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EVALUATION OF PROPHAGES AND ANTI MICROBIAL RESISTANCE PROFILE OF BACILLUS SUBTILIS USING IN SILICO APPROACH

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ABSTRACT

The main objective of this study was to identify the prophages and antimicrobial resistance profile of Bacillus subtilis using computational methods. Prophages are potential therapeutic antimicrobial agents against antibiotic- resistant pathogens. The potential of prophage to induce antimicrobial resistance and increase pathogenicity in bacterial genomes resulted in renewed interest in the identification of prophage sequences and their associated antimicrobial resistant genes in bacterial genomes. Sequences of Bacillus subtilis retrieved from the sequence raw archive (SRA) of the National Centre for Biotechnology Information was submitted to bacterial viral bioinformatics resource centre for genome assembly. Prophage sequences were identified using the PHASTER server. The region length, region position and GC percentage of nucleotides of prophage sequences were identified. The antimicrobial resistance gene family, drug class, resistance mechanism and length of reference sequences were identified using the resistance gene identifier protocol of the comprehensive antimicrobial resistance database. It can be concluded that the Bacillus subtilis strain evaluated contained prophage sequences associated with antimicrobial resistance genes, there is, therefore, the need to assess the safety of bacteriophages before they are utilized as alternative to antibiotics.

Keywords: Bacillus, Bacteriophages, Antibiotic resistance, In silico

INTRODUCTION

Bacteriophages or phages are bacterial viruses that can disrupt bacterial metabolism, the antibacterial potential of bacteriophages has been documented (Alexander *et al*., 2001). The emergence of antibiotic resistant pathogenic microbes is a global threat to public health. The development of alternative antimicrobial agents is therefore, one of the important priorities of medical research. The threat of antimicrobial resistance to human health has become recognized globally, it is associated with the death of millions of people annually (Neill, 2016). The urgent need for alternative and effective antibiotics has generated interest in prophages as alternative means of treatment of infections caused by antibiotic resistant bacteria. A large number of phages are reported to be present in the digestive system and may play a significant role in modifying immune functions (Gorski *et al*., 2006). Prophages are capable of bypassing the epithelial layer thereby influencing immune responses in different parts of the body.

Studies have indicated that prophages do not exert significant harmful effects on mammalian cells (Minasyan, 2017). Apart from antibacterial action, phages are reported to have anti inflammatory and immune modulatory effects The use of bacteriophages to combat antimicrobial resistance must comply with quality assurance and safety measures including elliminating the risk of prophage contamination with resistant bacterial cells. Bacterial lines used for the manufacture of bacteriophages must be assessed for phages and antimicrobial resistant genes to prevent the transfer of virulent genes and antimicrobial resistance to other bacterial strains in the patient. The main objective of this study was to identify prophages and antimicrobial resistant genes in Bacillus subtilis strain using computational methods.

MATERIALS AND METHODS

Data retrieval and genome assembly

Data on Bacillus subtilis was retrieved from sequence raw achieve (SRA) of the National Centre for Biotechnology Information. The retrieved sequence was submitted for genome assembly using the database of the Bacterial Viral Bioinformatic Resource Centre (BV-BRC). The database is an information system designed to support research on bacteria and infectious diseases (Olson *et al*., 2023).

Prophage identification using the PHASTER server

The assembled genome file obtained from BV-BRC was submitted for prophage identification using Phage Search Tool Enhanced Release. The PHASTER database is reliable for rapid identification and annotation of prophage sequences within bacterial genomes and plasmids (Amdt *et al*., 2016).

Evaluation of antimicrobial resistance profile

Identification of antimicrobial resistance genes was done using the resistance gene identifier protocol of the Comprehensive Antimicrobial Resistance Database (CARD). It is a bioinformatic database of resistance genes, their products and associated phenotypes (Alcock *et al*., 2023). The database combines antibiotic resistance ontology with curated antimicrobial resistance gene sequences and mutations that provide a framework for annotation and interpretation of resistomes.

RESULTS AND DISCUSSION

Prophage sequences identified in Bacillus subtilis using PHASTER server are presented in Table1.

Table 1: Prophage sequences of Bacillus subtilis identified using PHASTER server

Region length	Score	Region position	Phage	GC %
6.2 Kb	20	302060- 308273	PHAGE_Bacil_vB_BsuM_Goe3_NC_048652	44.27%
8.5 Kb	40	346291 -354878	PHAGE_Bacil_AR9_NC_031039	43,06%
10.7 Kb	30	16561 - 27338	PHAGE_Gordon_Hedwig_NC_031099	44.43%
6.8 Kb	10	316359 - 323198	PHAGE_Escher_vB_EcoM_Schickermooser_NC_048196	40.61%
32.5 Kb	100	50616 - 83168	PHAGE_Bacil_SPbeta_NC_001884	46.81%
13.1 Kb	20	27178 - 40308	PHAGE_Bacil_G_NC_023719	45.54 %
8.3 Kb	10	203060 - 211415	PHAGE_Prochl_Syn33_NC_015285	50.34 %

Intact score > 90
Questionable (score 70-90)
incomplete (score < 70)
Region Length: The length of the sequence of that region (in bp).
Score**:** The score of the region based on the above criteria.
Region Position**:** The start and end positions of the region on the bacterial chromosome.
Most Common Phage**:** The phage(s) with the highest number of proteins most similar to those in the region.
GC %: The percentage of GC nucleotides of the region.
Antimicrobial gene family, drug class and resistance mechanism identified in Bacillus subtilis are presented in table 2.

Table 2: Antimicrobial resistance profile of Bacillus subtilis strain

AMR Gene family	Drug class	Resistance mechanism	% Identity of matching region	% Length of reference sequence
Cfr 23S ribosomal RNA Methyltranferase	Lincosamide antibiotic, streptogramin antibiotic, oxazolidinone antibiotic, phenicol antibiotic pleormutinine antibiotics	Antibiotic target alteration	97.42	100
Class A Bacillus ccereus BC bet-lactamase	Cephalosporin	Antibioticinactivation	62.42	100
Fosfomycin thiol transferase	Phosporic acid antibiotic	Antibiotic inactivation	64.23	100
Glycopeptide resistance gene cluster, Van T	Glycopeptide antibiotic	Antibiotic target alteration	33.75	55.20
Major facilitator superfamily (MFS) antibiotic efflux pump	Tetracycline antibiotic	Antibiotic efflux	75.49	100
Small multidrug resistance (SMR) antibiotic efflux pump	Disinfecting agents and antiseptic	Antibiotic efflux	44.9	100

The prophages and antimicrobial resistance profile of Bacillus subtilis evaluated are shown in tables 1 and 2. The high rate of development of antimicrobial resistance compared to the level of antibiotic discovery represent a global public health challenge (Futaro *et al*., 2018). There is therefore, a need to give special attention to the development of phages as potential therapeutic antimicrobial agents against antimicrobial resistant pathogens. One of the advantages of bacteriophage is that very few doses are required because of the increase concentration of bacteriophage at site of infection after the initial administration, their effects are also limited to the site of infection (Pouilot *et al*., 2012). Significant reduction in the cost of treatment has also been associated with use of bacteriophages. Prophages induce antimicrobial resistance and increase pathogenicity in bacterial species leading to renewed interest in identification of prophage sequences in bacterial genomes.

CONCLUSION

Findings of this study indicated the presence of prophages associated with antimicrobial resistance genes in Bacillus subtilis strain evaluated. There is therefore a need for quality assessment of bacteriophages before they are used as alternative to antibiotics. Prophage screening is essential measure to prevent the transfer of antibiotic resistant genes to bacterial genomes.

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