

### 3.6 Summary and Conclusions

Using the *Chromobacterium violaceum* based bioassay, we have isolated a molecule from the crude extract of isolate 671 (GenBank Accession number KX548356). The molecule has been purified to the single HPLC peak purity using XAD-2 and XAD-4 resins column chromatography for partial purification followed by separation on ODS2 semi-preparative HPLC. The isolated pure molecule is having pinkish-red color and it has been identified by applying various spectroscopic techniques like UV-VIS absorption maximum, FTIR and HRMS analysis. From the spectroscopic analysis data and the literature survey it was found that the spectroscopic data is showing similarity with a known molecule i.e. Cu (III)- coproporphyrin (Figure 3.9). The high-resolution mass spectrum of the pink colored isolated compound is found exactly matching the HRMS of Cu-coproporphyrin. Further, the HRMS has shown the presence of stable isotopes of copper i.e. at  $m/z$  716.1072, 718.1073 and 720.1058 Da, showing the presence of Cu complexed coproporphyrin.

This presence of Cu complexed coproporphyrin is also supported by the data obtained from UV and FTIR studies where the similarity pattern observed in the absorbance maxima of both our isolated molecule and Cu-coproporphyrin which showing spectrophotometric absorbance maxima at 405 nm along with characteristic peaks at 535 nm and 570 nm. Further, the FTIR analysis also showing the presence of secondary amine, aromatic C=C, and alkyl C-H functional groups in the framework of the isolated compound.

The pure isolated molecule has been found to show potent quorum sensing inhibition activity against *Chromobacterium violaceum*, *Agrobacterium tumefaciens* and *Pseudomonas aeruginosa* with an  $IC_{50}$  of 8.15  $\mu$ M for the violacein production and effective biofilm inhibition at 20  $\mu$ M. Hence the isolated molecule is having broad spectrum QS inhibition activity acting against the CviR, TraR and LasR receptor based quorum sensing. The identity of the actinomycete isolate 671 was found related to *Streptomyces xanthochromogenes* with similarity less than <94% indicating the likelihood of being novel *Streptomyces* species involved in the quorum sensing inhibitor production. This is the first time where we have shown the probability of production of Cu-coproporphyrin from a novel isolate 671 i.e. a close relative of *Streptomyces xanthochromogenes* on the basis of 16S rDNA gene sequencing showing quorum sensing inhibition activity

---

**In conclusions,**

- Using *Chromobacterium violaceum* based bioassay, we have isolated a microbial strain that produce quorum sensing inhibitor molecule.
- The isolate showed 93% homology with the *Streptomyces xanthochromogenes* on the basis of 16S rRNA gene sequence.
- The quorum sensing inhibitor compound was isolated and purified using XAD-2 and XAD-4 resins, and HPLC using Spherisorb column.
- The FTIR analysis revealed that some of the characteristics bonds of Coproporphyrin have been found to occur in the isolated quorum sensing inhibitor compound. On the basis of LC-MS/MS, the compound has been putatively identified as Cu-Coproporphyrin III.
- The isolated compound have shown an  $IC_{50}$  of  $8.15\ \mu\text{M}$  for the violacein production against *Chromobacterium violaceum* and effective biofilm inhibition of *Pseudomonas aeruginosa* at  $20\ \mu\text{M}$  concentrations. This purified compound also found to inhibit quorum sensing of the *Agrobacterium tumefaciens* in both cross-feeding agar plate assay as well as liquid bioassay.