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### Isolation and Structural Determination of Bioactive Metabolites Produced by a Soil Bacterium, *Arthrobacter* sp. TAJX1902

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# Isolation and Structural Determination of Bioactive Metabolites From a soil bacterium, *Arthrobacter* sp. TAJX1902



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## Abstract

As antimicrobial resistance persistently disrupts the treatment of microbial infection, natural product chemistry has played a significant role in identifying novel drugs with novel modes of action critical to getting ahead of resistance. The primary goal of this project is to extract and identify potential novel antimicrobial natural products produced by *Arthrobacter* sp. TAJX1902. Although underexplored, *Arthrobacter* sps. have been known to produce bioactive compounds with versatility; one such is a depsipeptide with quorum-sensing inhibitory activity.<sup>1</sup> In this research, an *Arthrobacter* sp. TAJX1902 isolated from a soil sample showed inhibitory activity against a filamentous indicator bacterium and a violacein-producing *Janthinobacterium* sp. A. sp. TAJX1902 was cultured using rich medium broth and agar. Isolated compounds were characterized via spectroscopic techniques, including 1D and 2D-NMR spectroscopy, as well as GCMS analysis. A. sp. TAJX1902 produces pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methyl propyl), and five other bioactive cyclic dipeptides (CDP).

## Background

➤ Antifungal, antiviral, anti-inflammatory, antibacterial, antitumor agents, Toxins, etc have been isolated from living organism.

➤ Noteworthy, are compounds useful in the treatment of microbial infections. However, some pathogenic cells develop resistance to the active agents/ drugs.

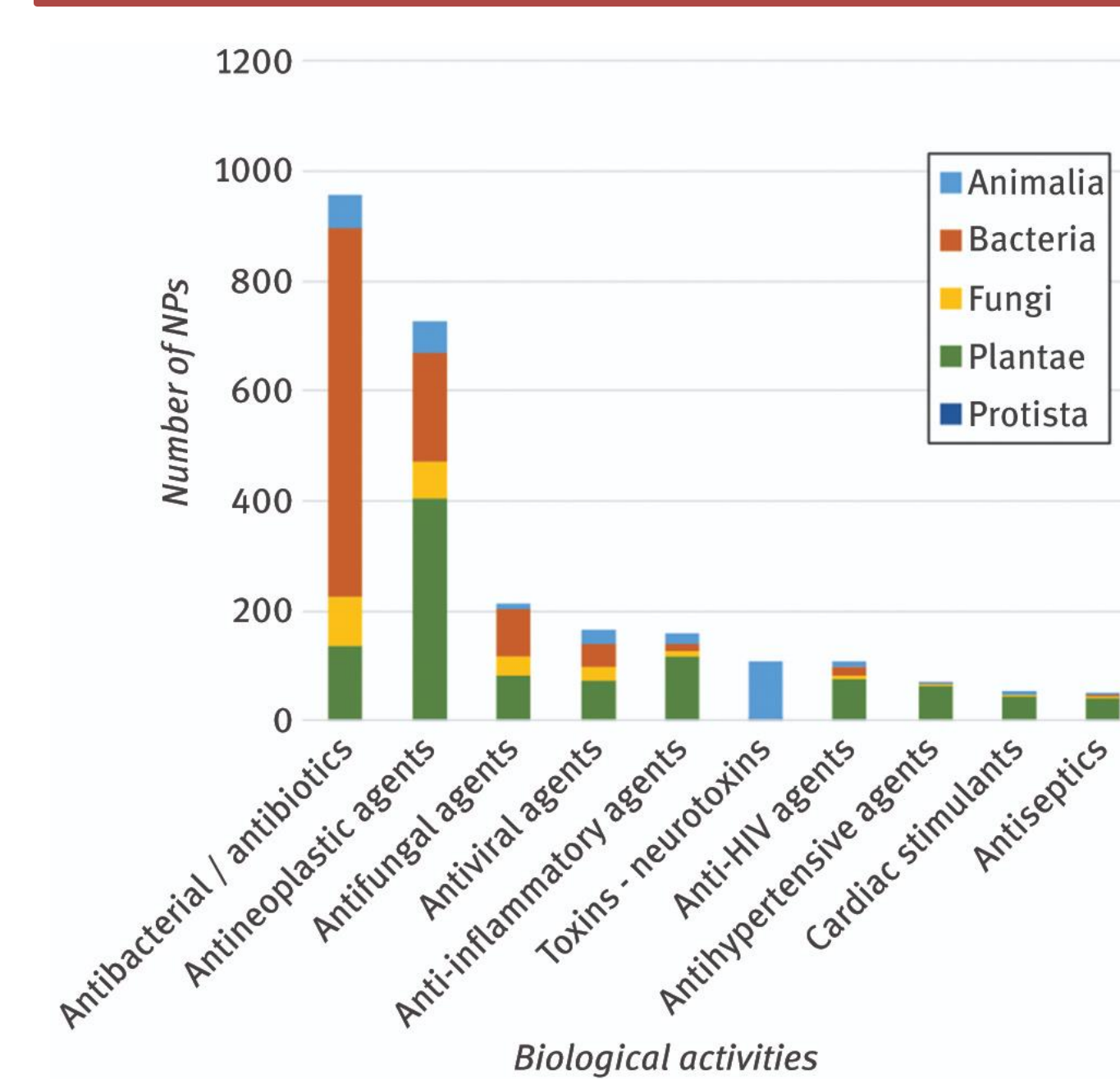


Fig. 1. An analysis of bioactivity of compounds produced by different kingdoms of life.<sup>4</sup>

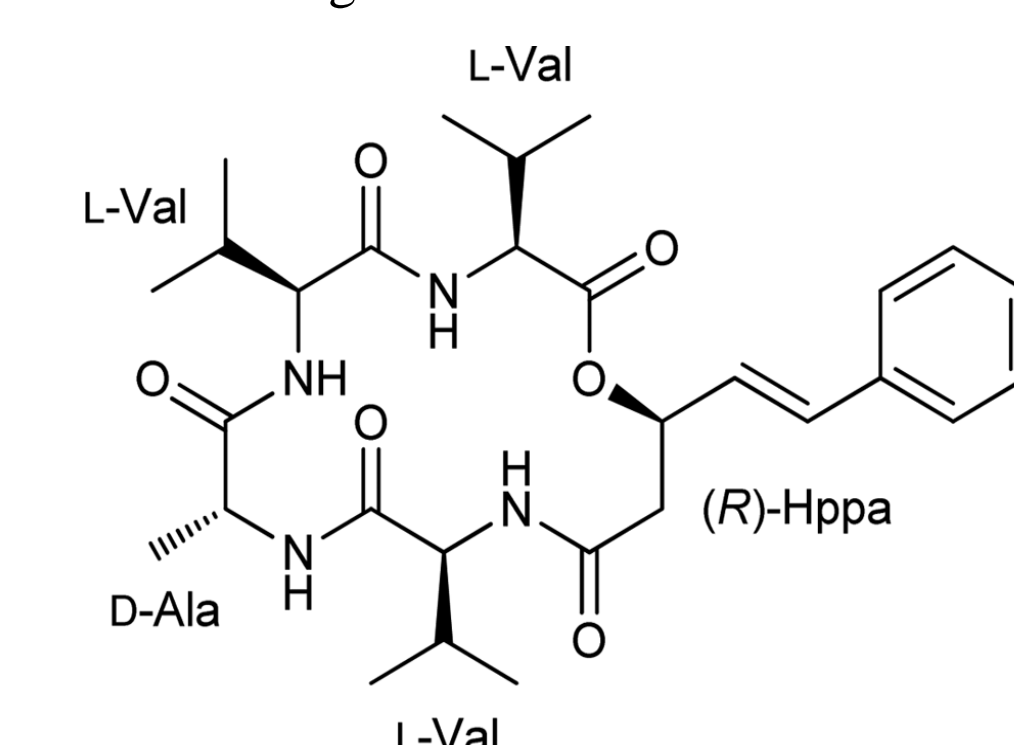


Fig. 2. Structure of Arthroamide. Inhibits a signaling pathway of quorum sensing in *Staphylococcus aureus* isolated from an *Arthrobacter* sp.<sup>1</sup>

Quorum Sensing (QS) is a key method by which bacteria cells communicate. Modulating the the QS system of pathogenic bacteria cells can provide advantages over inhibiting bacterial growth, such as

- minimize selection pressure to drive the revolution of a resistant phenotype
- and potentially reduce the aggregation into biofilms allowing immune systems to fight the more susceptible infection.<sup>5</sup>

## Objective

➤ To extract and identify potential novel antimicrobial natural products produced by *Arthrobacter* sp. TAJX1902.

## Experimental Methods

### Isolation and Inhibitory Activity of Bacterium

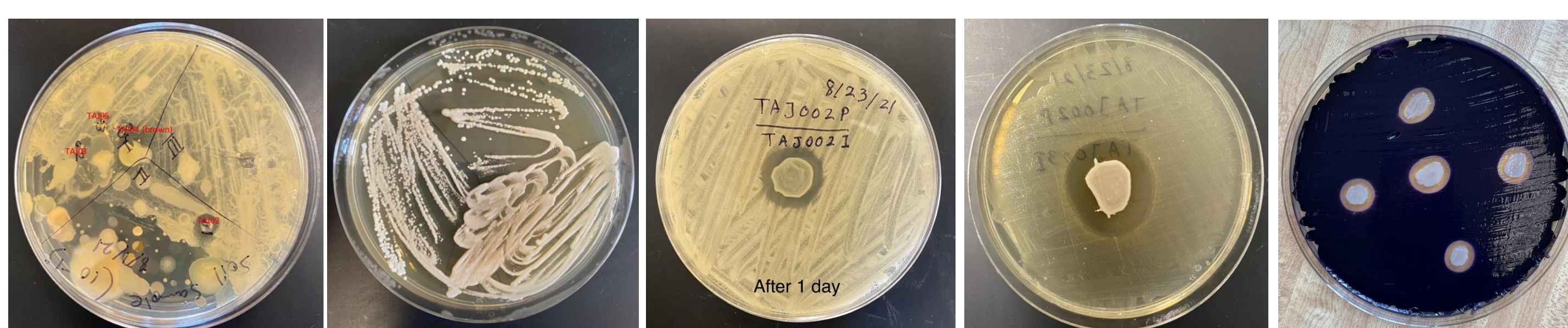
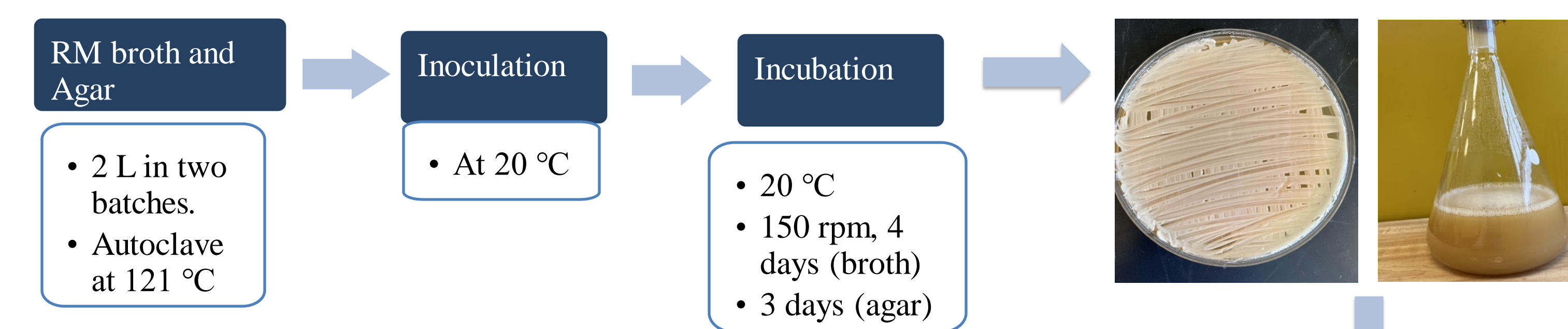


Fig. 3. Images of bacteria isolation and inhibition of three indicator bacteria, the third indicator bacterium has an active QS system

## Experimental Methods

Bacterial Identification was carried out by 16S rRNA PCR analysis.

### Cultivation, Fermentation and Extraction.



Scheme 1. Flow diagram of large scale cultivation and fermentation of TAJX1902 and extraction of metabolites.

### Isolation and purification of compound from crude extract.

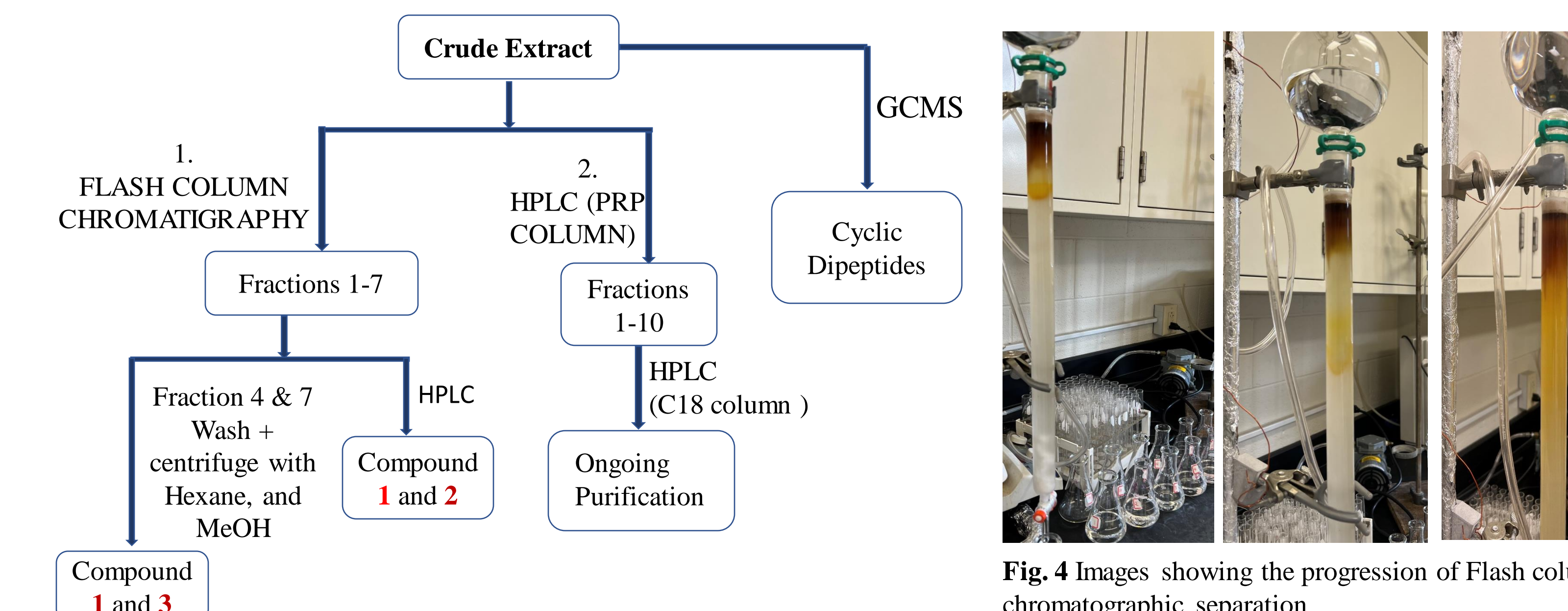


Fig. 4 Images showing the progression of Flash column chromatographic separation

### Characterization of Metabolites

Structures of isolated compounds were determined by <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectral analysis. NMR analysis was performed using an Oxford AS400 NMR spectrometer.

## Results

### Identification - Results indicated that the identity of the microbe is *Arthrobacter* sp.

#### GCMS analysis of *Arthrobacter* sp. TAJX1902 crude extract

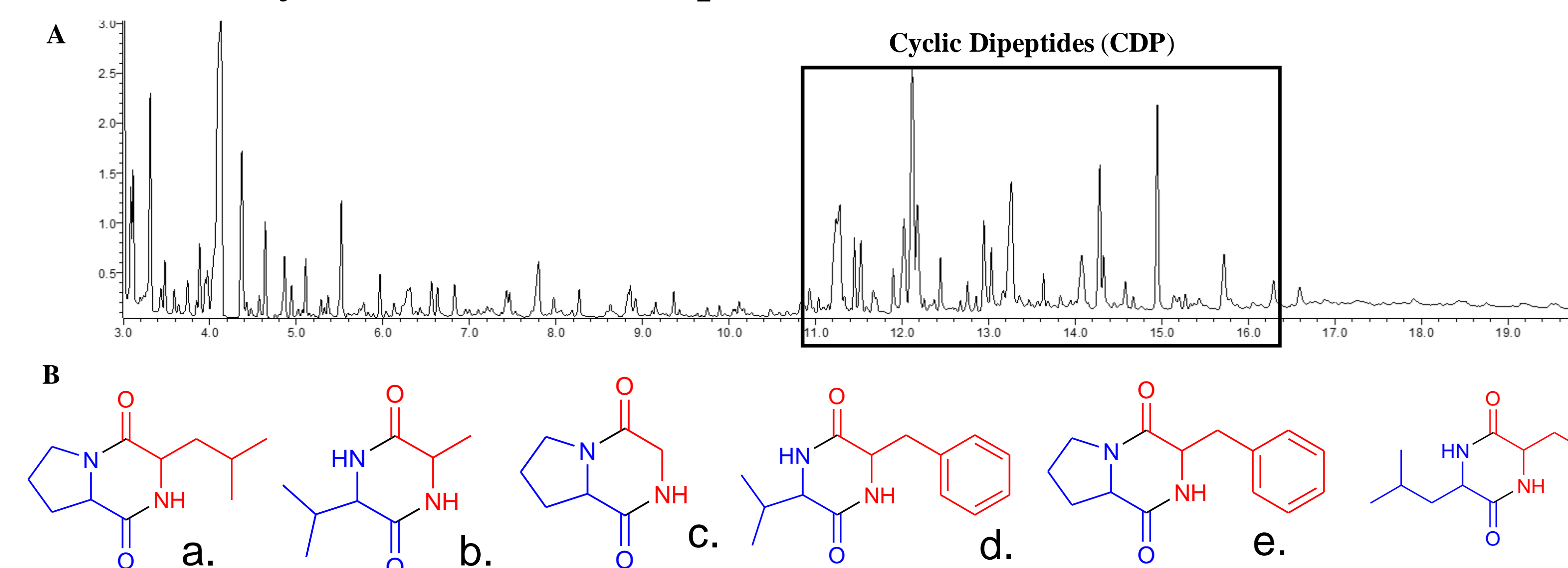


Fig. 5. A. Gas Chromatogram of *Arthrobacter* sp. TAJX1902 crude extract. B. Structures of cyclic dipeptides present in the crude extract (a) Cyclo- Pro-Leu (11.52 min) (b) Cyclo- Val-Ala (11.15 min) (c) Cyclo- Pro-Gly (11.28 min) (d) Cyclo- Leu-Leu (13.26 min) (e) Cyclo- Pro-Phe (14.07 min) (f) Cyclo- Val-Phe (15.43 min)

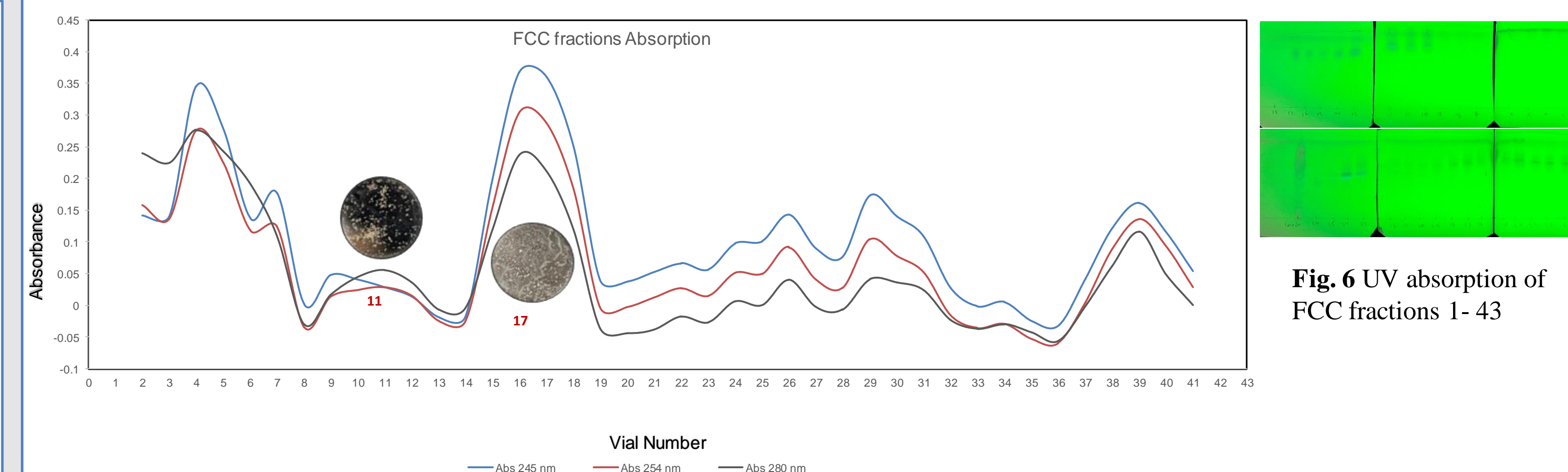


Fig. 6 UV absorption of FCC fractions 1- 43

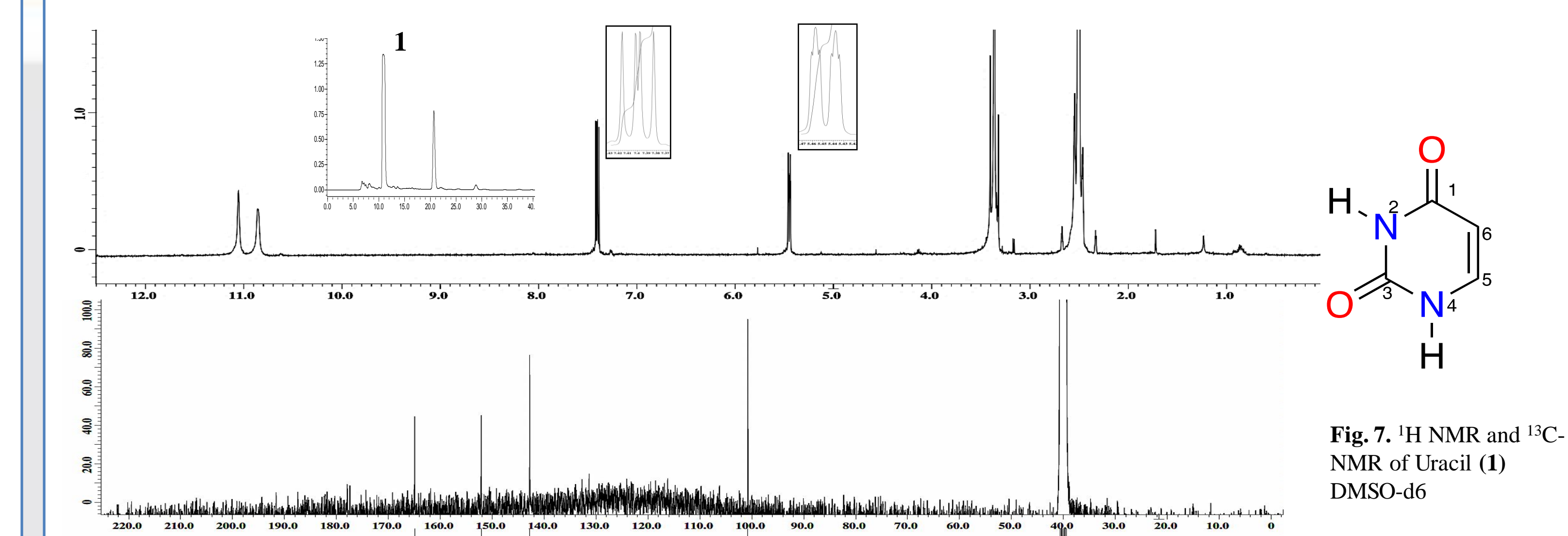


Fig. 7. <sup>1</sup>H NMR and <sup>13</sup>C-NMR of Uracil (1) DMSO-d<sub>6</sub>

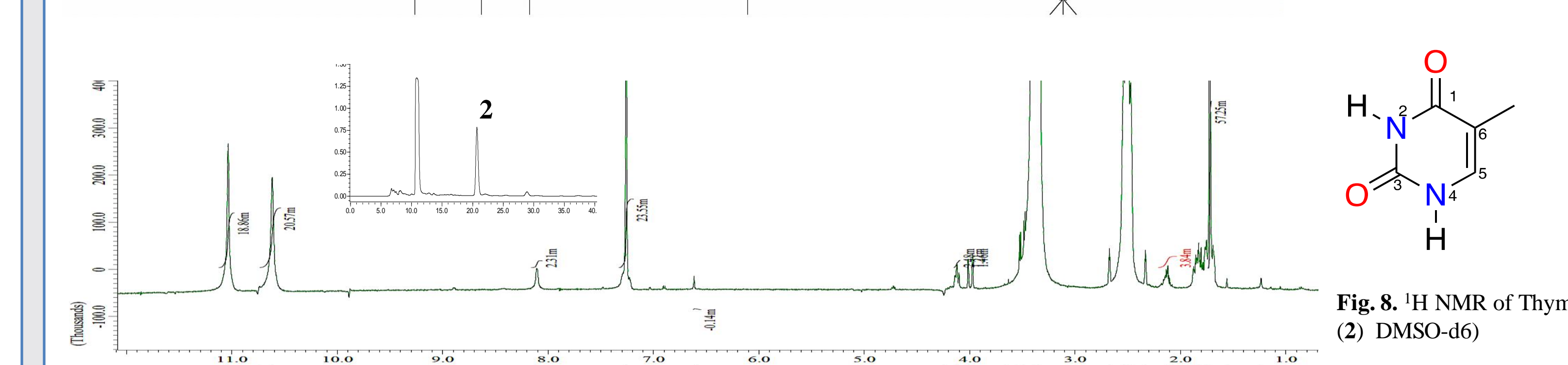


Fig. 8. <sup>1</sup>H NMR of Thymine (2) DMSO-d<sub>6</sub>

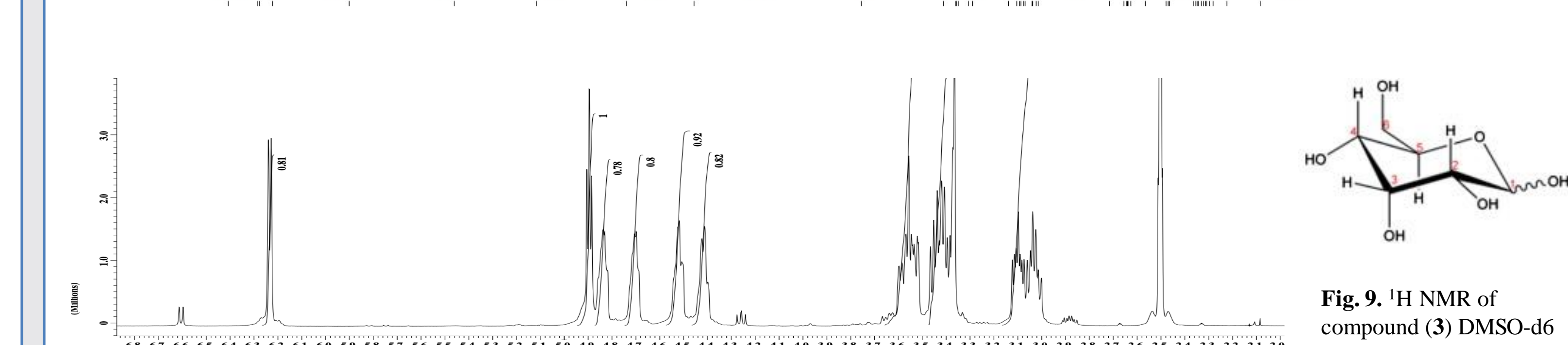


Fig. 9. <sup>1</sup>H NMR of compound (3) DMSO-d<sub>6</sub>

## Discussion

- The GCMS analysis of crude extracts suggested the presence of Cyclic dipeptides (CDP) which are known to have varying bioactivities such as antibacterial, antifungal, antitumor, antiviral activities. However, insufficient quantities were produced by the bacteria to proceed with further analysis.
- Although, the isolated uracil and thymine show no antibacterial activity, the bacteria provides a biosynthetic preparation pathway for the production of (1), an important intermediate of the antitumor 5-fluorouracil.

## Future Work

- Optimization of *Arthrobacter* sp. TAJX1902 culture conditions to increase the production of bioactive metabolites.
- Purification of cyclic dipeptides and other compounds by High Performance Liquid Chromatography (HPLC).
- Structural elucidation of compounds using 1D, 2D-NMR, and antimicrobial bioassay of purified compounds.

## Acknowledgements

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