



Purification of Flexirubin Pigments from *Chryseobacterium*

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Abstract

The taxonomy of bacteria belonging to the Flavobacteriaceae, Cytophagaceae, and Chitinophagaceae families of the Bacteroidetes phylum has been widely debated and modified in the past few decades. The presence of flexirubins, a modestly studied class of pigments, has been used to differentiate related genera of the Bacteroidetes. The purification and structural determination of forty-two flexirubins isolated from 5 bacterial species has already been accomplished. This project focuses on purifying flexirubins from multiple *Chryseobacterium* species and deducing their chemical structures. Initially, acetone extraction of pigments from *Chryseobacterium oranienense* was performed in methanol for separation of related flexirubin pigments utilizing high performance liquid chromatography (HPLC) at a preparative scale. After months of optimization, a longer column, a more extended solvent gradient, and the use of a low pH solvent system, baseline separation of the *C. oranienense* flexirubins was achieved. The collection of preparative fractions is currently underway so that structural analysis can be completed for the two major flexirubin peaks of *C. oranienense*. In addition, the variation of 25 flexirubin-producing bacteria was investigated. The observed variation in the species chromatographs suggests substantial structural variation as expected. These unique structural variations will be studied in the future with the optimized method designed for this project.

Background

A key difference between the Flavobacteriaceae genera is yellow-orange pigmentation

Figure 2. General chemical structure and variation of a flexirubin. A, General structure with R groups and scale of determined structural variations. B, Flexirubin structural variation.

Purification and structural determination of flexirubins has only been completed for:

- *Chitinophaga filiformis* Fx 1.c.
- *Flavobacterium* sp. strain C-5.
- *Flavobacterium* sp. strain Samoa
- *Flavobacterium johnsoniae* Cy 3j.
- *Sulfosyrillium delatoyanum* 5175.

Methods

Figure 3. Cell Pellet and Solvent Before (A) and After (B) Acetone Extraction.

Extraction → HPLC Purification → Structural Analysis

Figure 5. Reverse-phase HPLC chromatograms. A, Gradient and isocratic solvent as described for analytical runs. B, Gradient and isocratic solvent as described for preparative runs.

Results

Figure 6. Time axis and 10 peaks of Analytical HPLC from 20 *Chryseobacterium* species. A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z.

Figure 7. Time axis and 10 peaks of Analytical HPLC from 20 *Chryseobacterium* species. A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z.

Figure 8. Time axis and 10 peaks of Analytical HPLC from 20 *Chryseobacterium* species. A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z.

Figure 9. Neighbor joining 16S rRNA tree of species of which flexirubin variation was investigated and other related species. Made using MEGA5.

Conclusion

- Successful optimization of flexirubin preparative runs
- Enough material in *C. oranienense* fractions to analyze
- Similar flexirubin profiles observed for similar species
- Flexirubin variation may cluster by genera
- The optimized method to collect flexirubin profiles will be a useful technique for flexirubin-producing bacteria

Future Work

- Analyze structures of *C. oranienense* purified fractions
- Choose other species to purify
 - *F. granulii*, *E. diehli*, *E. tenax*
 - *C. luteum*, *C. pippei*, *C. soli*, *C. aquaticum*
- Test Antioxidant Capacity of purified fractions
- Investigate color shift
- Repeat variation experiments
- Publish structures

Figure 9. Color of colonies of Flavobacterium, Chryseobacterium, Lyconia, Cytophaga, and Kaistella species. A, Color of colonies before 40% KOH treatment. B, Color of colonies after 40% KOH treatment. Color of colonies of each species are each letter or sets of letters as indicated below figures.

Figure 10. Neighbor joining 16S rRNA tree of species of which flexirubin variation was investigated and other related species. Made using MEGA5.

References

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