



Genomic analysis of endophytic *Bacillus altitudinis* strain VUMS1 from *Kappaphycus alvarezii* and its inhibitory effect against *Vibrio parahaemolyticus*

Vernon Vest Mangun, Wilson Thau Lym Yong and Nur Athirah Yusof*

Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, Kota Kinabalu, 88400, Sabah, Malaysia.
Email: nrathirah.yusof@ums.edu.my

ABSTRACT

Aims: Sabah's red algae, *Kappaphycus alvarezii* is facing a problem whereby the production of seaweed is declining over the years due to a disease called ice-ice disease caused by *Vibrio* spp. Endophytic *Bacillus* strains have been widely studied for their potential as biocontrol agents against harmful pathogens. This study reports the genome sequence of the beneficial endophytic *Bacillus* strain VUMS1 isolated from the healthy *K. alvarezii* at Semporna Island in Sabah, attempting to determine its full biocontrol potential.

Methodology and results: The whole genome sequence showed that VUMS1 genome size is 3,754,982 bp with 3,854 protein-coding where 2,535 are genes with assigned functions. The analysis revealed the presence of genes that are involved in antimicrobial and antifungal activity such as fengycin, bacillibactin, bacilysin and lichenysin. The biocontrol potential of VUMS1 was evaluated against *Vibrio parahaemolyticus* isolated from the diseased *K. alvarezii*. Results showed that the inhibition zone of VUMS1 by cross-streaking method against *V. parahaemolyticus* was 21 ± 0.71 mm and the growth of *V. parahaemolyticus* treated with VUMS1 in a co-culture experiment decreased by 98% on day 5 of treatment.

Conclusion, significance and impact of study: The results of this work indicate that VUMS1 is affiliated as *Bacillus altitudinis* and it may contribute to the biocontrol activity against *Vibrio* spp. infection in *K. alvarezii*. This is the first report of endophytic *Bacillus altitudinis* from *K. alvarezii* with biocontrol properties. Future studies will determine the potential application of the *B. altitudinis* VUMS1 strain in biological control and growth promotion for sustainable seaweed farming.

Keywords: *Bacillus altitudinis*, biocontrol potential, endophytic bacteria, genomic analysis, *Kappaphycus alvarezii*

INTRODUCTION

Kappaphycus alvarezii is a commercially important species of seaweed, commonly known as "cottonii" or "*Eucheuma cottonii*". It is valued for its carrageenan content, which is a type of phycocolloid extracted from seaweeds. Carrageenan is a polysaccharide that is widely used in various commercial applications in the food, pharmaceutical and cosmetic industries due to its unique gelling, thickening and stabilising properties (Loureiro *et al.*, 2017). Recently, *K. alvarezii* is facing a problem whereby the production of the seaweed is declining over the years due to environmental stresses which leads to an infestation of pathogenic bacteria species known as *Vibrio* spp. such as *Vibrio parahaemolyticus* which causes ice-ice disease (IID) (Mahmud *et al.*, 2007; Tahiluddin and Terzi, 2021).

Vibrio parahaemolyticus is mainly known to be present in sea creatures such as shrimps but studies have shown that they are also known to be more abundant in seaweeds than in seawater. Experts warn people from getting into contact with water containing seaweed as it

has a high chance to get infected by *Vibrio* infection (Mahmud *et al.*, 2007; Prieur, 2023). These bacteria spoil the seaweed by lysing the seaweed's epidermal cells and cytoplasm causing the seaweed tissues to turn white. Unfortunately, little effort has been made to overcome the problem faced in the seaweed industry (Largo *et al.*, 1995; Hurtado *et al.*, 2006; Azizi *et al.*, 2018; Geraldine, 2021). *V. parahaemolyticus* not only infect seaweeds but also humans. The symptoms of *Vibrio* infection may include and not be limited to skin rash, nausea, cramping, high fever, chills and diarrhoea (Mahmud *et al.*, 2007). In recent years, districts in Sabah such as Tawau and Tenom had reported massive cases of diarrhoea, vomiting and abdominal discomfort due to unknown reasons and *Vibrio* infection might be one of the reasons behind the cases (Inus, 2022; Fong, 2023).

Endophytic bacteria are good candidates for promoting the growth and overall health of their host plants. In recent studies, endophytic bacteria are used as a biocontrol agent against harmful pathogens (Senthilkumar *et al.*, 2011; Morales-Cedeño *et al.*, 2021; An *et al.*, 2022). Endophytic bacteria are microorganisms

that live within the tissues of plants without causing any apparent harm to the host. Instead, they form a mutually beneficial relationship with the plant, providing various benefits that can positively impact plant growth and development. Even in the early stage of plant development, a stable endophytic bacterial community may have already colonised the host plant (Mocali *et al.*, 2003; Wu *et al.*, 2021). Endophytes have evolved to live inside the host plant's endosphere, which refers to the internal tissues and spaces within the plant, to create a specialised niche that offers them protection from the external environment. Since pathogenic bacteria and endophytic bacteria inhabit the same niche, endophytic bacteria are the best candidate to biologically control plant pathogens (Senthilkumar *et al.*, 2011). *Bacillus altitudinis* has been found in a variety of settings, including the southern Indian Ocean, deep freshwater in Manasbal Lake, soil and silt (Kumar *et al.*, 2010; Mao *et al.*, 2013; Halder *et al.*, 2017; Shafi *et al.*, 2017). This species has been considered a potential factor for biological control due to its broad-spectrum antimicrobial activity against *Phytophthora sojae*, *Streptomyces scabies*, *Magnaporthe oryzae*, *Colletotrichum gloeosporioides*, *Corynespora cassicola*, *Fusarium verticillioides*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* (Jin *et al.*, 2011; Lu *et al.*, 2017; Goswami and Deka, 2019; Li *et al.*, 2019). The current study was driven to identify the genes involved in antimicrobial activity as supported in previous research that *Bacillus* spp. possessed biocontrol properties against *Vibrio* spp. (Vaseeharan and Ramasamy, 2003; Gu *et al.*, 2021; Leal *et al.*, 2021). In this study, we report the genome sequence of the endophytic *B. altitudinis* strain VUMS1 and analyse its genome properties, to expand our knowledge of *B. altitudinis* benefits, as well as design efficient and sustainable biocontrol strategies for seaweed farming.

MATERIALS AND METHODS

Revival of VUMS1 and *V. parahaemolyticus* V2

The healthy and diseased *K. alvarezii* was collected from Kampung Baru-Baru, Kota Belud (6.30228, 116.29455) and around Bum-Bum Island, Semporna (4.44747, 118.68691). VUMS1 was isolated from healthy *K. alvarezii* meanwhile *V. parahaemolyticus* was isolated from diseased *K. alvarezii*. These two bacteria were stored at -80 °C in glycerol stocks. *B. altitudinis* VUMS1 and *V. parahaemolyticus* were revived from glycerol stock by culturing 10 µL of glycerol stock in sterile Luria Bertani (LB) agar (Sigma-Aldrich, USA) in 28 °C incubator for 24 h. A single colony was inoculated into 10 mL of LB broth (Sigma-Aldrich, USA) in a conical tube and incubated at 28 °C, 180 rpm overnight. Then, 1 mL of overnight culture was suspended into fresh 9 mL of LB broth in a conical tube and incubated until approximately OD₆₀₀ is 0.8 to 1.0. Cultures were stored at 4 °C until further use.

gDNA extraction

A single colony of VUMS1 was inoculated into 10 mL of LB broth in a conical tube and incubated at 28 °C, 180 rpm overnight. Wizard Genomic DNA Purification Kit (Promega, USA) was used to extract the genomic DNA (gDNA). DNA extraction was performed based on the manufacturer's protocol (Rupert *et al.*, 2022). The extracted gDNA was sent to NeoScience Sdn. Bhd. (Selangor, Malaysia) for whole genome sequencing.

Genome sequencing, assembly, annotation and bioinformatic analysis

Sequencing and assembly of VUMS1 was outsourced to NeoScience Sdn. Bhd. Based on the whole genome sequence report from NeoScience Sdn Bhd, the VUMS1 draft sequences were produced by BGISEQ and genome assembly was assembled by using De Bruijn graph v1.2.2. Genome annotation was obtained from NCBI Prokaryotic Genome Annotation Pipeline (PGAP). 16S rRNA gene sequence homology searches were performed against sequences maintained in the NCBI GenBank database using the BLASTN algorithm while 16S-based ID algorithm using EZBioCloud database for Average Nucleotide Identity (ANI) calculations. High identity of 16S rRNA gene sequence hits were used to identify closely related bacteria to VUMS1. OrthoANlu, ANIb and ANIm were used to identify the Average Nucleotide Identity (ANI) values for VUMS1 genome sequence against 15 similar genome sequences with high identity >99.9% and one random *Bacillus* sp. genome sequence downloaded from NCBI GenBank database. OrthoANlu was calculated by using EZBioCloud ANI calculator web server (Yoon *et al.*, 2017) meanwhile ANIb and ANIm were calculated using JSpeciesWS pairwise genome comparisons analysis (Richter *et al.*, 2015).

Prediction of secondary metabolite biosynthetic gene clusters (BGCs)

The antiSMASH v7.0.0 webserver (Blin *et al.*, 2019) was used to analyse the BGCs of VUMS1. Strict detection strictness was used to detect only well-defined clusters and cluster blast was added as an extra feature to identify similar BGCs in other species.

Tests for biocontrol potential against *Vibrio parahaemolyticus*

VUMS1 was tested for its ability to inhibit the growth of *V. parahaemolyticus* isolated from diseased Sabah red algae from Semporna and Kota Belud. 30 µL of VUMS1 culture with approximately OD₆₀₀ 0.8 to 1.0 was cultured in the centre of Mueller Hinton (MH) agar (Oxoid, UK) to create a single vertical streak culture and incubated at 28 °C for 48 h. *V. parahaemolyticus* was inoculated perpendicular to VUMS1 and incubated at 28 °C for 5

Table 1: General features of *B. altitudinis* VUMS1.

Category	<i>Bacillus altitudinis</i> VUMS1
Number of contigs	17
G + C content (%)	41.21
Protein-coding genes	3,854
Genes with assigned function	2,535
tRNAs	50
rRNAs (5S, 16S, 28S)	1,1,1
ncRNAs	5

days. The inhibition zone of the VUMS1 was determined and tabulated in a table for day 1 and day 5. This experiment was done in 3 replicates.

Co-culture was performed to further test the biocontrol potential of VUMS1 against *V. parahaemolyticus*. VUMS1 and *V. parahaemolyticus* were pre-cultured separately in LB broth at 28 °C for 24 h. Overnight culture of *V. parahaemolyticus* was added to fresh LB broth at OD₆₀₀ of 0.09. Then, overnight VUMS1 was added to the mixture at OD₆₀₀ of 0.8. Overnight *V. parahaemolyticus* added in fresh LB broth at OD₆₀₀ of 0.09 was the control for this test. The total volume of all mixtures was 200 mL and incubated at 28 °C. The growth of *V. parahaemolyticus* with and without VUMS1 was observed for 5 days and ten-fold serial dilution was performed each day by taking 30 µL of the mixture and cultured on thiosulfate citrate bile salts sucrose (TCBS) agar plates to count the colony forming unit (CFU) of *V. parahaemolyticus*. The growth of *V. parahaemolyticus* was determined in CFU/mL for 5 days and reported in a graph.

RESULTS AND DISCUSSION

Revival of bacteria and preparation of VUMS and *V. parahaemolyticus*

The revival of VUMS1 and *V. parahaemolyticus* from glycerol stocks were successful. The DNA of VUMS1 and *V. parahaemolyticus* was sent to Neoscience Sdn. Bhd. to further identify the bacteria. BLASTN was used to identify the species for *V. parahaemolyticus* using 16S rRNA gene sequence and deposited to NCBI under the accession number OQ866406.1, VUMS1 genome sequence was discussed further in this study.

B. altitudinis VUMS1 genome properties

The complete genome sequence of VUMS1 is available in NCBI with the accession number CP124520.1 and the accession numbers for VUMS1 BioProject and BioSample are PRJNA954470 and SAMN34146841, respectively. Table 1 shows the general features of VUMS1. The adapted and quality trimmed sequence for VUMS1 contained a circular chromosome that produced 17 scaffolds with a genome size of 3,754,982 bp distributed in 17 contigs and has 41.21% of G + C content. Out of 3,854 protein coding genes, 2,535 protein coding genes are assigned with functions and the remainder 1,319 are

hypothetical proteins. VUMS1 also has 58 RNAs in total that consists of tRNAs, rRNAs and ncRNAs.

Comparison of other related *Bacillus* strains genomes

The comparison of VUMS1 genome sequence to other related *Bacillus* strain genome sequences is crucial to identify the intergenomic distances between genomes and which species or subspecies they belong to. BLASTN results showed that VUMS1 16S rRNA gene sequence is similar to *Bacillus altitudinis* based on the GenBank database. A similar result showed in the EZBioCloud database where the 16S rRNA gene sequence was 100% similar to type-strain *B. altitudinis* 41KF2b. BLAST and EZBioCloud results were used for genome-level identification by ANI. ANI is a type of computer study that can be used to establish archaea and bacteria species boundaries. All 15 strains' genome sequences have an identity percentage of ≥99.94%, query coverage of 100% and other parameters such as E-value and gaps of 0.0. This is to ensure that the genome level identification is significantly F reliable (Pearson, 2014). In this study, three types of ANI values were used such as OrthoANIu (USEARCH), ANIb (BLAST+) and ANIm (MUMmer) to compare the difference between different algorithms (Lee *et al.*, 2016; Yoon *et al.*, 2017).

For VUMS1 to be affiliated with an assigned species, the ANI value must be equal to or higher than the cut-off values for species delimitation which is >95% (Chun *et al.*, 2018). Based on Table 2, ANI values showed significant results whereby all ANI values against genome sequence VUMS1 were >95% and the values from different ANI algorithms were close and consistent. Hence, the ANI values obtained were reliable. Among all the strains, the closest strain to VUMS1 is *B. altitudinis* strain HQ-51-Ba with an ANI average value of 98.52% and as expected, *B. valenasensis* ATR2 is the most distant strain with an average ANI value is 74.97%. Hence, VUMS1 is assigned as *B. altitudinis*, a Gram-positive, rod-shaped bacteria known to be a plant growth-promoting bacteria and has biocontrol potential against bacteria and fungi (Sunar *et al.*, 2015; Zhang *et al.*, 2021; Jankoski *et al.*, 2023).

Secondary metabolite biosynthetic gene clusters (BGCs)

The antiSMASH software v7.0.0 was used to predict the gene clusters to evaluate the genes that are present in

Table 2: Genome comparison of VUMS1 with known related strains 16S rRNA ≥99.94%.

Strains	Genbank accession	GC (%)	OrthoANIu (%)	ANIb (%)	ANIm (%)
<i>B. altitudinis</i> P-10	CP024204.1	41.52	98.48	98.28	98.50
<i>B. altitudinis</i> 11-1-1	CP054136.1	41.30	98.03	97.85	98.07
<i>B. aerophilus</i> 232	CP026008.1	41.42	98.54	98.39	98.52
<i>B. altitudinis</i> D9_B_49	CP128109.1	41.06	98.17	97.82	98.19
<i>B. altitudinis</i> GQYP101	CP040514.1	41.31	98.04	97.82	98.04
<i>B. cellulasensis</i> Ku-bf1	CP014165.1	41.64	98.57	98.30	98.55
<i>B. cellulasensis</i> NJ-V2	CP012482.1	41.30	98.38	98.13	98.40
<i>B. altitudinis</i> SCU11	CP038517.1	41.40	98.34	98.15	98.45
<i>B. altitudinis</i> SGAir0031	CP022319.2	41.40	98.11	97.91	98.12
<i>B. pumilus</i> SH-B11	CP010997.1	41.32	98.06	97.86	98.05
<i>B. pumilus</i> TUAT1	AP014928.1	41.42	98.52	98.30	98.56
<i>B. altitudinis</i> HQ-51-Ba	CP040747.1	41.40	98.56	98.44	98.56
<i>B. altitudinis</i> DSM 103869	CP120625.1	41.28	98.28	98.08	98.28
<i>B. altitudinis</i> CHB19	CP043559.1	41.34	98.17	97.95	98.13
<i>B. altitudinis</i> UKM RB11	CP094654.1	41.23	98.17	97.86	98.27
<i>B. valezensis</i> ATR2	CP018133.1	46.34	70.65	69.78	84.48

Table 3: Biosynthetic gene clusters (BGC) analysis in VUMS1.

Type	Genomic location (nt)		Most similar known cluster	Similarity (%)
Betalactone	498,962	527,272	Fengycin	53
NRP-metallophore, NRPS	26,381	78,107	Bacillibactin	80
other	305,440	346,861	Bacilysin	85
NI-siderophore, terpene	306,302	335,058	Schizokenin	60
NRPS	146,649	230,374	Lichenysin	85

the VUMS1 genome sequence. Secondary metabolites biosynthetic gene clusters predicted numerous types of BGCs for each genome, only those having a percentage of resemblance to clusters in the database equal to or greater than 50% were reported as shown in Table 3.

Based on the antiSMASH server result, 5 gene clusters were observed with similarity ranges from 53% to 85%. There were two non-ribosomal peptide synthase (NRPS) gene clusters, one beta lactone gene cluster, one non-ribosomal peptide-metallophore gene cluster, one non-ribosomal peptide synthase-independent siderophores gene cluster and lastly, one 'other' type of gene clusters. Gene cluster 1 had 53% similarity with fengycin BGC and 100% similarity found in *B. altitudinis* Ku-bf1. Gene cluster 2 had 80% similarity with bacillibactin BGC and 100% similarity found in *B. pumilus* PDSL2g-1. Next, gene cluster 3 had 85% similarity with bacilysin BGC and was found in *B. altitudinis* GR-8 with 100% similarity. Other than that, gene cluster 4 had 60% similarity with schizokenin BGC and had 100% similarity found in *B. altitudinis* ZAP62. Gene cluster 5 had 85% similarity with lichenysin BGC and found 100% similarity in *B. altitudinis* SCU11. Based on the percentage of similarity of BGC, VUMS1 has high potential in producing a novel fengycin, bacillibactin, bacilysin, schizokenin and lichenysin.

Fengycin is a cyclic lipopeptide that is used as a fungicide in agriculture. Lipopeptides are a type of antibiotic that may efficiently combat a wide range of disease-causing organisms. *Bacillus subtilis* produces it

as an immunological response to fungal infection and it works by destroying the target's cell membrane. Many are expressed by bacteria and contain antibacterial and antifungal properties (Ongena and Jacques, 2008; Sur *et al.*, 2018). Bacillibactin is a siderophore based on catechol via the utilisation of ABC transporters. It engaged in the chelation of ferric iron (Fe³⁺) from the environment before being carried into the bacterial cytoplasm (Hotta *et al.*, 2010). Siderophore bacillibactin is a promising antibacterial agent owing its ability to cut pathogen's iron intake resulting in cell death (Khan *et al.*, 2018). Another related metal-chelator is schizokenin, which is a hydroxamate-type siderophore schizokenin and it is known for its ability to promote plant growth (Nascimento *et al.*, 2019). Unfortunately, there is limited information about schizokenin as little research has been done. Moreover, lichenysin is a biosurfactant grouped under the surfactins, a cyclic heptapeptide linked to a β-hydroxy fatty acid. Other than surfactants, lichenysin is also known to have antimicrobial properties (Coronel-León *et al.*, 2015; Gudiña and Teixeira, 2022). As a dipeptide antibiotic, bacilysin demonstrated efficacy against a variety of infections by rupturing the microbial cell wall's structure. *Bacillus* species that produce bacilysin can prevent plant diseases including potato ring rot and bacterial infections of rice (Wu *et al.*, 2015; Wang *et al.*, 2021; Islam *et al.*, 2022). In summary, VUMS1 has a high potential to have biocontrol potential against bacteria and fungi as well as plant growth-promoting bacteria.

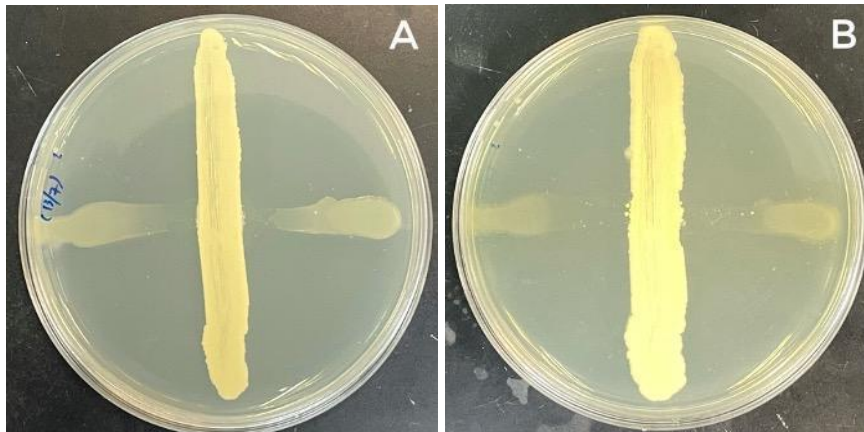


Figure 1: The inhibition of *V. parahaemolyticus* by VUMS1 on day 1(A) and day 5 (B) on MH agar.

Table 4: Biocontrol potential of *B. altitudinis* VUMS1 on MH agar.

<i>Vibrio</i> sp.	Inhibitory activity (mm)	
	Day 1	Day 5
<i>V. parahaemolyticus</i>	15.0 ± 0.71	21.0 ± 0.71

Biocontrol potential of VUMS1 against *V. parahaemolyticus*

The antagonism by VUMS1 against *V. parahaemolyticus* was assessed by using the cross-streaking method to evaluate the biocontrol potential of VUMS1. As shown in Figure 1, the vertical culture was VUMS1 and the horizontal cultures, left and right, was *V. parahaemolyticus*. According to studies, the cross-streaking method produced better inhibition zones on indicator bacteria than the agar well diffusion method (Lertcanawanichakul and Sawangnop, 2011). Other than that, the cross-streaking method usually shows inhibition zones on day 1 of inhibition compared to agar well diffusion which usually shows inhibition zones on day 7. Hence, the cross-streaking method is chosen as an antagonism assay method.

The result of this antagonism assay is shown in Table 4. On day 1, the inhibition zone of VUMS1 against *V. parahaemolyticus* was 15 mm with a low standard deviation of 0.71 mm. After 5 days of incubation, the inhibition zone increased by 6 mm more which made the final inhibition zone 21 mm. The incubation stopped on day 5 because the inhibition zone did not increase any further.

MH agar is often used as antimicrobial susceptibility testing (AST) and was used for the cross-streaking method because of its 'loose' agar that allows the secondary metabolites of VUMS1 to diffuse throughout the media based on the Kirby-Bauer method, resulting in more accurate results (Nassar *et al.*, 2019). These results showed significant findings that highlight the ability of VUMS1 to inhibit the growth of *V. parahaemolyticus*. The biocontrol potential assessment was continued by testing the ability of VUMS1 to inhibit the growth of *V.*

parahaemolyticus in liquid media by using a co-culture experiment.

As shown in Figure 2, the *V. parahaemolyticus* has a yellow and shiny colony. The *V. parahaemolyticus* in Figure 2A and Figure 2C, the control of this experiment has more CFU compared to the *V. parahaemolyticus* in Figure 2B and Figure 2D, the treated *V. parahaemolyticus* v2. Figure 2B and Figure 2D have less CFU compared to the control because *V. parahaemolyticus* v2 in Figure 2B and Figure 2D are treated with VUMS1 which is known to inhibit their growth. Figure 3 shows the growth of *V. parahaemolyticus* for five days. The straight line indicates the growth of the control for this experiment which is the untreated *V. parahaemolyticus* meanwhile the dotted line indicates the growth of *V. parahaemolyticus* treated with VUMS at OD₆₀₀ of 0.8.

The growth of *V. parahaemolyticus* was determined in CFU/mL and plotted in a trendline graph with error bars. The growth of the control increased from day 1 to day 5. Meanwhile, the growth of treated *V. parahaemolyticus* increased from day 1 to day 3. Interestingly, the growth of treated *V. parahaemolyticus* started to decline significantly at 98% and 99.8% on day 4 and day 5, respectively. The co-culture experiment showed that the VUMS1 was able to decrease the growth of *V. parahaemolyticus*. Although the treated *V. parahaemolyticus* growth increased from day 1 until day 3, the growth of control *V. parahaemolyticus* until day 3 were higher than the treated. This shows that VUMS1 still inhibits the growth of *V. parahaemolyticus* but at a slower rate.

Based on a similar study done by Vaseeharan and Ramasamy (2003) on the biocontrol potential of *B. subtilis* BT23 against *Vibrio* spp., their study showed similar results whereby the increasing growth of treated *Vibrio*

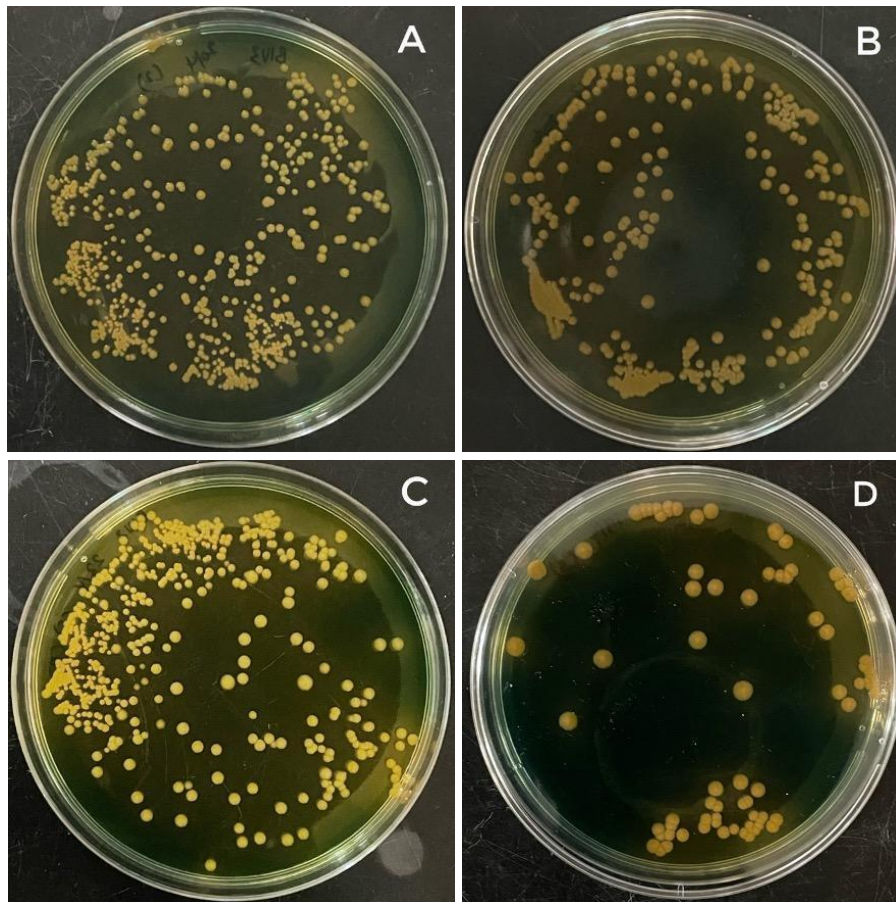


Figure 2: Colonies of *V. parahaemolyticus* on TCBS agar plate on day 1 (A) and day 5 (C) and the colonies of *V. parahaemolyticus* on TCBS agar plate treated with VUMS1 on day 1 (B) and day 5 (D).

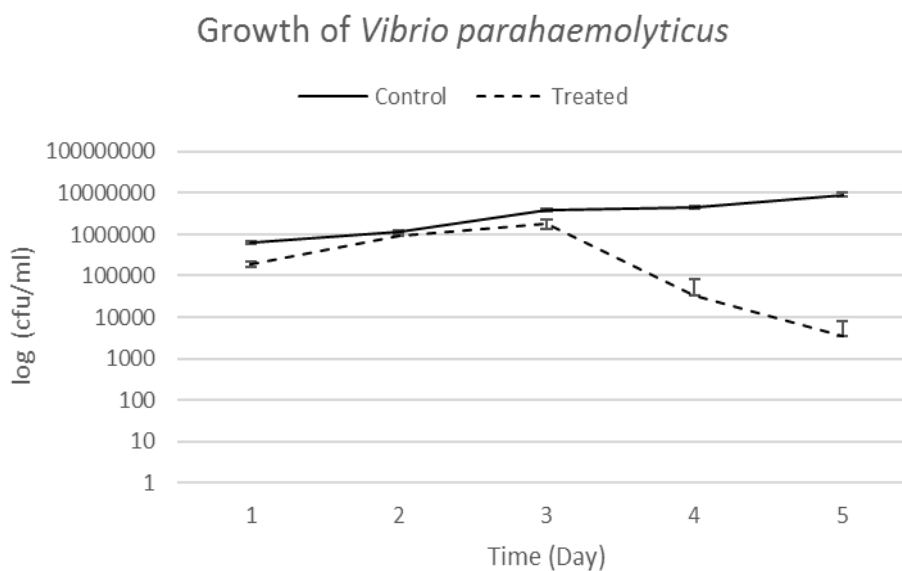


Figure 3: Growth of *V. parahaemolyticus* with and without VUMS1 at 28 °C. The growth of *V. parahaemolyticus* without any co-culture with B1V was the control (—) and V2 was treated with endophyte B1V (----).

spp. on day 1 until day 3 did not exceed the growth of their control *Vibrio* spp. and treated *Vibrio* spp. only started to decline on day 4. Vaseeharan and Ramasamy (2003) also stated that 10^7 to 10^9 CFU/mL is the concentration of bacteria required to inhibit the growth of *Vibrio* spp. This suggests that a high concentration of VUMS1 was required to inhibit the growth of *V. parahaemolyticus*. VUMS1 was able to inhibit the growth of *V. parahaemolyticus* because the concentration of VUMS1, which was at OD₆₀₀ of 0.8, is equal to 8.0×10^8 of bacteria cells. *Bacillus* spp. produce a wide range of chemicals involved in the biocontrol of plant diseases making them attractive candidates for a wide range of agricultural and biotechnological uses. They are often used for this purpose because they excrete extracellular metabolites such as antibiotics, cell wall hydrolases and siderophores, demonstrating antagonistic action (Miljaković *et al.*, 2020). Based on the result present in this study, it is speculated that the predicted genes such as fengycin, bacillibactin, bacilysin and lichenysin are the responsible genes for the biocontrol potential possessed by VUMS1. However, more comprehensive and deeper research must be performed in order to understand the mechanism and correlation of the biocontrol potential of VUMS1 and the antimicrobial genes predicted.

CONCLUSION

In this study, the VUMS1 genome is affiliated with *B. altitudinis* based on the genome analysis done. Other than that, the analysis suggests that the predicted gene clusters responsible for antimicrobial correlates with the biocontrol potential of VUMS1 against *V. parahaemolyticus*. Lastly, VUMS1 revealed its potential as a suitable candidate to be used as a probiotic to *K. alvarezii* to mitigate ice-ice disease owing to the fact that VUMS1 possessed antimicrobial, antifungal and plant growth-promoting genes.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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