Berberine at non-lethal concentrations attenuates virulence of *Chromobacterium violaceum*

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Background

- Berberine is a bioactive plant compound.

- *Chromobacterium violaceum* is primarily a soil-dwelling gram-negative bacterium, which is inherently resistant to many antibiotics. Though not frequently involved in human infections, whenever it infects humans, mortality is high. This bacterium is being employed as a model of bacterial quorum sensing since many years.

- This study attempted to investigate berberine's potential anti-virulence effect at non-lethal concentrations against *C. violaceum,* wherein the nematode worm *Caenorhabditis elegans* was employed as a model host, and berberine's effect on test bacterium was investigated at whole transcriptome level.

Table 1. Test pathogen

Bacterium	Source and Media	Resistant to which antibiotics	Remarks	Incubation Temp. (°C)
Chromobacterium violaceum	MTCC 2656 Nutrient Agar/Broth (HiMedia)	Co-Trimxazole, Colistin, Cefixime, Clindamycin, Ampicillin	MDR; Emerging pathogen, Hemolytic, Violacein producer	35

Antibiotic susceptibility profile of the bacterium was generated using the antibiotic discs- Icosa G-I PLUS and Icosa G-I Minus (HiMedia, Mumbai) through disc diffusion assay as per NCCLS guidelines (10.1177/001857870403900608)

MDR - Multi Drug Resistant

MTCC - Microbial Type Culture Collection

Table 2: Model host

Nematode worm	Strain	Media	Incubation Temperature
Caenorhabditis elegans	N2 Bristol (wild type)	Nematode Growing Media (NGM)	22°C

- Can be infected with many human-pathogenic bacteria.
- Shorter life cycle and self -fertilizing hermaphrodite
- 38% of the *C. elegans* protein-coding genes have predicted orthologs in the human genome; 60-80% of human genes have an ortholog in the *C. elegans* genome; and 40% of genes known to be associated with human diseases have clear orthologs in the *C. elegans* genome.
- *E. coli* OP50 (LabTIE, Netherlands) was used as food for *C. elegans.*

Lifecycle of *Caenorhabditis elegans*

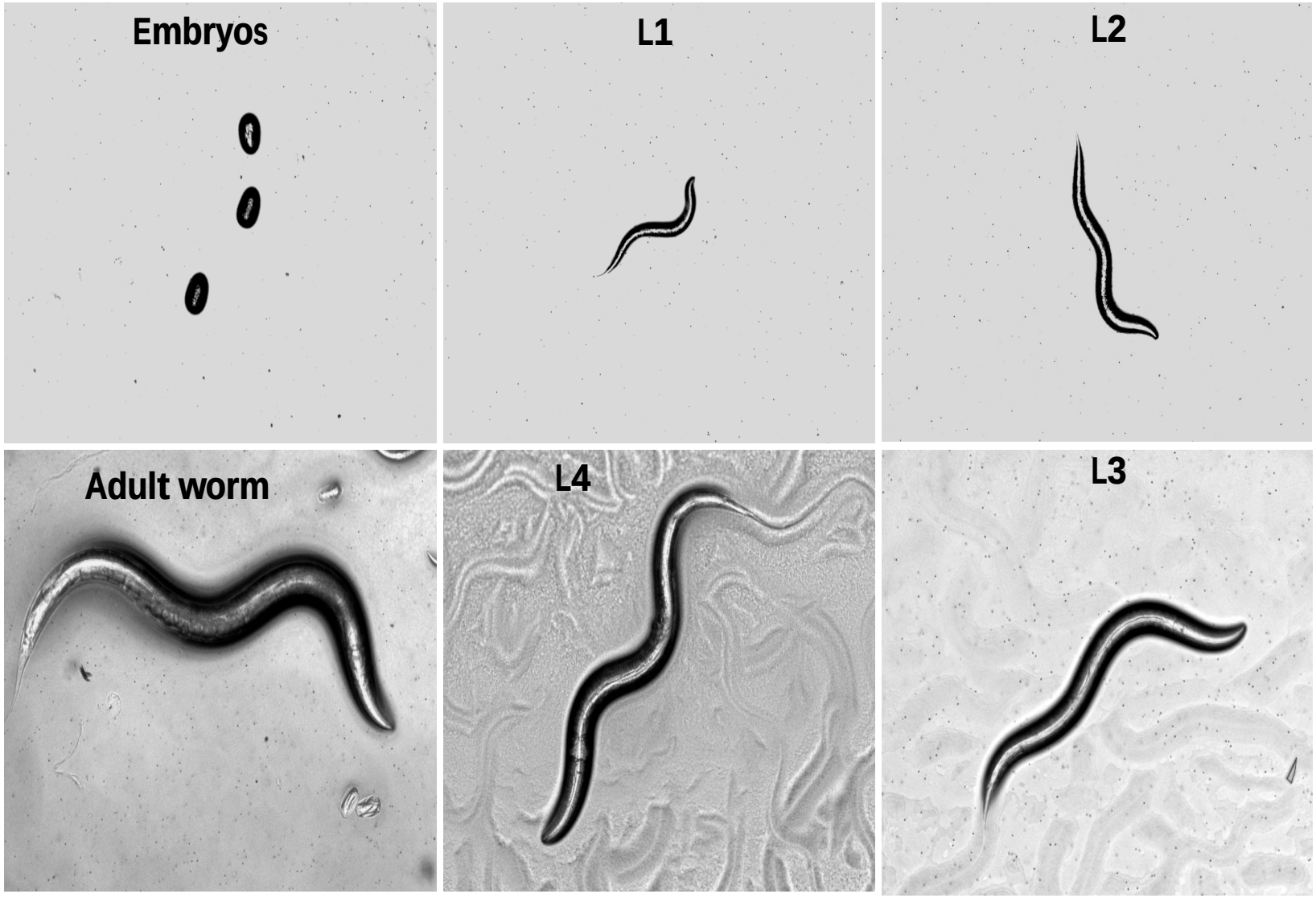


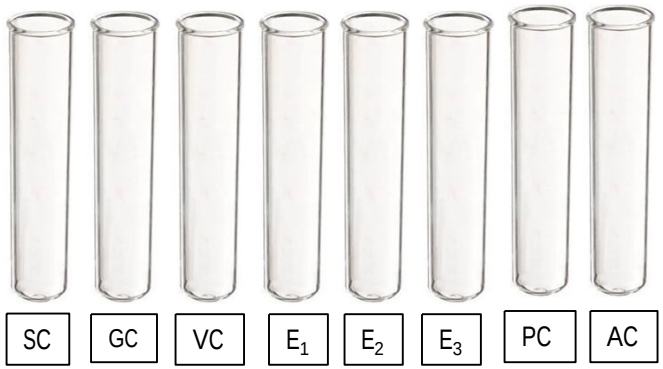
Table 3. Test compound

Sr No.	Test materials	Procured from	Solubility
1	Berberine chloride	Sigma-Aldrich	100% soluble in DMSO

100 mg of berberine was dissolved in 5 mL of DMSO, and stored in refrigerator for further use in experiments.

virulence assay

Broth dilution assay



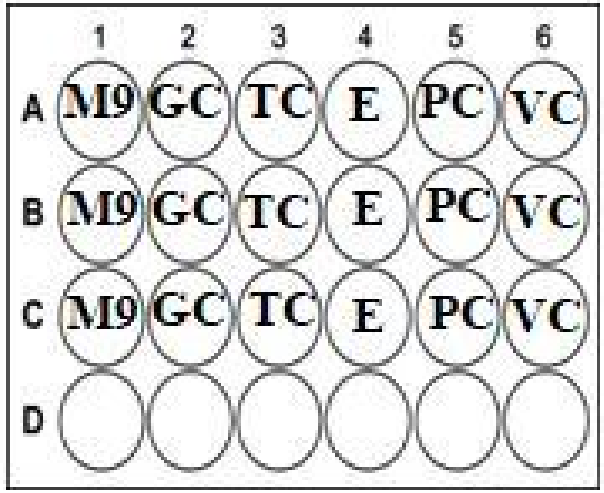
IC	Infection control
TC	Toxicity control
E	Experimental
PC	Positive control
VC	Vehicle control

The test organism (standardized to 0.5 McFarland turbidity standard) was inoculated in nutrient broth supplemented with different concentrations of berberine in the range of 0.5- 20 µg/mL.

After inoculation, intermittent shaking was provided, and incubation was carried out at 35^0C for 22-24 h.

In vivo

Post incubation, 100 µL (OD_{764} = 1.50 ± 0.05) of the culture broth was mixed with 900 µL of M9 buffer containing 10 worms (L3-L4 stage) in a 24-well plate.



- M9- 1mL M9 buffer + 10 worms
- IC- 900 µl M9 buffer + 100 µl (Bacterial culture)
- VC- 900 µl M9 buffer + 100 µl (DMSO treated bacterial culture)
- E- 900 µl M9 buffer + 100 µl (Plant extract - treated bacterial culture)
- PC- 900 µl M9 buffer + 100 µl (Antibiotic - treated bacterial culture)
- TC- 995 µl M9 buffer + 5 µl plant extract (Test concentrations)

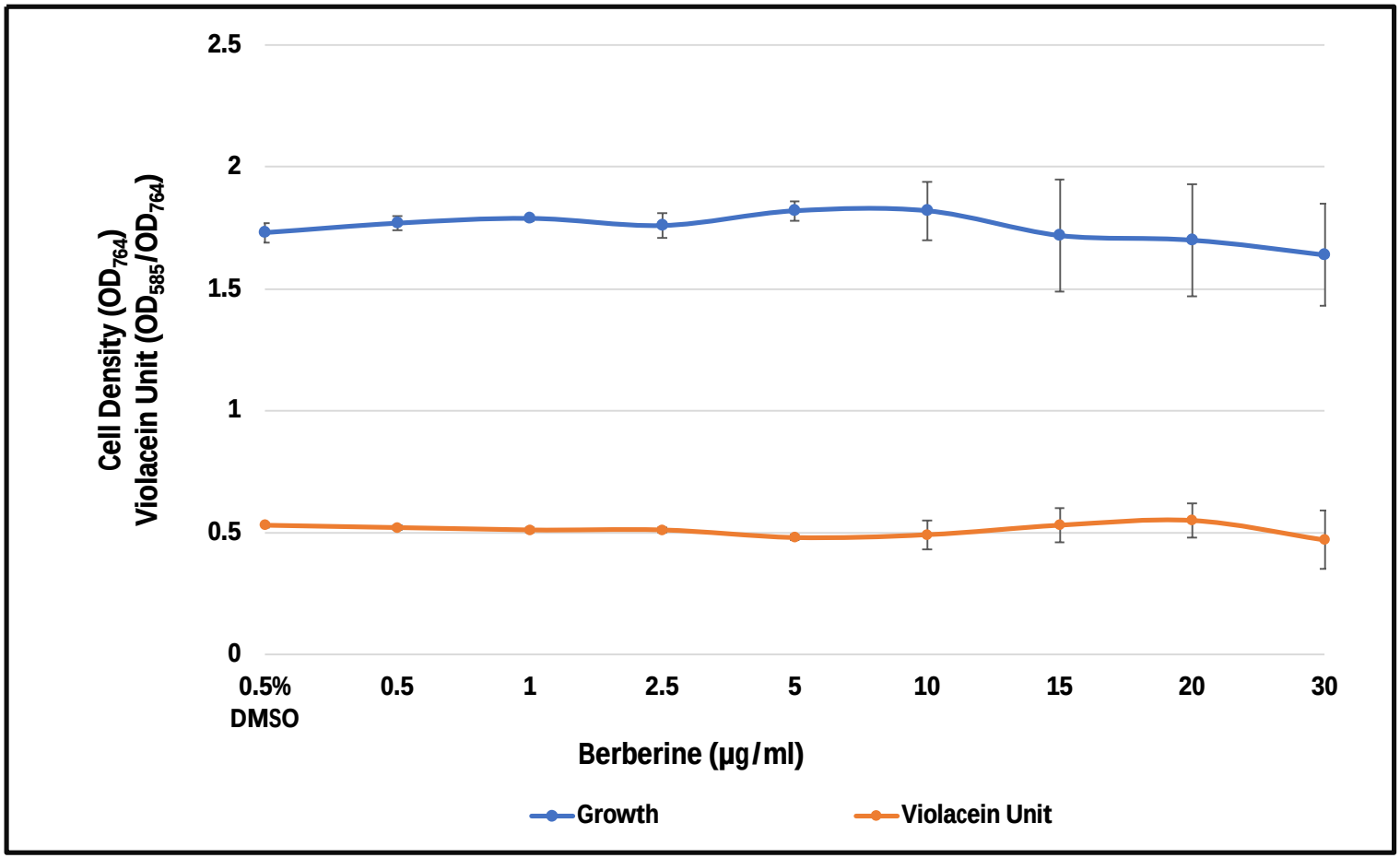


Figure 1. Berberine had no effect on growth and pigment of *C. violaceum*. OD of violacein was measured at 585 nm, and Violacein Unit was calculated as the ratio OD_{585}/OD_{764} (an indication of violacein production per unit of growth)

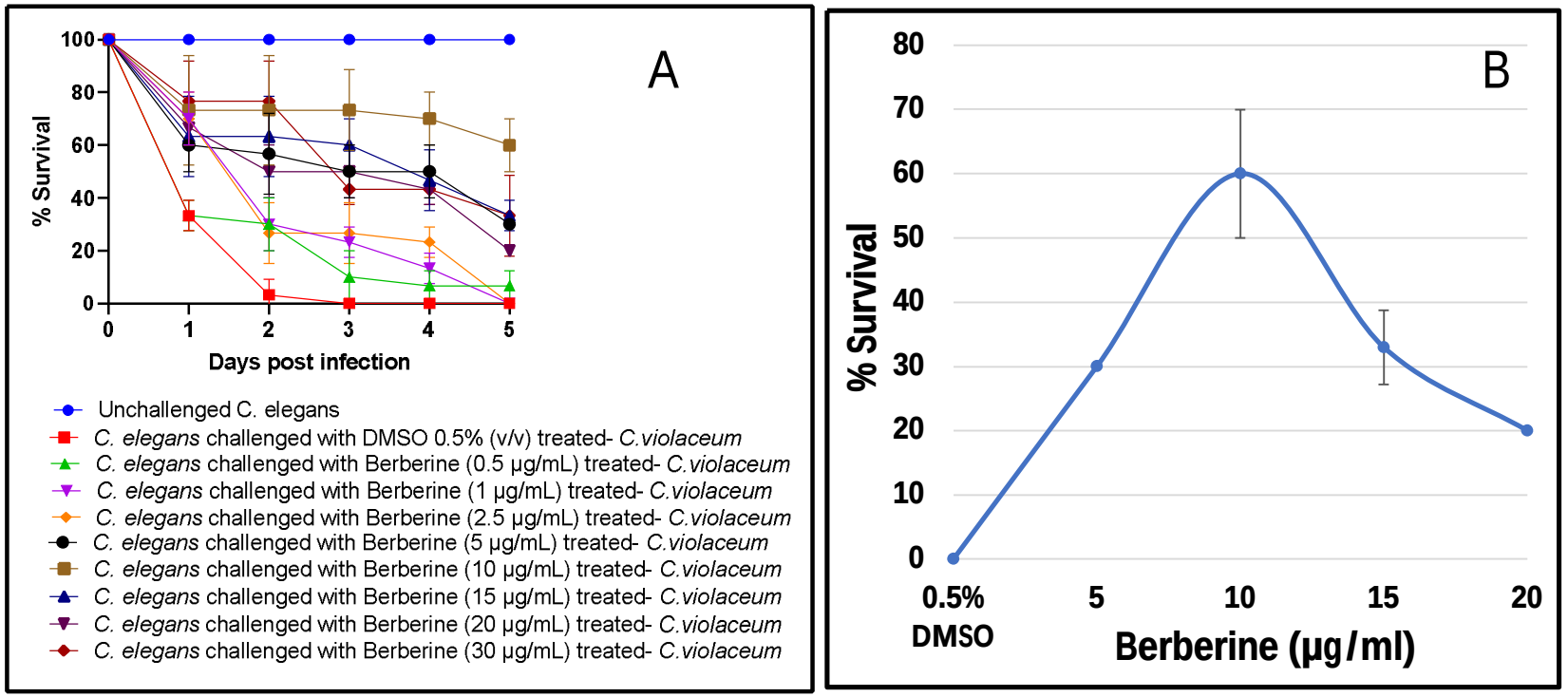
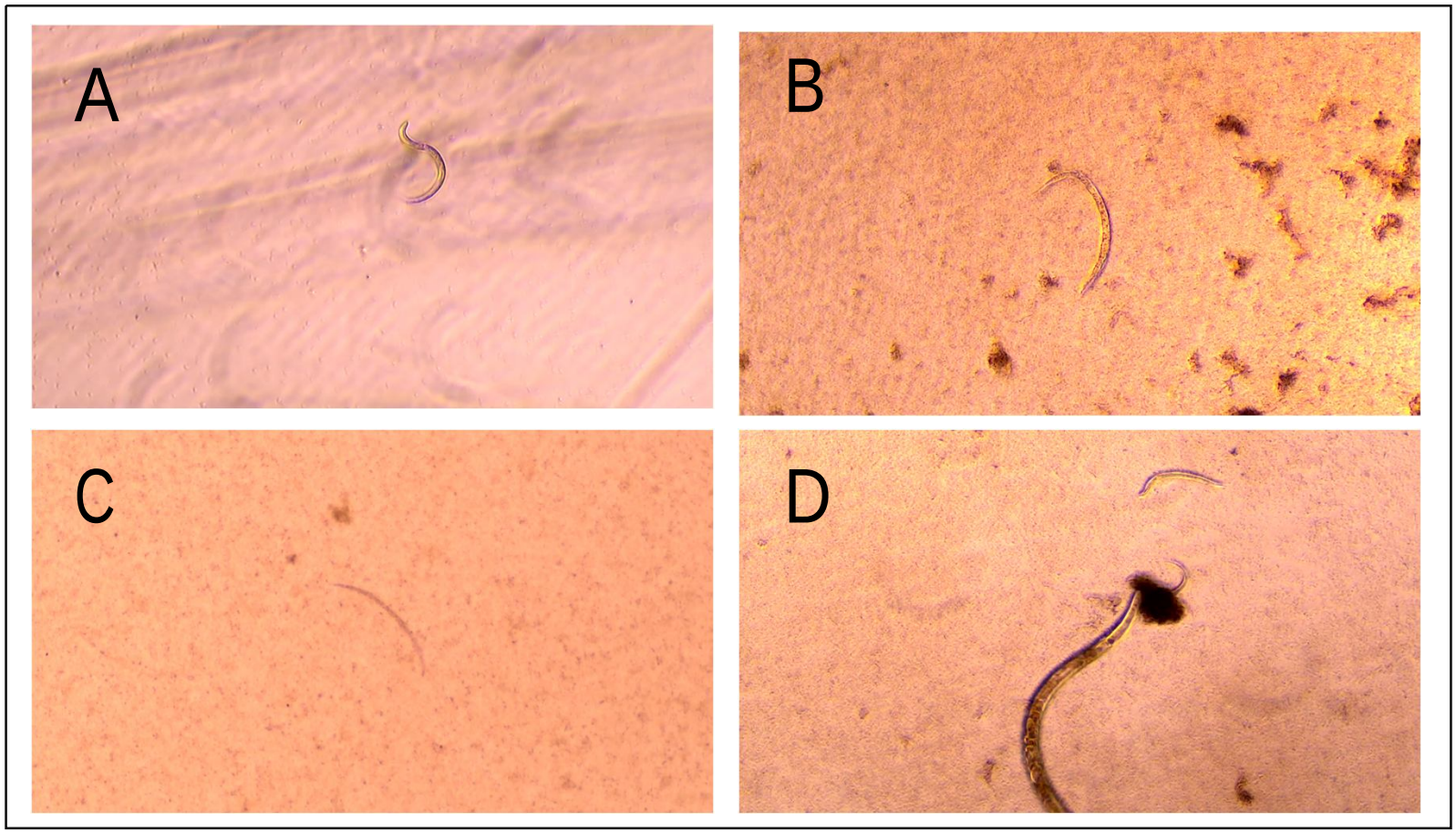


Figure 2. Berberine pre-treatment reduced bacterial virulence towards *C. elegans*. (A) Pre-treatment of bacteria with berberine at 10 µg/mL reduced bacterial virulence towards host worm by 60 % ± 10 (p<0.001). These % values refer to the difference between number of worms surviving in experimental and control wells. Progenies were observed on third day in experimental wells. Gentamicin and Vancomycin were employed as positive control at IC_{50} but could not reduced bacterial virulence towards *C. elegans*. (B) Berberine's anti-virulence effect follows an inverted-U shaped dose-response pattern.

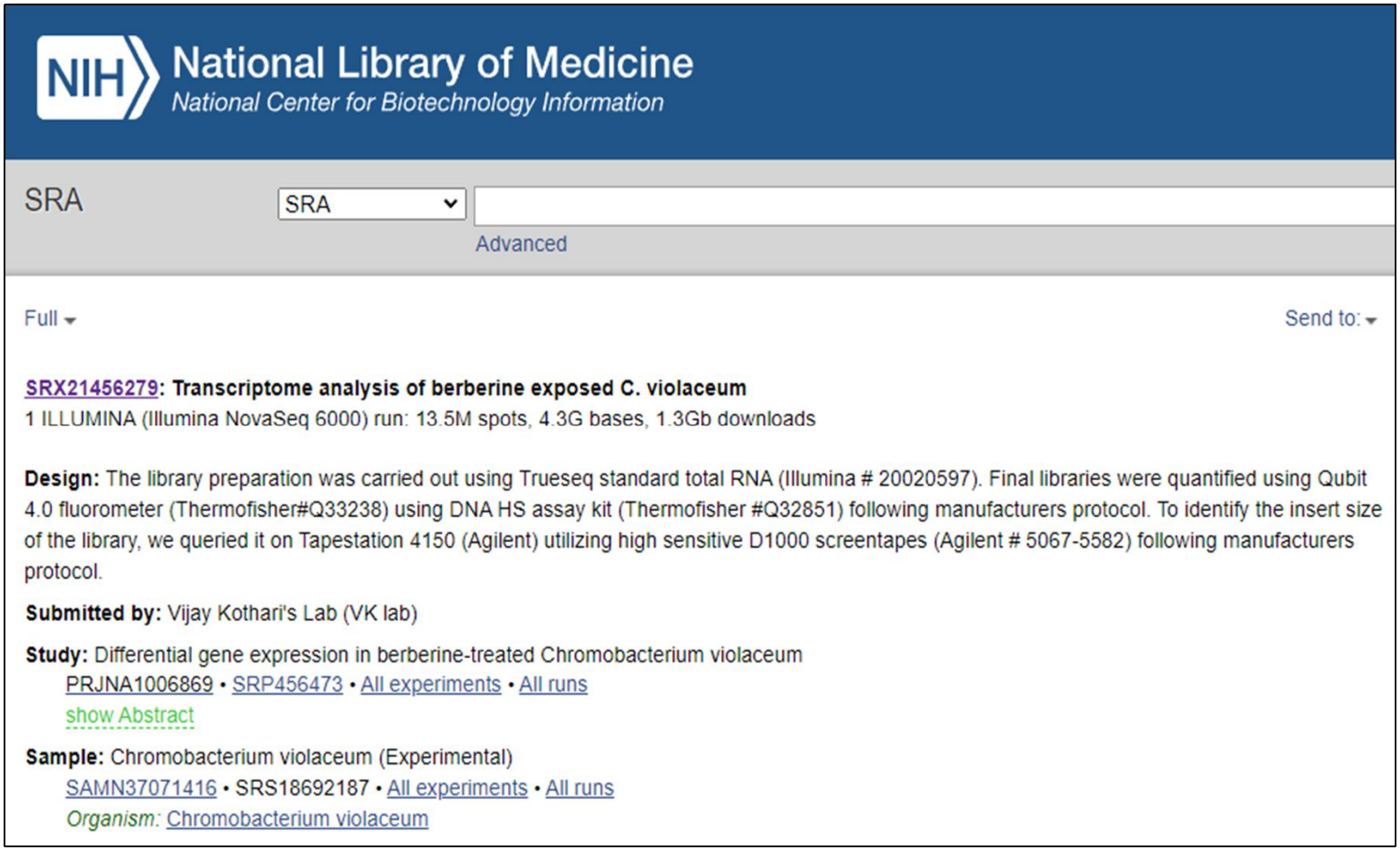
Videos : Berberine pre-treatment reduced bacterial virulence towards C. elegans



A. Control worm in M9 buffer.
B. Infection Control: Worms challenged with *C. violaceum* could kill all worms within 96 hours.
C. Vehicle Control: DMSO (0.5% v/v)-pre-treated *C. violaceum* could kill all worms within 96 hours.
D. Experimental: Worms challenged with berberine (10 µg/ml)-pre-treated *C. violaceum* registered better survival. Active movement and progeny formation after 96 hours of incubation are visible.

Whole transcriptome analysis of berberine-treated *C. violaceum*

- Total number of genes: 4365
- Total number of genes differentially expressed: 9 (**Log FC≥2; FDR≤0.01)**
- Total number of genes up-regulated: 7
- Total number of genes down-regulated: 2
- Relevant accession of Sequence Read Archive **:** PRJNA1006869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SRX21456279: Transcriptome analysis of berberine exposed C. violaceum
1 ILLUMINA (Illumina NovaSeq 6000) run: 13.5M spots, 4.3G bases, 1.3Gb downloads
Design: The library preparation was carried out using Trueseq standard total RNA (Illumina # 20020597). Final libraries were quantified using Qubit 4.0 fluorometer (Thermofisher#Q33238) using DNA HS assay kit (Thermofisher #Q32851) following manufacturers protocol. To identify the insert size of the library, we queried it on Tapestation 4150 (Agilent) utilizing high sensitive D1000 screentapes (Agilent # 5067-5582) following manufacturers protocol.
Submitted by: Vijay Kothari's Lab (VK lab)
Study: Differential gene expression in berberine-treated Chromobacterium violaceum
PRJNA1006869 • SRP456473 • All experiments • All runs
show Abstract
Sample: Chromobacterium violaceum (Experimental)
SAMN37071416 • SRS18692187 • All experiments • All runs
Organism: Chromobacterium violaceum

Table 4. List of DEG satisfying the dual criteria of log fold-change ≥2 and FDR≤0.01

Sr. No	Feature ID	Gene	Gene encoded for/function	log FC
1	CV_RS12005	NorM	MATE family efflux transporter	5.70
2	CV_RS05670	ZntA	heavy metal translocating P-type ATPase	4.81
3	CV_RS12000	Q7NV90	Xaa-Pro peptidase family protein	4.72
4	CV_RS12010		TetR family transcriptional regulator	3.39
5	CV_RS05695	A0A202BDG5	GtrA family protein	3.22
6	CV_RS22050		putative metalloprotease CJM1_0395 family protein	3.20
7	CV_RS05680	A0A202BDM3	acyl-CoA thioesterase	2.90
8	CV_RS09795	nirK	copper-containing nitrite reductase	-3.13
9	CV_RS17225	norB	nitric-oxide reductase large subunit	-4.88

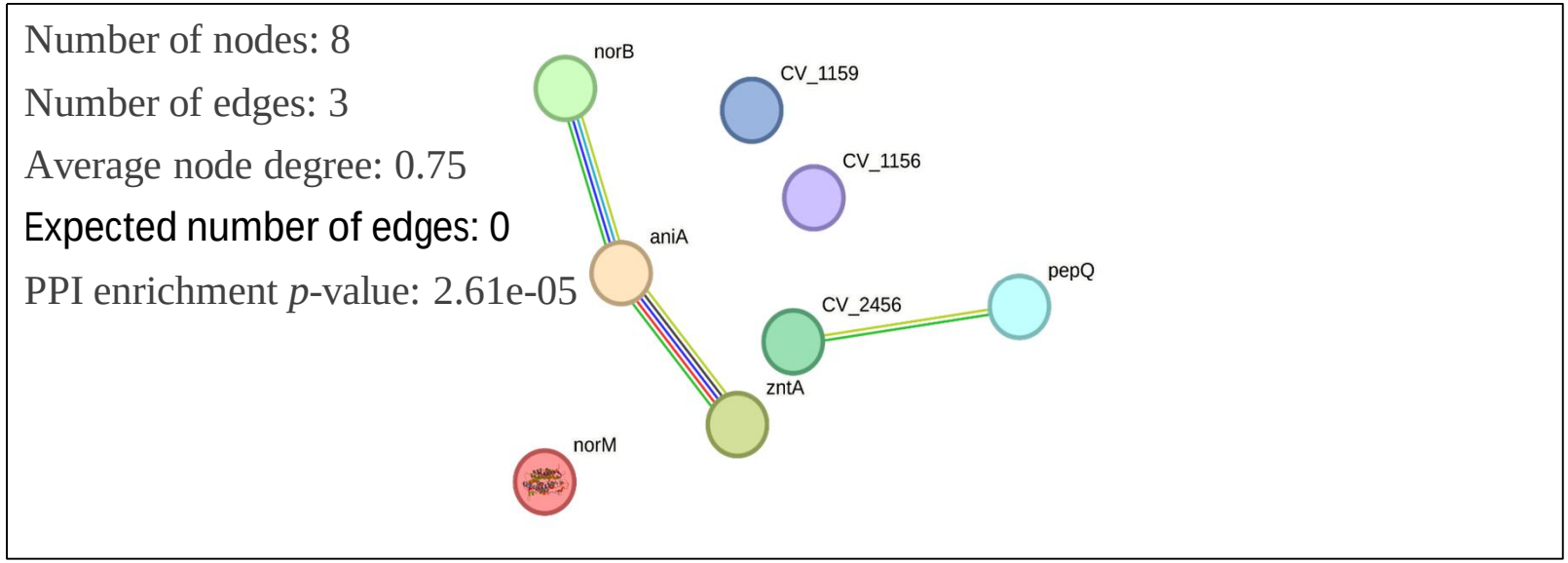


Figure 3. Protein-Protein Interaction (PPI) network of DEG following the dual criteria of fold change ≥ log 2 and FDR ≤ 0.01 in berberine-treated *C. violaceum*

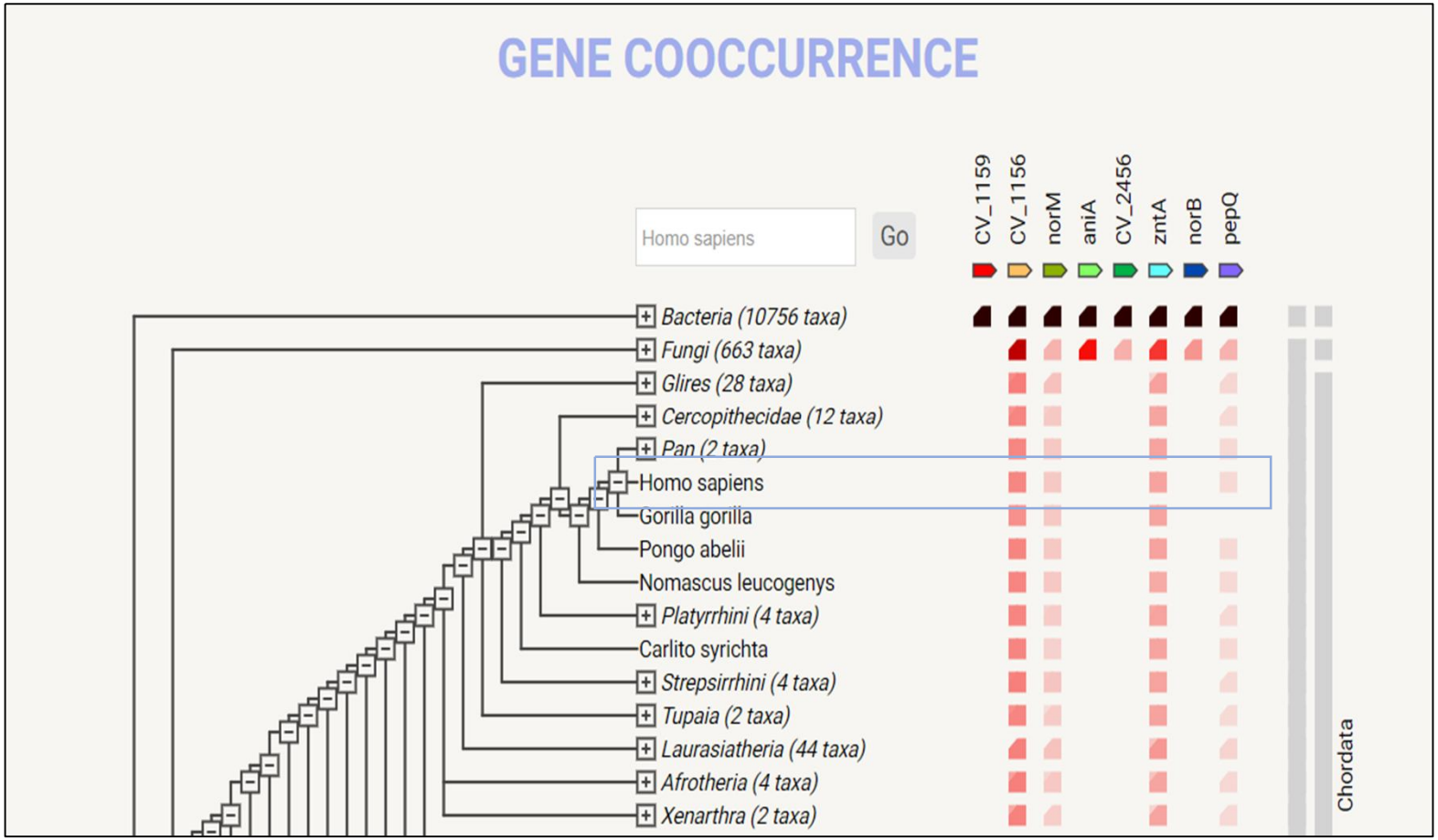
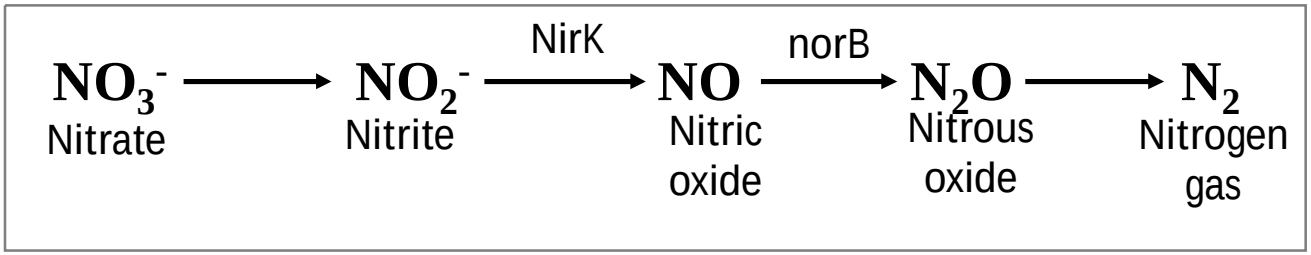


Figure 4. Co-occurrence analysis of genes coding for potential targets in *C. violaceum*

The darker the shade of the squares, higher is the homology between the genes being compared.

Denitrification process: A part of Nitrogen metabolism



- NirK and norB, both are involved in denitrification pathway. Their downregulation in berberine-exposed *C. violaceum* indicates disturbance of nitrogen metabolism, which needs to be validated through *in vitro* assay.

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Further Work

- Further investigation on targetability of these genes (NirK and norB) with respect to development of novel anti-virulence agents, and correlation of nitrogen metabolism with virulence of *C. violaceum* is warranted.

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