kanamycin, gentamycin, tetracycline, ciprofloxacin and streptomycin.[8] Another study, Ming-Feng Lin *et al.,* (2014) found that many efflux pump systems contribute to antibiotic resistance in Acinetobacter baumanii strains.[11]

To conclude, this study demonstrated the virulence and antibiotic resistance data-based on draft genome and antimicrobial susceptibility testing. The obtained data could be used to assess the bacterial community (as microbial flora) and detect bacterial infections which has important applications in the clinic and disease diagnosis. However, many resistance genes of *Bacillus mycoides sp.* 20 might not be expressed under the idea that many mutations do not led

to resistant phenotype.[12] For this reason, this research used antimicrobial databases, such as CARD and RAST tools to detect the resistance genes. These genes may be hazardous to immunocompromised patients.

References

1. Abdelhameed A, Alqaysi R, Hilal HA, Alharbi N. Purification of L-Glutaminase from Bacillus sp. B12 and study its properties. Indian Journal of Forensic Medicine & Toxicology. 2020;14(2):1161.
2. Quinn GA, Abdelhameed AM, Banat AM, Alharbi NK, Baker LM, Castro HC, et al. Streptomyces Isolates from the Soil of an Ancient Irish Cure Site, Capable of Inhibiting Multi-Resistant Bacteria and Yeasts. Applied Sciences. 2021;11(11):4923.
3. AL-Dulaimi M, Algburi A, Abdelhameed A, Mazanko MS, Rudoy DV, Ermakov AM, *et al*. Antimicrobial and Anti-Biofilm Activity of Polymyxin E Alone and in Combination with Probiotic Strains of Bacillus subtilis KATMIRA1933 and Bacillus amyloliquefaciens B-1895 against Clinical Isolates of Selected Acinetobacter spp.: A Preliminary Study. Pathogens. 2021;10(12):1574.
4. Silva LN, Zimmer KR, Macedo AJ, Trentin DS. Plant natural products targeting bacterial virulence factors. Chemical Reviews. 2016;116(16):9162-236.
5. Tuazon CU. Bacillus species. Infectious Disease & Antimicrobial Agents. 2010.
6. Abdelhameed A, Naser RH, Salman AD. Whole Genome Sequence and Development of a Transposon Mutagenesis System for Pseudomonas Putida sp. 12. Indian Journal of Forensic Medicine & Toxicology. 2020;14(3).
7. D'Costa VM, Griffiths E, Wright GD. Expanding the soil antibiotic resistome: exploring environmental diversity. Current Opinion in Microbiology. 2007;10(5):481-9.
8. Abdelhameed A, Alharbi N, Algburi A. Genome Sequence And Assessment Of The Virulence And Antibiotics Resistance Of A Novel Paenibacillus Sp. 6a Isolated From A Patient With Acute Otites Media In Diyala City, Iraq.
9. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, *et al.* The comprehensive antibiotic resistance database. Antimicrobial agents and chemotherapy. 2013;57(7):3348-57.
10. Chau KM, Van Quyen D, Fraser JM, Smith AT, Van TTH, Moore RJ. Broad spectrum antimicrobial activities from spore-forming bacteria isolated from the Vietnam Sea. PeerJ. 2020;8:e10117.
11. Lin M-F, Lin Y-Y, Lan C-Y. The role of the two-component system BaeSR in disposing chemicals through regulating transporter systems in Acinetobacter baumannii. PLoS One. 2015;10(7):e0132843.
12. Humphries RM, Pollett S, Sakoulas G. A current perspective on daptomycin for the clinical microbiologist. Clinical microbiology reviews. 2013;26(4):759-80.