

Characterization of Quorum Sensing and Proteolytic Activity of Spoilage *Pseudomonas* from Raw Milk

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1. Background

Quorum sensing (QS) is a mechanism for cell-to-cell communication between inter- and intra-bacterial species through secreted molecules called autoinducers (AIs) employed to regulate the gene expression. *Pseudomonas* are main spoilage species in dairy products and produce heat-stable protease during cold storage of raw milk, whose expression can be regulated by QS signals called acyl-homoserine lactones (AHLs) (1,2). Role of QS in milk spoilage by *Pseudomonas* is still under-researched.

3. Experimental methods

Isolation of *Pseudomonas*

- Selective medium (CFC, Sigma Aldrich, Germany) was used to grow and isolate *Pseudomonas* from raw milk.

Assessment of spoilage activity

- *Pseudomonas* isolates were inoculated in skim milk liquid and agar and incubated at 4°C for 2 days.

Screening of AHL-producing *Pseudomonas*

- *Chromobacterium violaceum* CV026 (CV026) was used for the detection of AHLs in “T” streak assay, which produces violet pigment in response to exogenous AHL.

AHL-degrading assay

- Applying QS inhibitory molecules and postbiotics from *Lactobacillus*.

5. Detection of AHL-QS system in *Pseudomonas* isolates

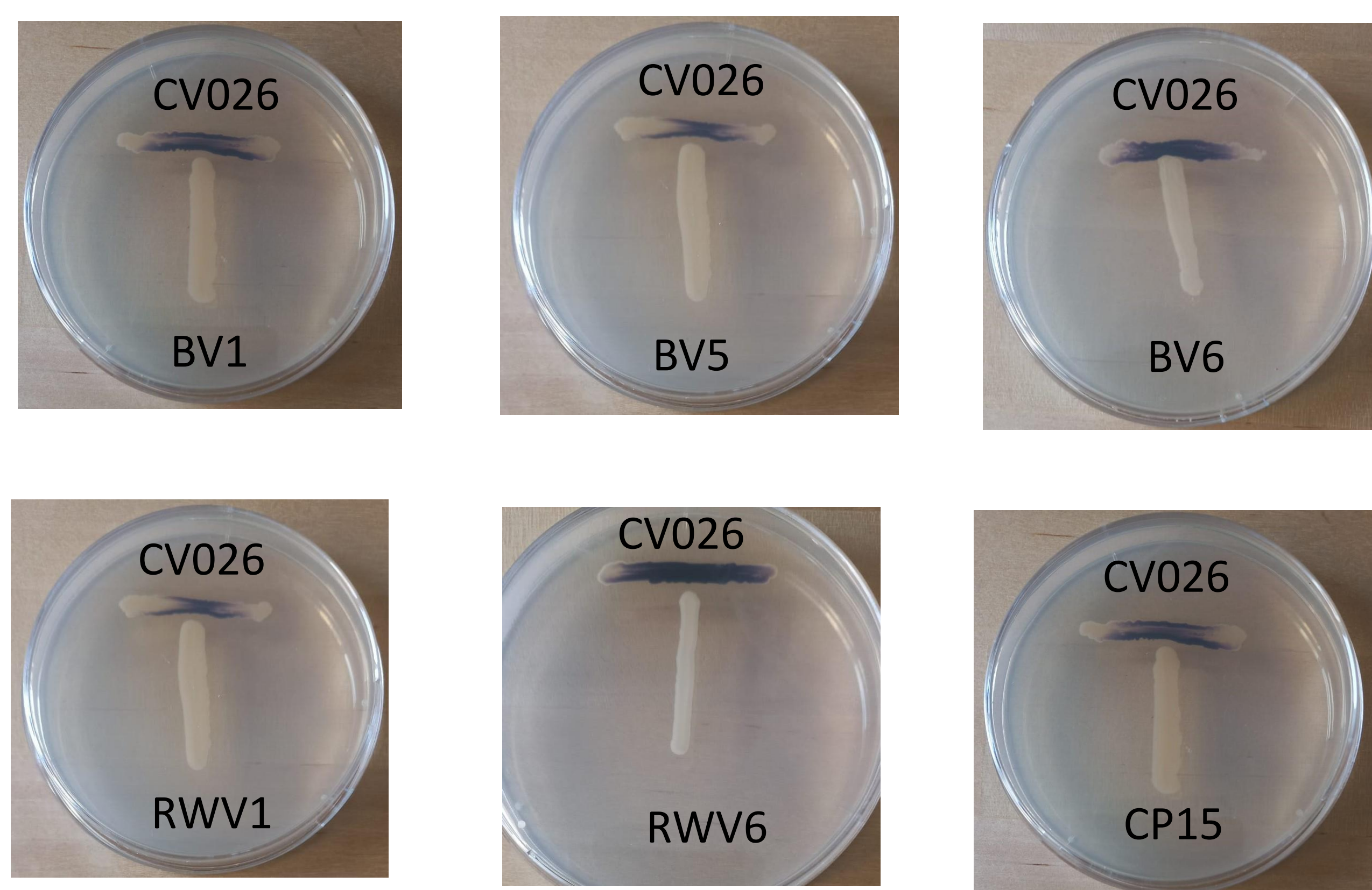
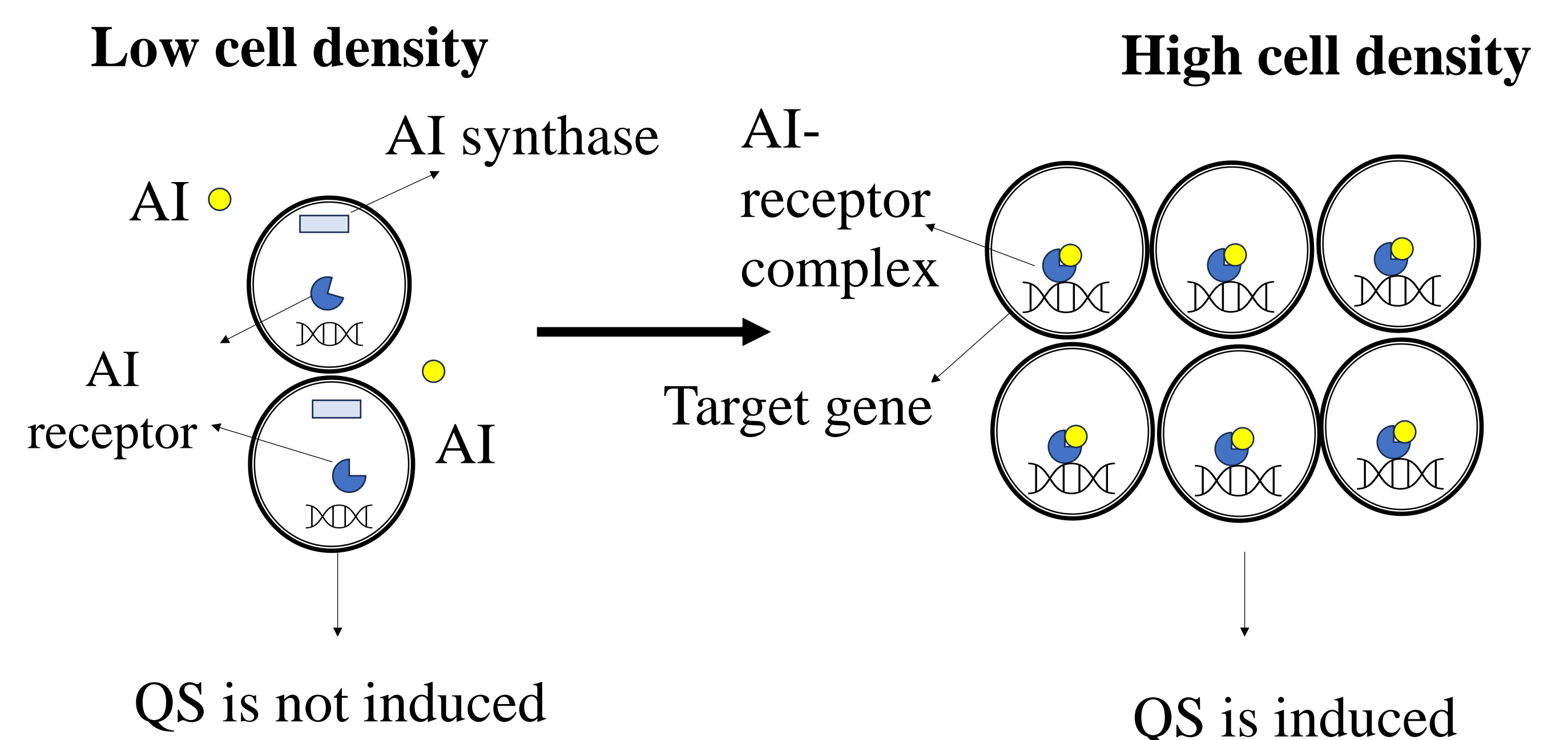


Figure 2. Induction of violacein production in CV026 by production of short chain AHLs by *Pseudomonas*

2. QS pathway



4. Identification of spoilage *Pseudomonas* species

Six isolates were found with high spoilage activity 4°C and identified as *P. Gessardi* by 16S rRNA gene sequencing.



Figure 1. *Pseudomonas* isolates spoilage activity in skim milk (left) and proteolytic activity on skim milk agar (right).

6. QS inhibitory effect of Salicylic acid (SA) on proteolytic activity

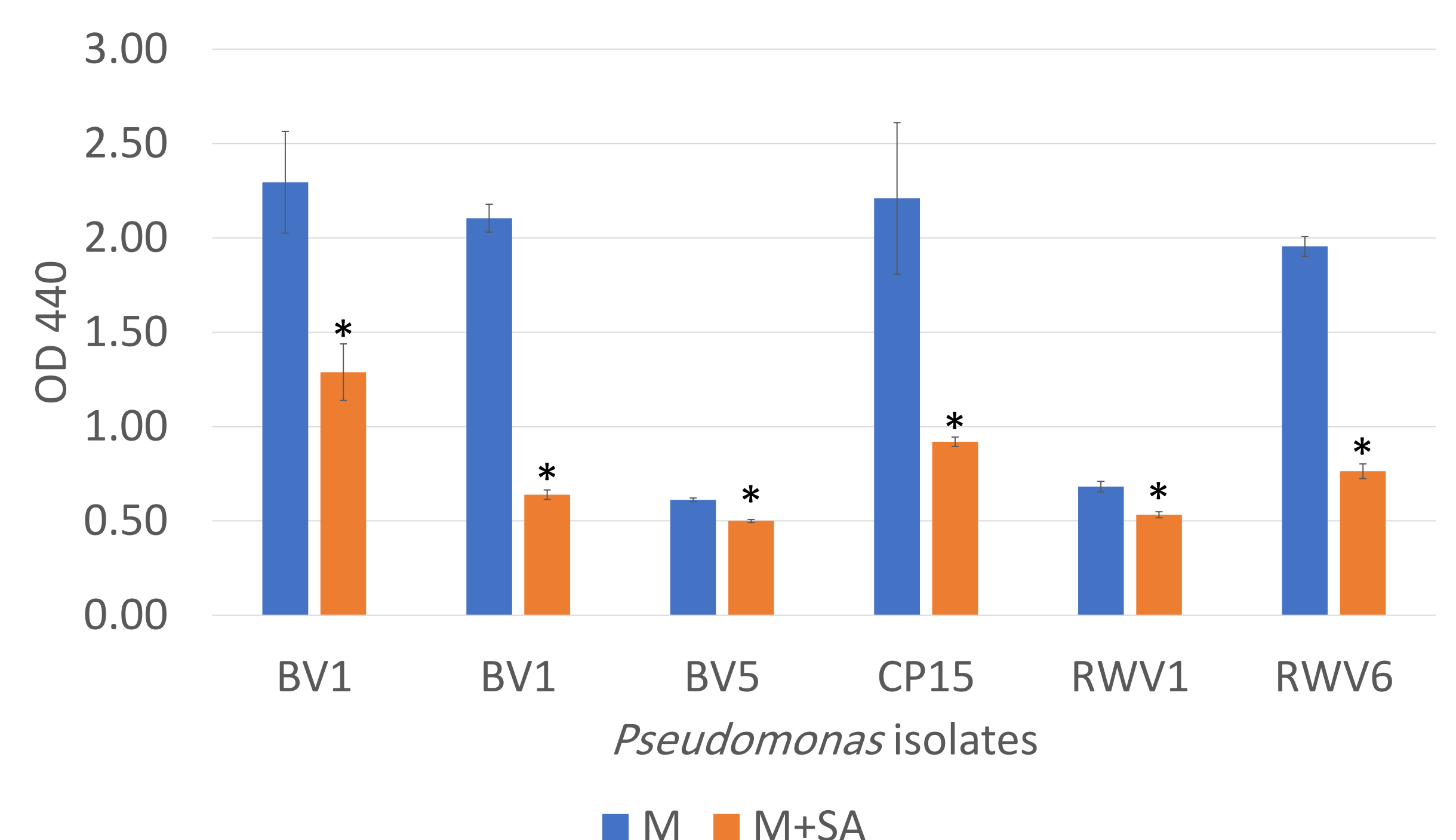


Figure 3. Proteolytic activity assessed by TNBS assay in milk inoculated with *Pseudomonas* only (M) and with *Pseudomonas* + SA 0.5 mg/ml (M+SA) and incubated at room temperature for 1 day. TNBS assay results presented by OD440. Results are shown as Mean ± S.D. Independent T-test was used for statistical analysis between control and test groups. * P-value < 0.05

References

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- 2) Salman, M. K., Abuqwider, J., & Mauriello, G. (2023). Anti-Quorum Sensing Activity of Probiotics: The Mechanism and Role in Food and Gut Health. *Microorganisms*, 11(3), 79.