Purification of Flexirubin Pigments from *Chryseobacterium*

TSA for 36 hrs @ 30°C

C = *Chryseobacterium angstadtii* KM^T
h = *Chryseobacterium aquaticum* KCTC 12483^T
r = *Chryseobacterium greenlandense* UMB 34^T
y = *Chryseobacterium jejuense* DSM 19999^T
s = *Chryseobacterium joostelii* DSM 18211^T
e = *Chryseobacterium luteum* DSM 18606^T
o = *Chryseobacterium oncorhynchi* DSM 24050^T
b = *Chryseobacterium piperi* CTM^T
s = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17072^T
t = *Chryseobacterium soli* DSM 19256^T
a = *Chryseobacterium vrystaatense* LMG 22846^T

After spraying with 40% KOH solution
red color = + for flexirubins

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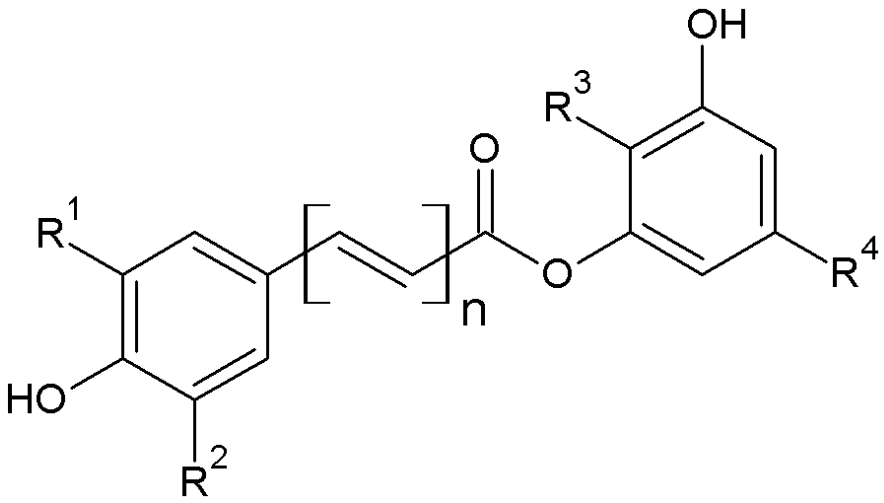
Andrew Gale,
Dr. Newman

Lycoming College

What is a Flexirubin?

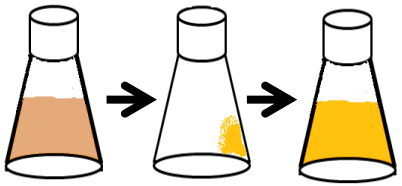
- By definition:
  - A class of polyene metabolites with a generalized 2,5-dialkylresorcinol (DAR) structure present as an aromatic ester

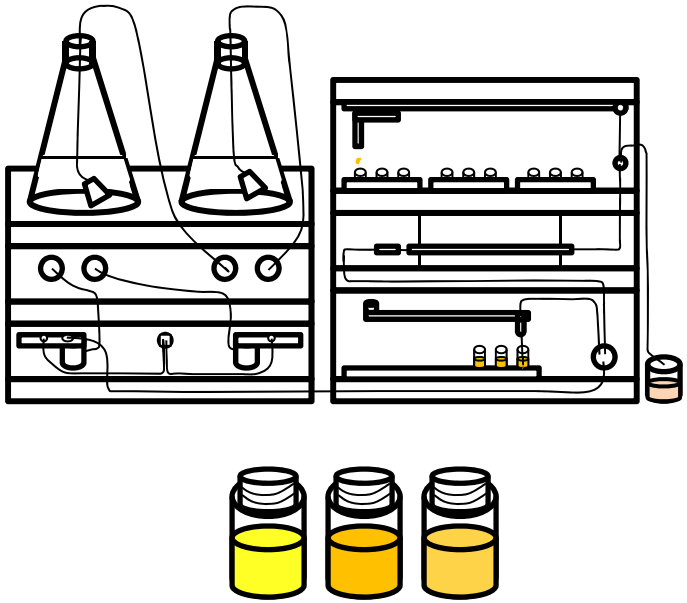


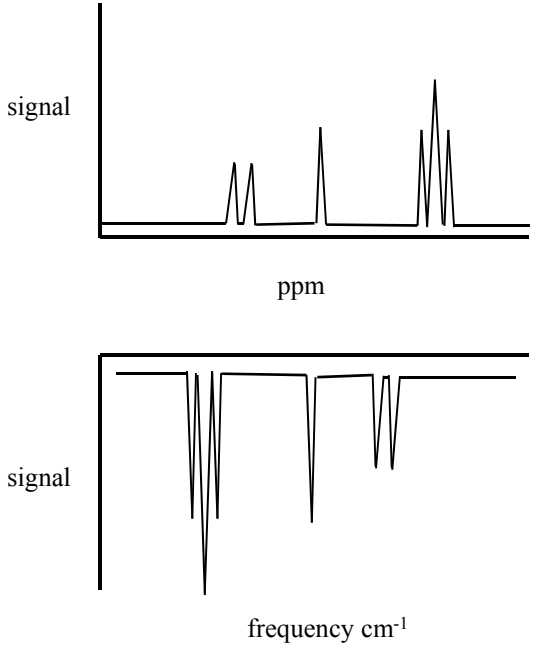
R^1	H, Cl, CH_3
R^2	H, Cl
R^3	different length and branching alkyl chains
R^4	
n	6 to 8 polyenes

Methods

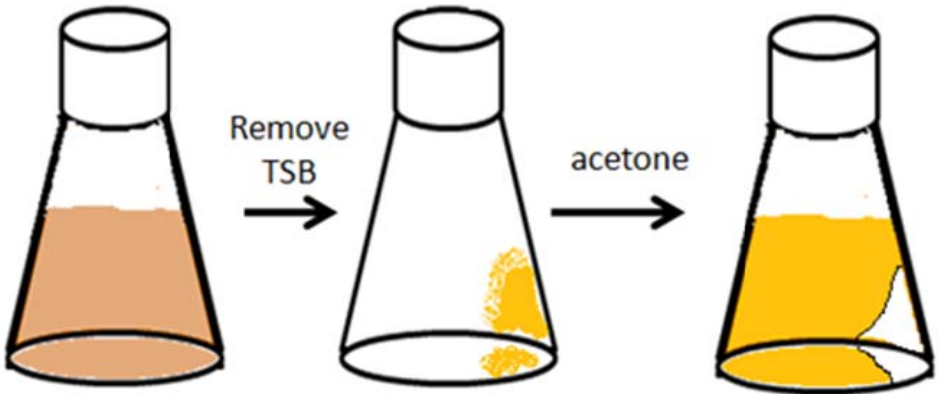
Extraction → Purification → Structural Analysis







Extraction



A

Before

B

After

Purfication

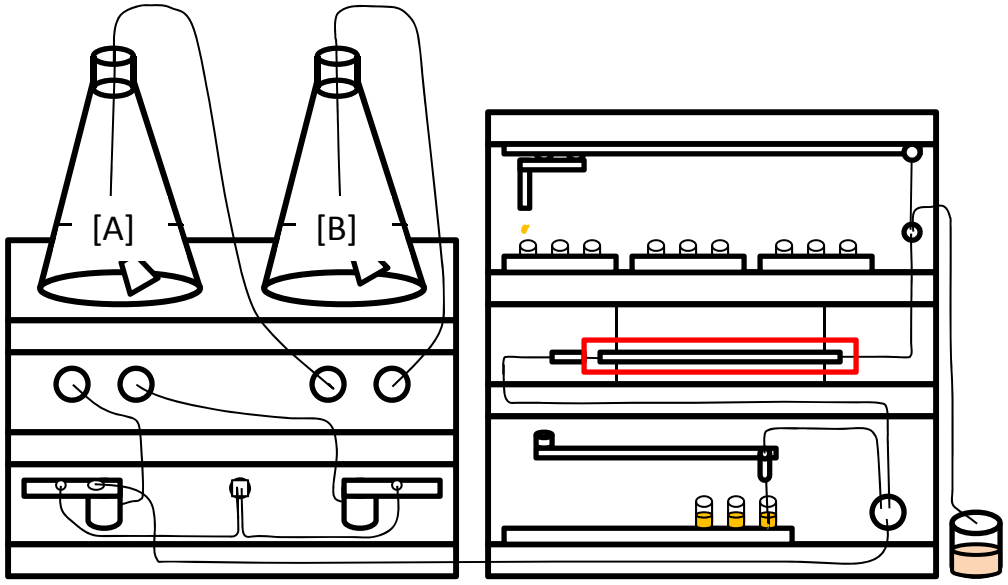
High-**P**erformance **L**iquid **C**hromatography

Reverse-phase HPLC

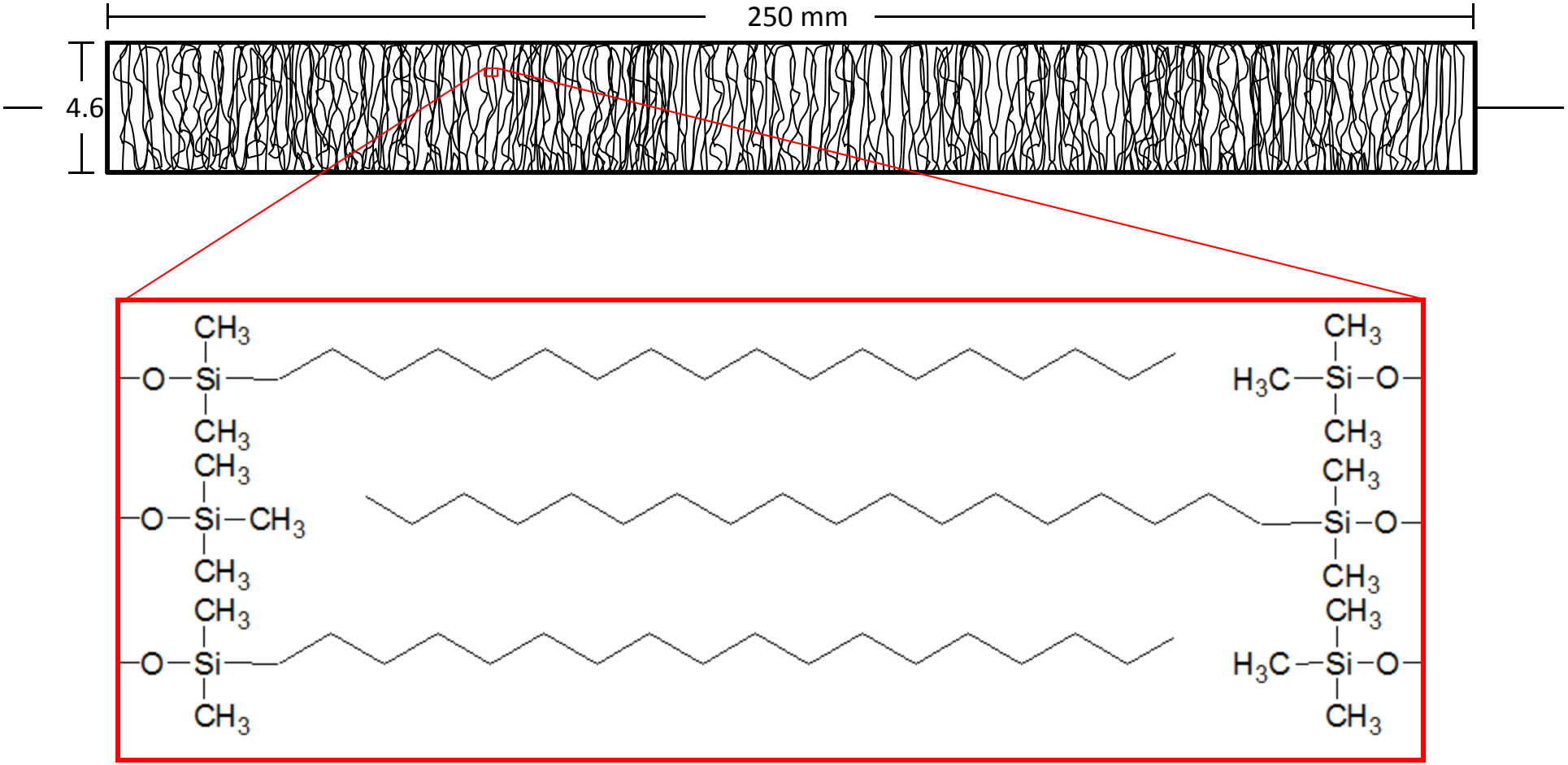
[A] = 50mM PO4 buffer (pH 2.4)

[B] = MeOH with 0.1% CH_3COOH

10% w/v acetone extract in CH_3COOH



C18 Column

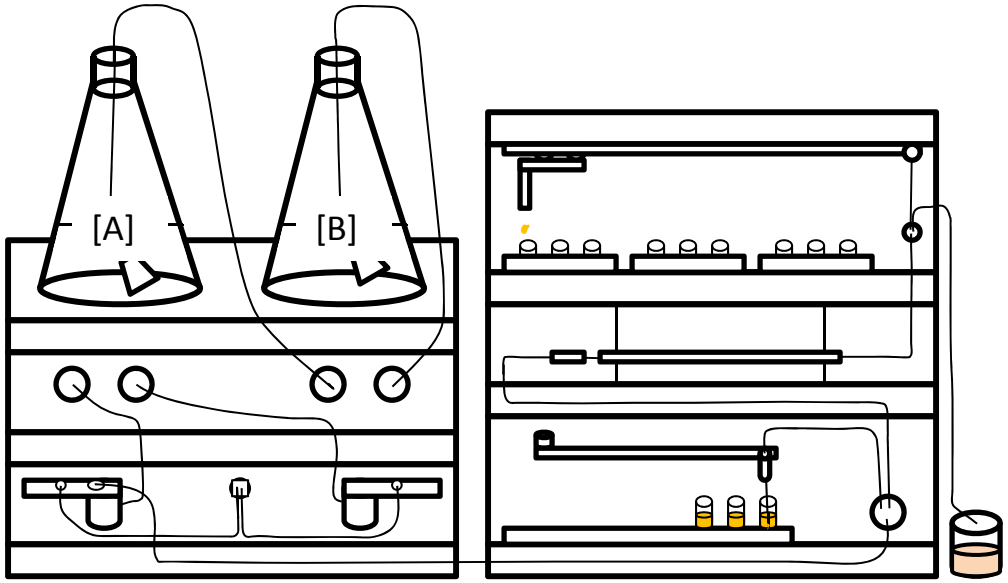


More Methods

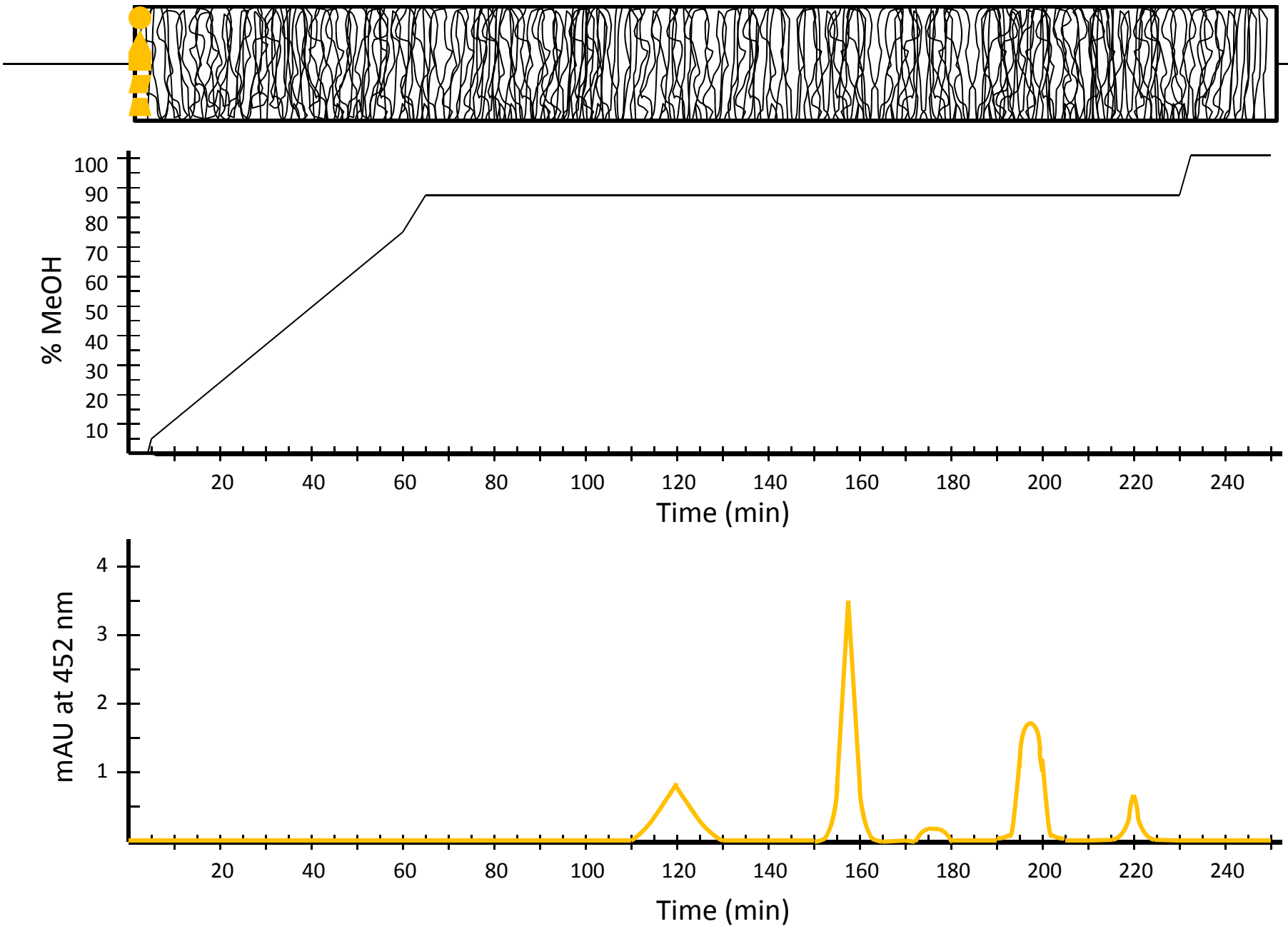
[A] = 50mM PO4 buffer (pH 2.4)

[B] = MeOH with 0.1% CH_3COOH

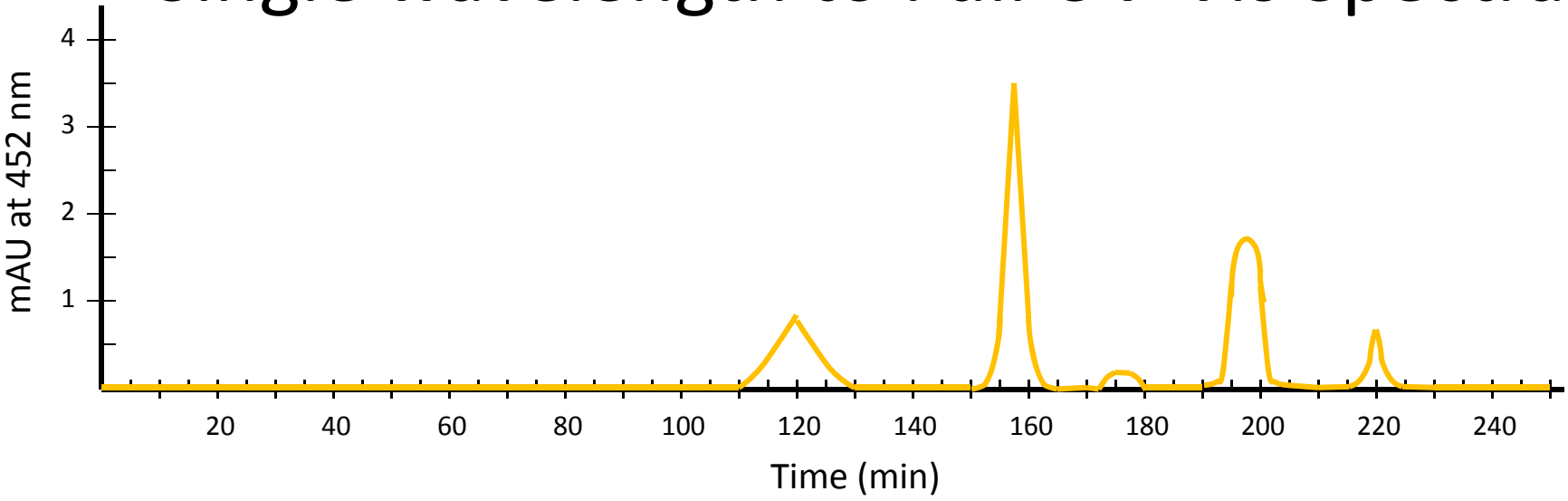
10% w/v acetone extract in CH_3COOH

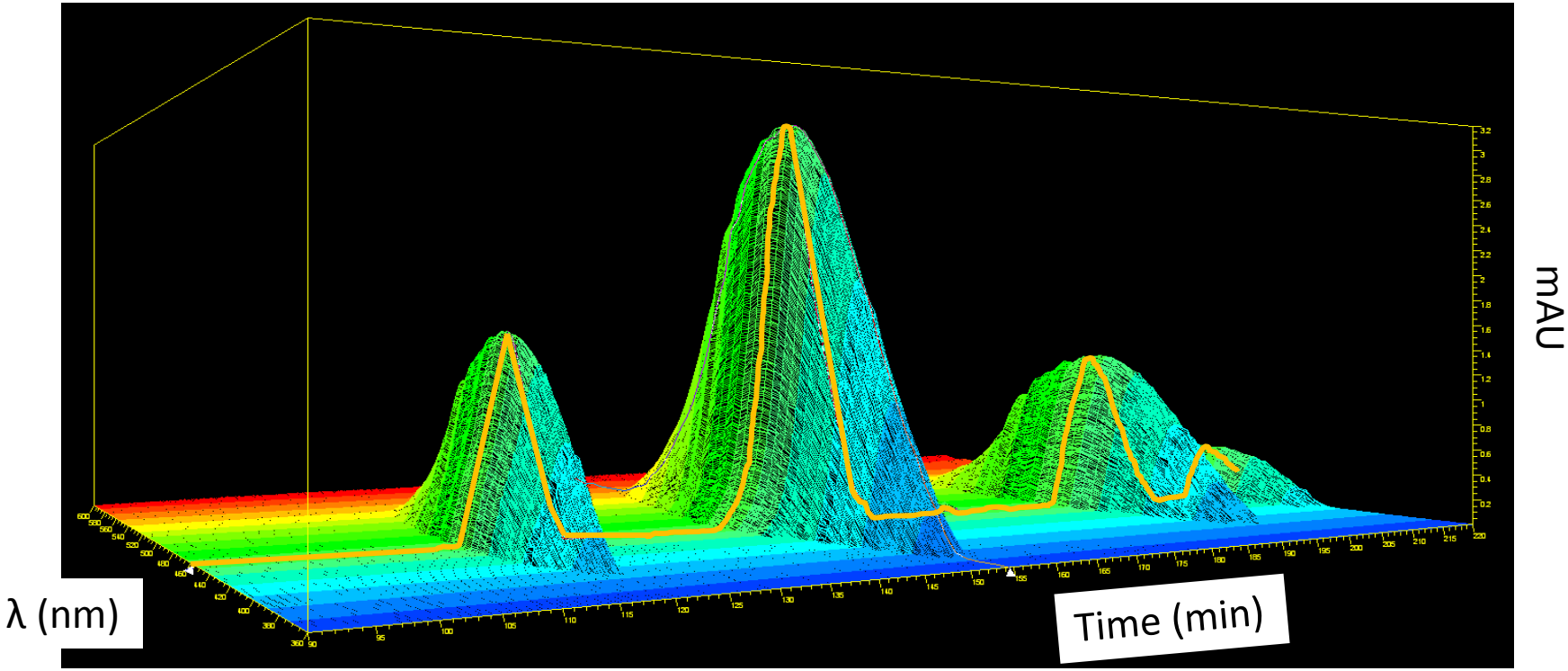


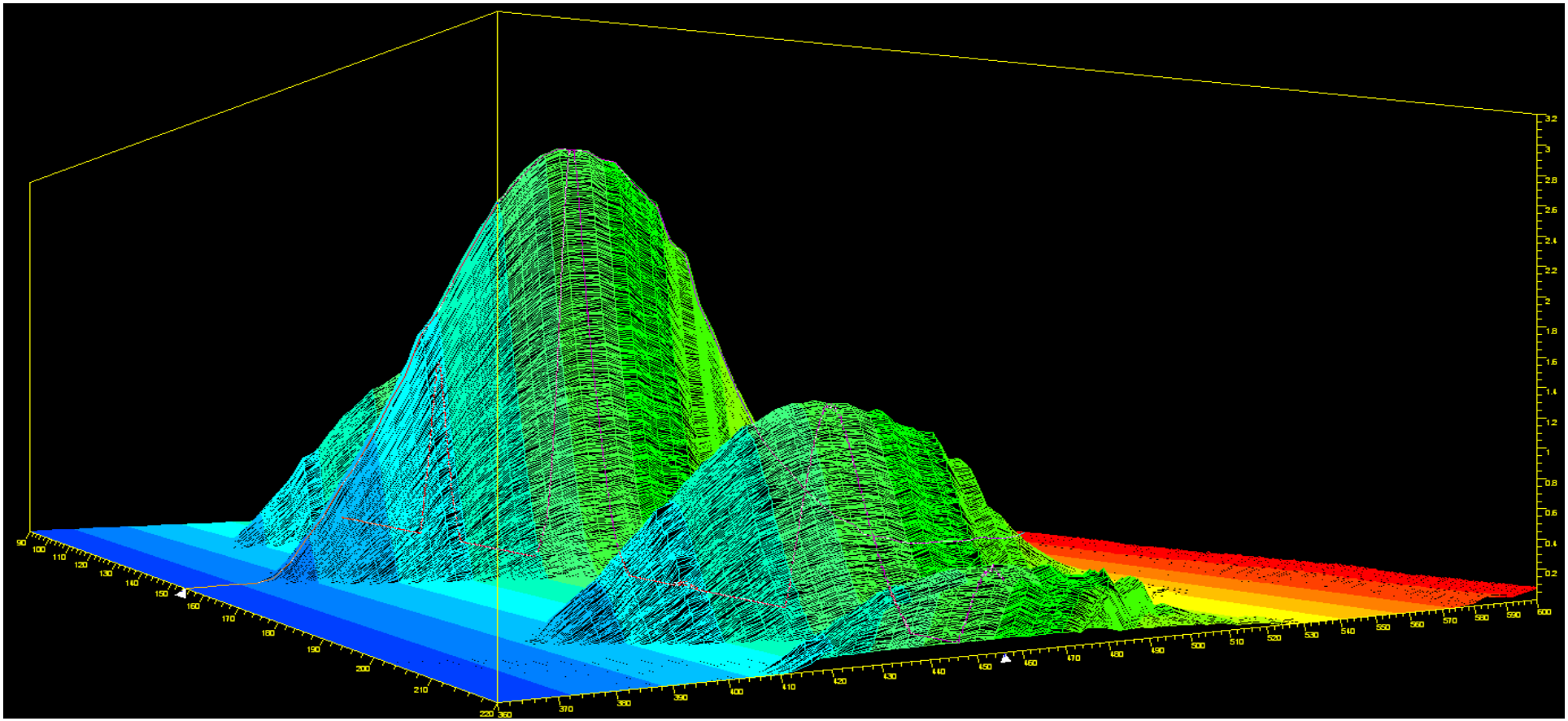
Gradient and Data Analysis



Single wavelength to Full UV-Vis Spectra






90
100
110
120
130
140
150
160
170
180
190
200
210
220
360
370
380
390
400
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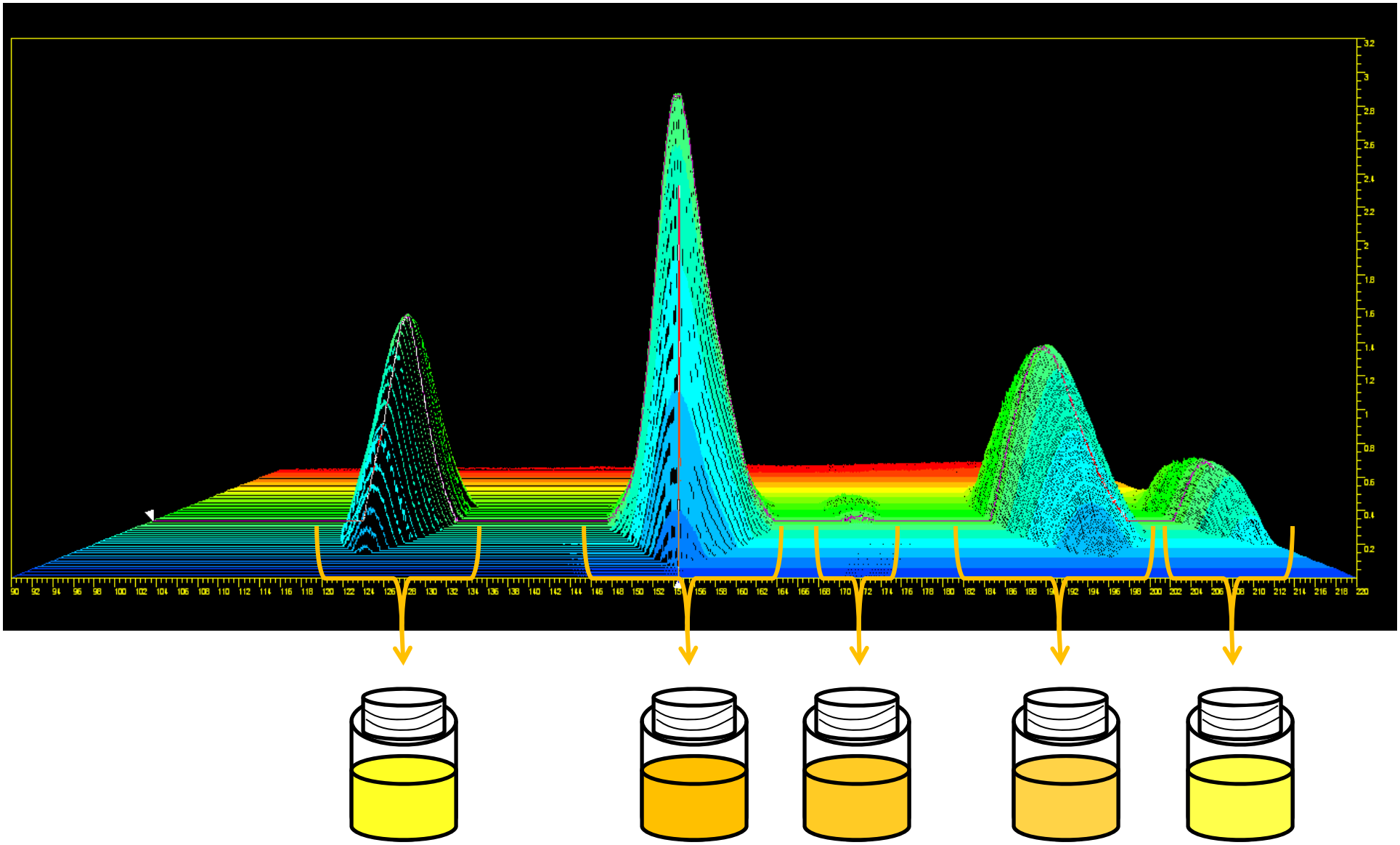
420
430
440
450
460
470
480
490
500
510
520
530
540
550
560
570
580
590
600
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2

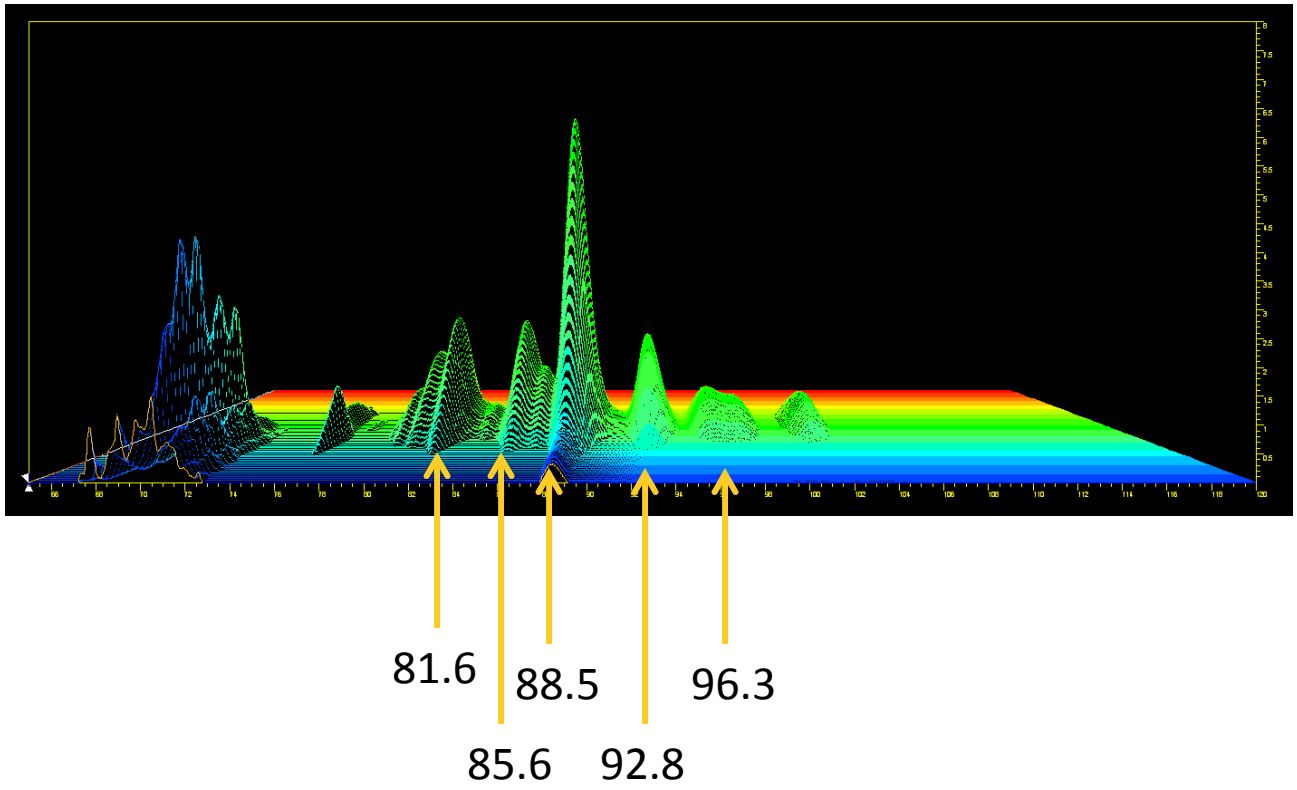
Preparative HPLC



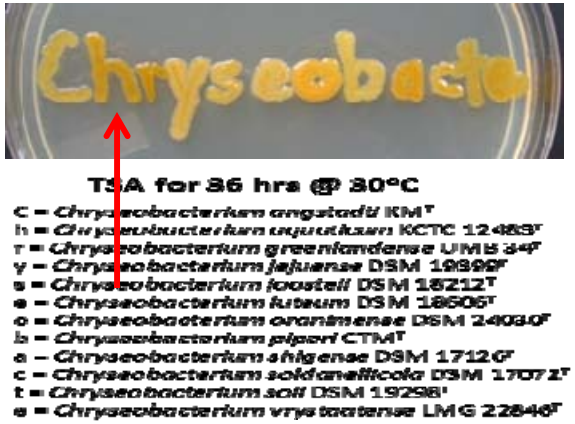
C. angstadti

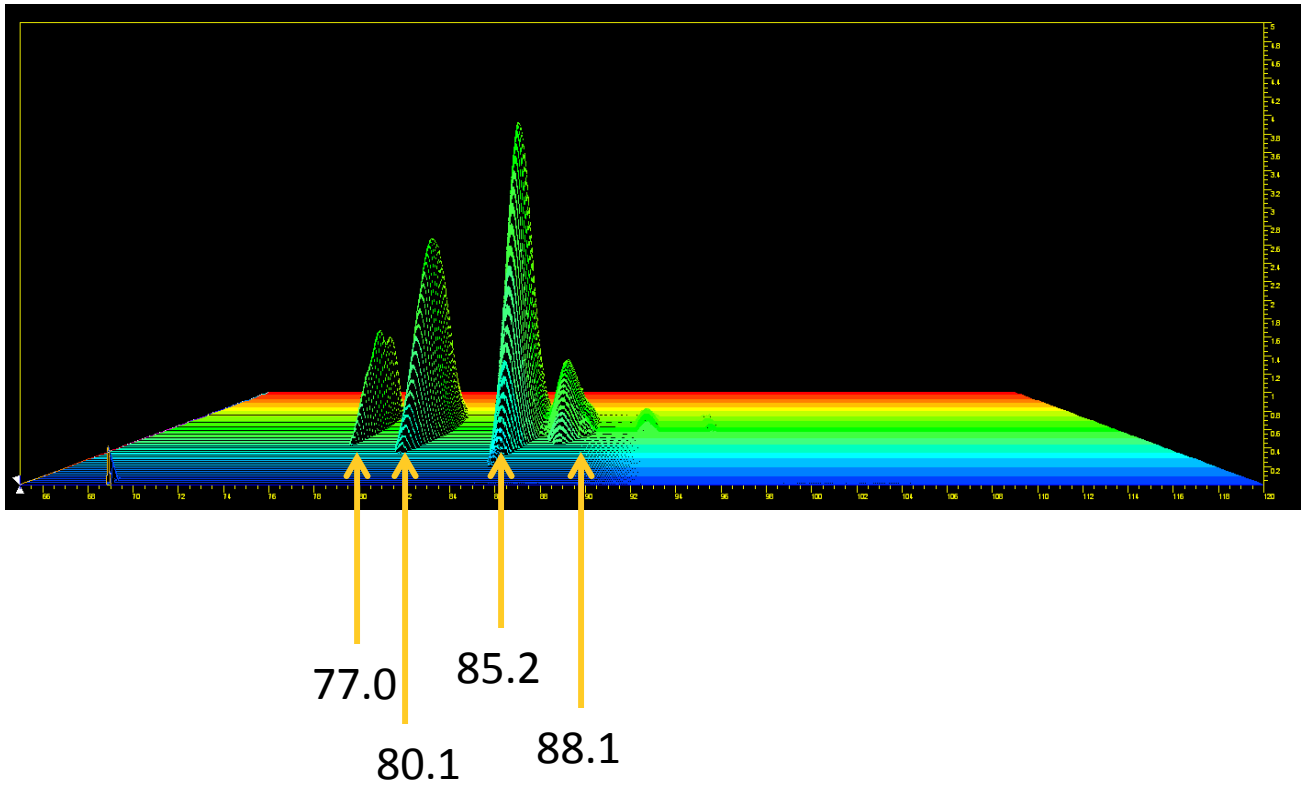
TSA for 36 hrs @ 30°C

c = *Chryseobacterium angstadti* KM^T
h = *Chryseobacterium aquaticum* KCTC 12483^T
r = *Chryseobacterium greenlandense* UMB 34^T
y = *Chryseobacterium jejuense* DSM 19999^T
s = *Chryseobacterium joostelli* DSM 18212^T
e = *Chryseobacterium luteum* DSM 18605^T
o = *Chryseobacterium oranimense* DSM 24030^T
b = *Chryseobacterium piperi* CTM^T
a = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17071^T
t = *Chryseobacterium soli* DSM 19298^T
e = *Chryseobacterium vrystaatense* LMG 22846^T



C. aquaticum

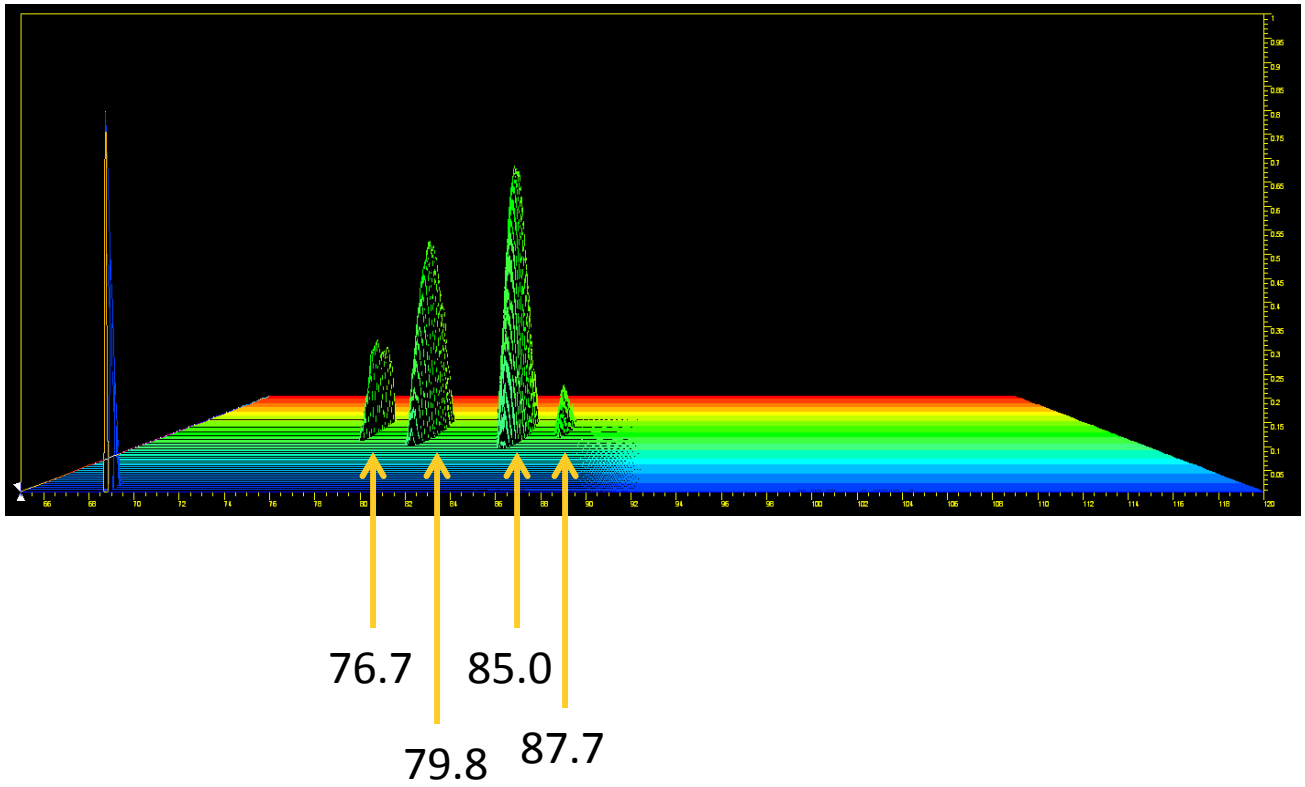




C. greenlandense

TSA for 36 hrs @ 30°C

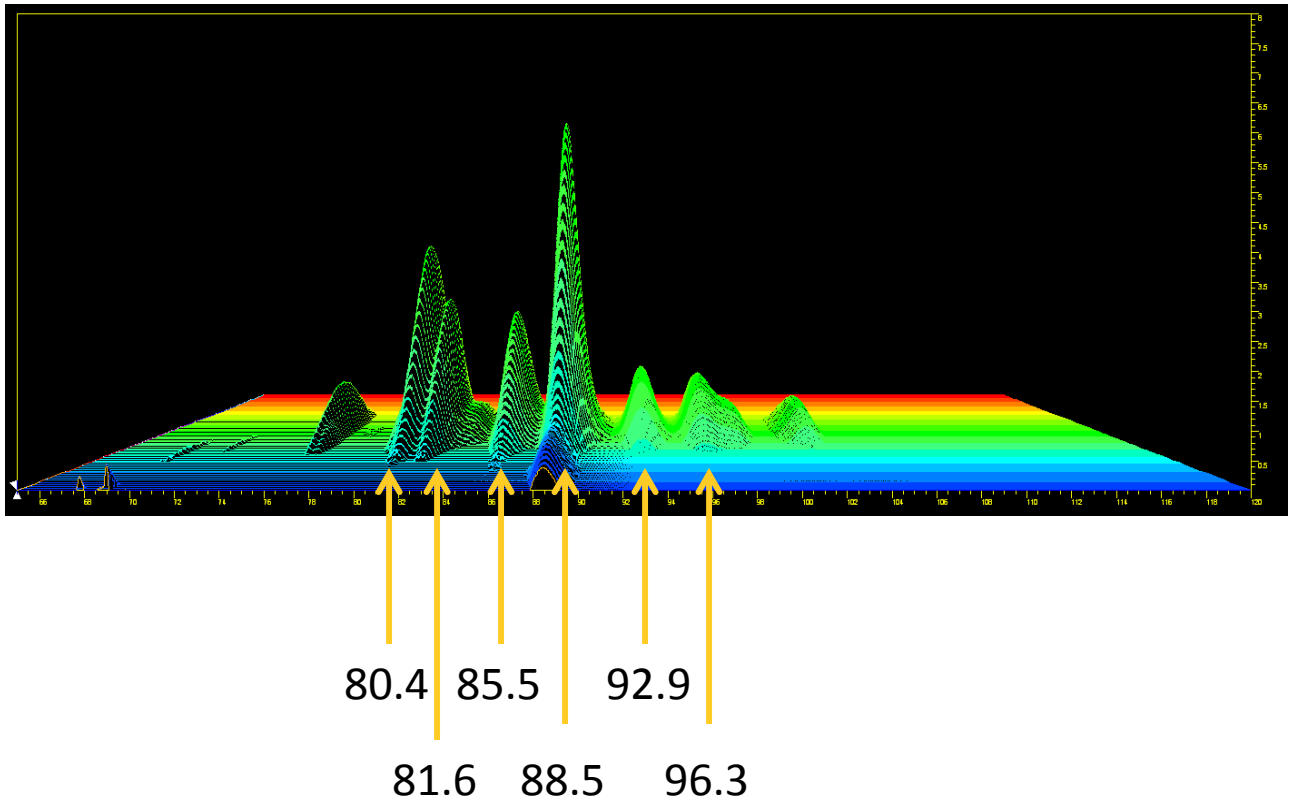
c = *Chryseobacterium angstadii* KM^T
h = *Chryseobacterium aquaticum* KCTC 12483^T
r = *Chryseobacterium greenlandense* UMB 34^T
y = *Chryseobacterium jejuense* DSM 19992^T
s = *Chryseobacterium joostelii* DSM 18212^T
e = *Chryseobacterium luteum* DSM 18605^T
o = *Chryseobacterium oranimense* DSM 24830^T
b = *Chryseobacterium piperi* CTM^T
a = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17071^T
t = *Chryseobacterium soli* DSM 19298^T
e = *Chryseobacterium vrystaatense* LMG 22846^T



C. jejuense

TSA for 36 hrs @ 30°C

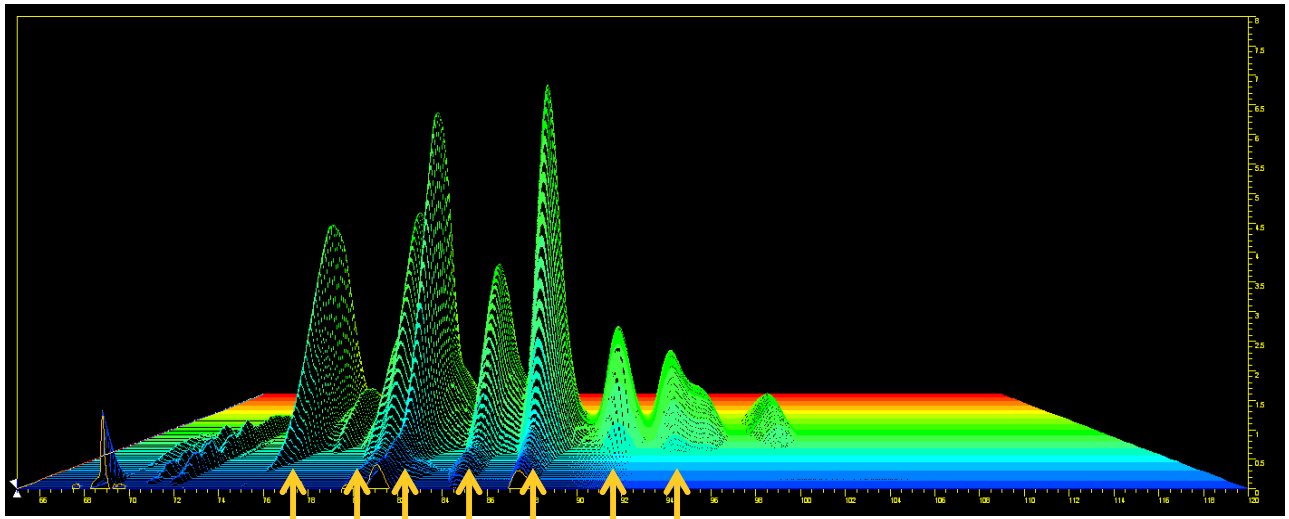
c = *Chryseobacterium angstadii* KM^T
h = *Chryseobacterium aquaticum* KCTC 12483^T
r = *Chryseobacterium greenlandense* UMB 34^T
y = *Chryseobacterium jejuense* DSM 19992^T
s = *Chryseobacterium joostelii* DSM 18212^T
e = *Chryseobacterium luteum* DSM 18605^T
o = *Chryseobacterium oranimense* DSM 24830^T
b = *Chryseobacterium piperi* CTM^T
a = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17071^T
t = *Chryseobacterium soli* DSM 19298^T
e = *Chryseobacterium vrystaatense* LMG 22846^T



C. joostei

TSA for 36 hrs @ 30°C

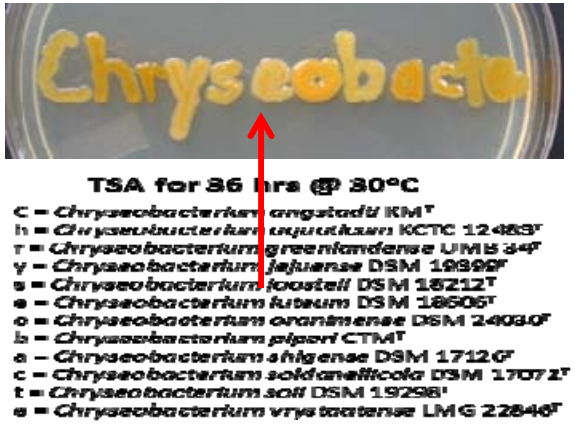
c = *Chryseobacterium angstadii* IM^T
h = *Chryseobacterium aquaticum* KCTC 12483^T
r = *Chryseobacterium greenlandense* UMB 34^T
y = *Chryseobacterium jejuense* DSM 19999^T
s = *Chryseobacterium joostei* DSM 18212^T
e = *Chryseobacterium luteum* DSM 18605^T
o = *Chryseobacterium oranimense* DSM 24030^T
b = *Chryseobacterium piperi* CTM^T
a = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17071^T
t = *Chryseobacterium soli* DSM 19298^T
e = *Chryseobacterium vrystaatense* LMG 22846^T

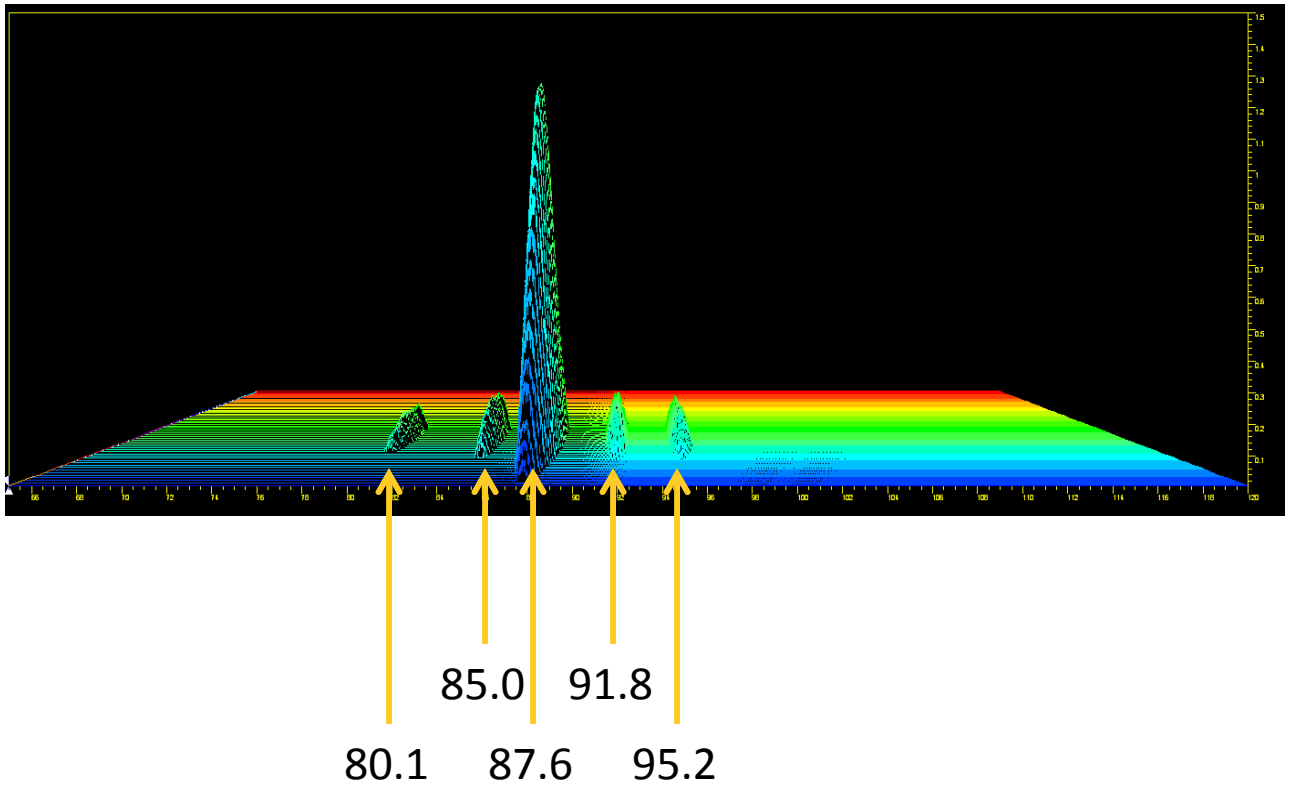


74.9 81.0 87.4 94.8

79.8 84.6 91.6

C. luteum

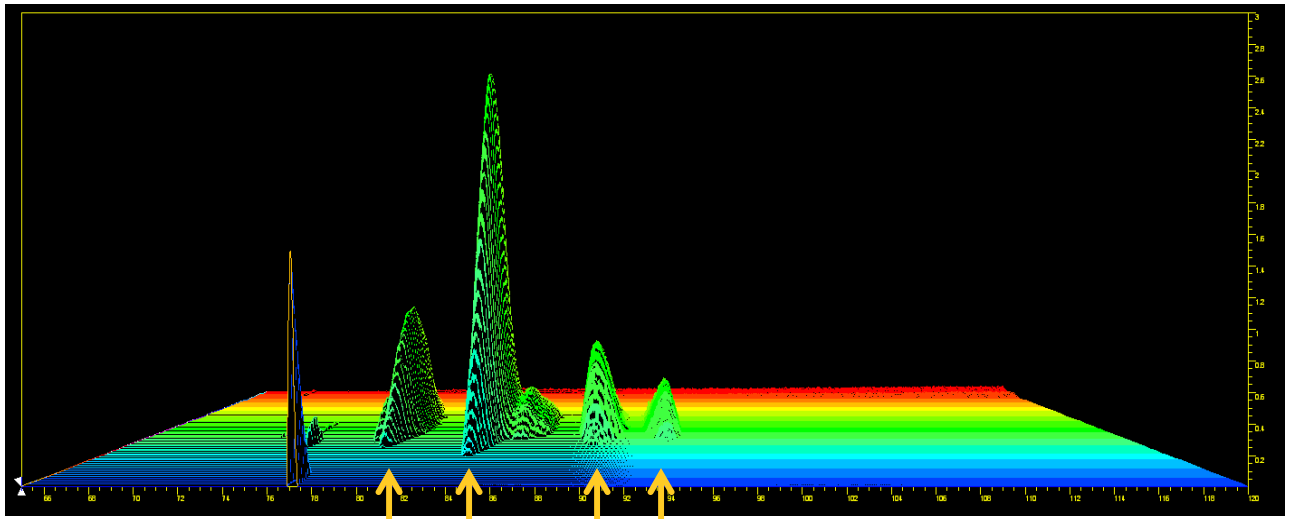




C. oranimense

TSA for 36 hrs @ 30°C

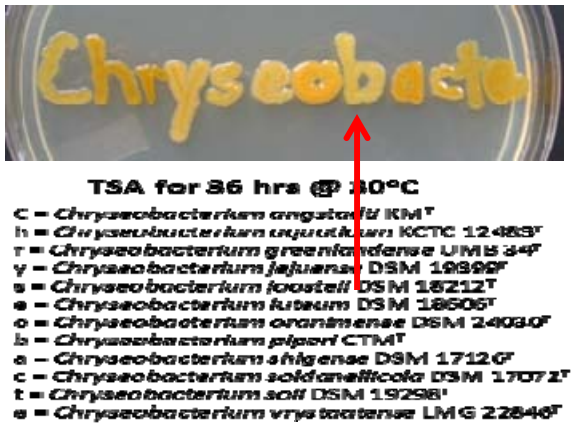
c = *Chryseobacterium angstadii* KM^T
h = *Chryseobacterium hyperatium* KCTC 12483^T
r = *Chryseobacterium greenlandense* UMB 34^T
y = *Chryseobacterium jejuense* DSM 19992^T
s = *Chryseobacterium foetidi* DSM 18212^T
e = *Chryseobacterium luteum* DSM 18605^T
o = *Chryseobacterium oranimense* DSM 24830^T
h = *Chryseobacterium piperi* CTM^T
a = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17071^T
t = *Chryseobacterium soli* DSM 19298^T
e = *Chryseobacterium vrystaatense* LMG 22846^T

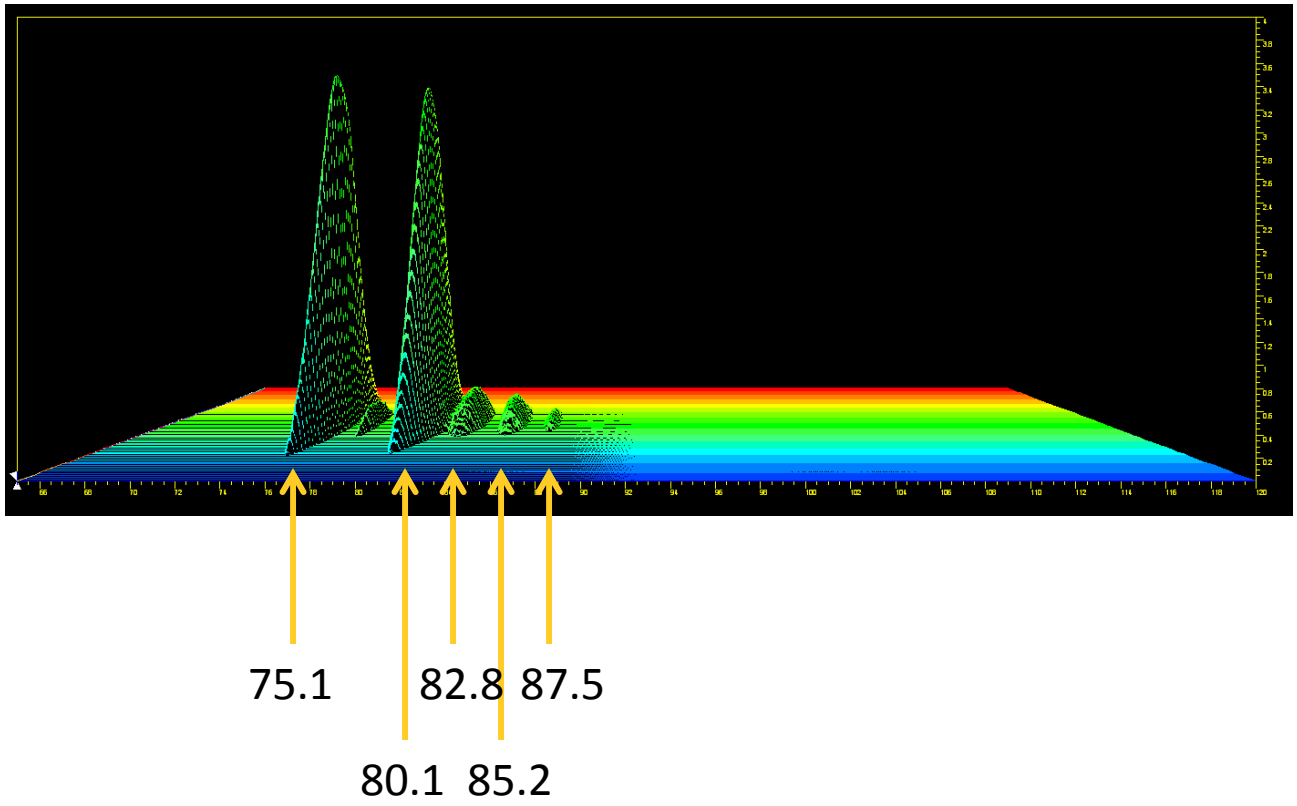


79.1 86.1

83.8 90.2

C. piperi

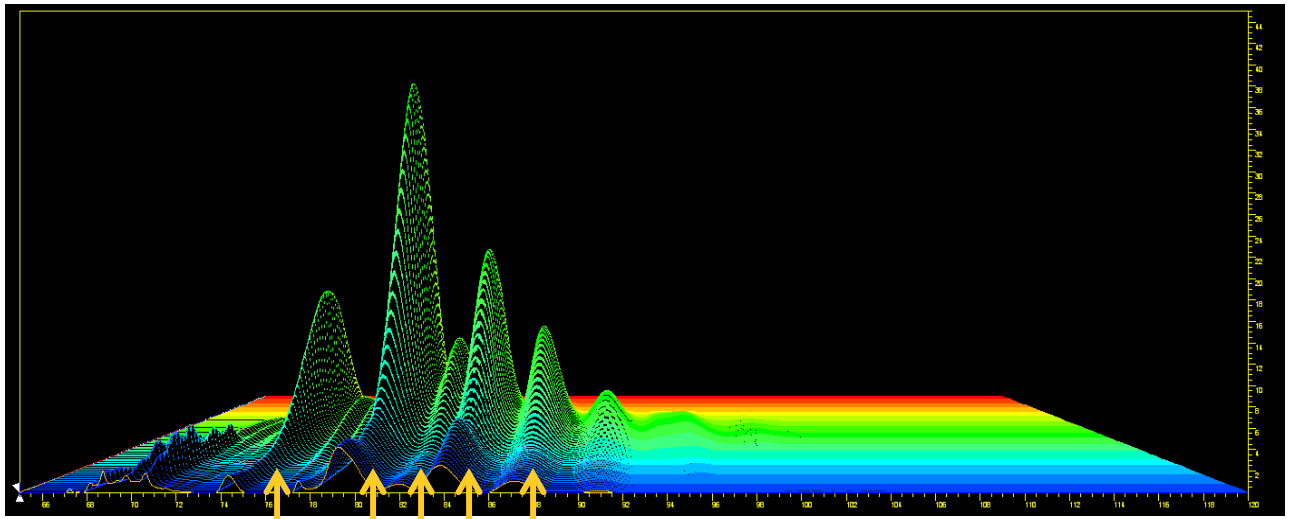

TSA for 36 hrs @ 30°C
c = Chryseobacterium angstadii KM^T
h = Chryseobacterium aquaticum KCTC 12483^T
r = Chryseobacterium greenlandense UMB 34^T
y = Chryseobacterium jejuense DSM 19999^T
s = Chryseobacterium joostelli DSM 18212^T
e = Chryseobacterium luteum DSM 18605^T
o = Chryseobacterium oranimense DSM 24030^T
b = Chryseobacterium piperi CTM^T
a = Chryseobacterium shigense DSM 17126^T
c = Chryseobacterium soldanellicola DSM 17071^T
t = Chryseobacterium soli DSM 19298^T
e = Chryseobacterium vrystaatense LMG 22846^T


75.1
82.8
87.5
80.1
85.2

C. shigense

TSA for 36 hrs @ 30°C

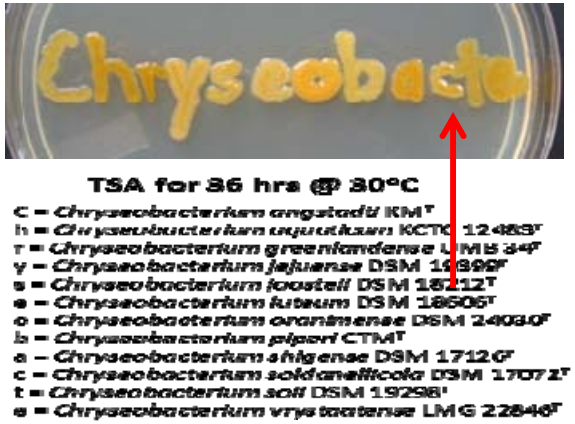
c = *Chryseobacterium angstadii* KM^T
h = *Chryseobacterium aquaticum* DCTC 12483^T
r = *Chryseobacterium greenlandense* UMB 34^T
y = *Chryseobacterium jejuense* DSM 19999^T
s = *Chryseobacterium joostelli* DSM 18212^T
e = *Chryseobacterium luteum* DSM 18605^T
o = *Chryseobacterium oranimense* DSM 24030^T
b = *Chryseobacterium piperi* CTM^T
a = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17071^T
t = *Chryseobacterium soli* DSM 19298^T
e = *Chryseobacterium vrystaatense* LMG 22846^T

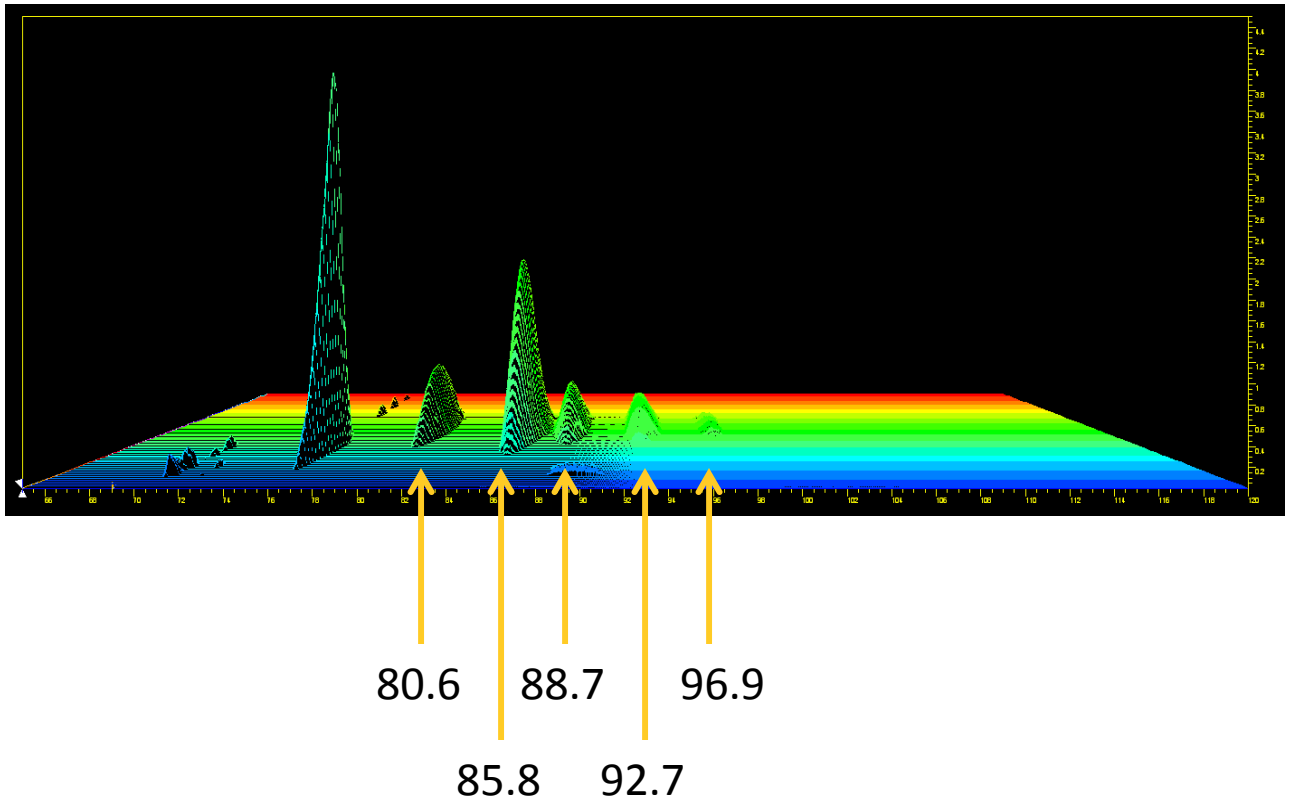


74.3 82.0 87.1

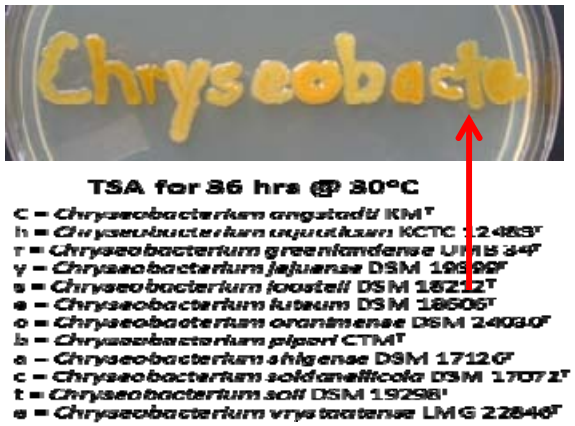
79.3 83.4

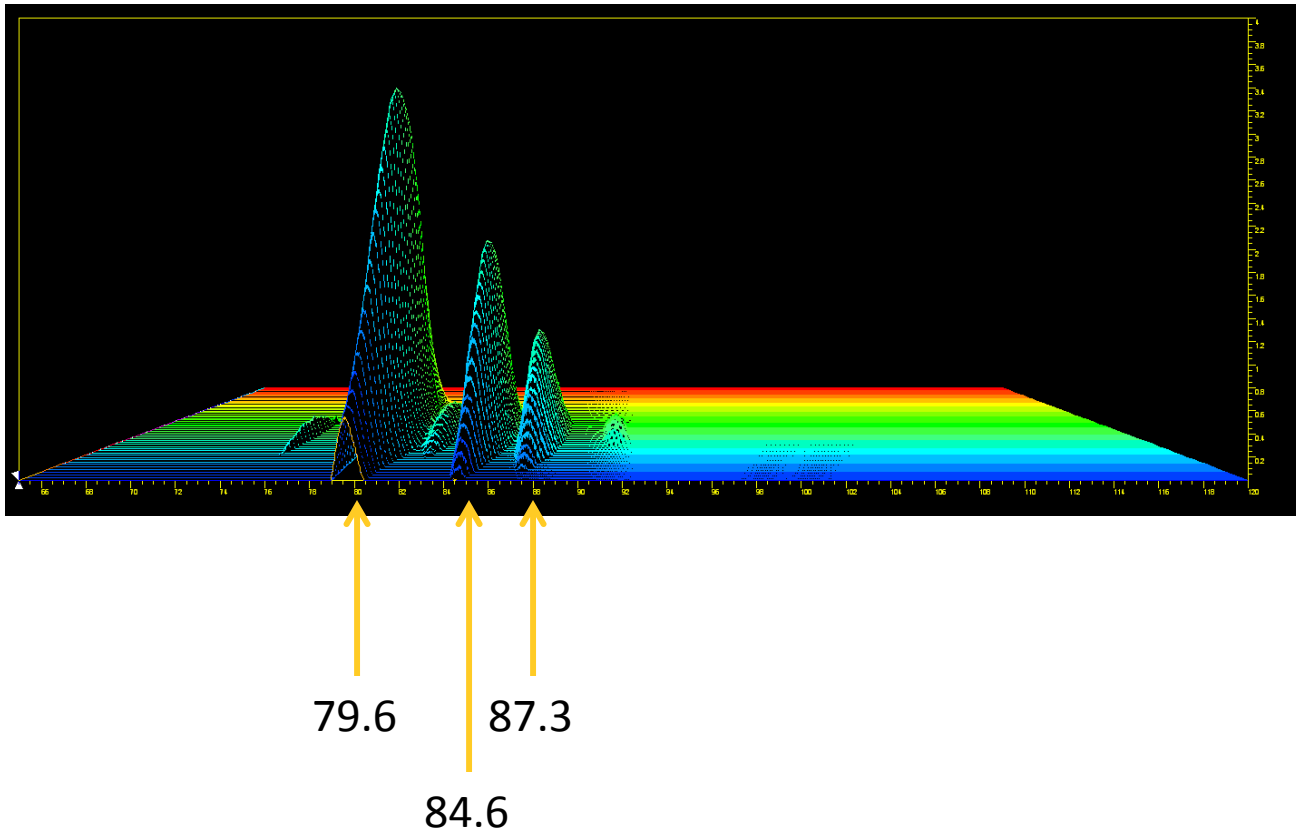
C. soldanellicola





C. soli

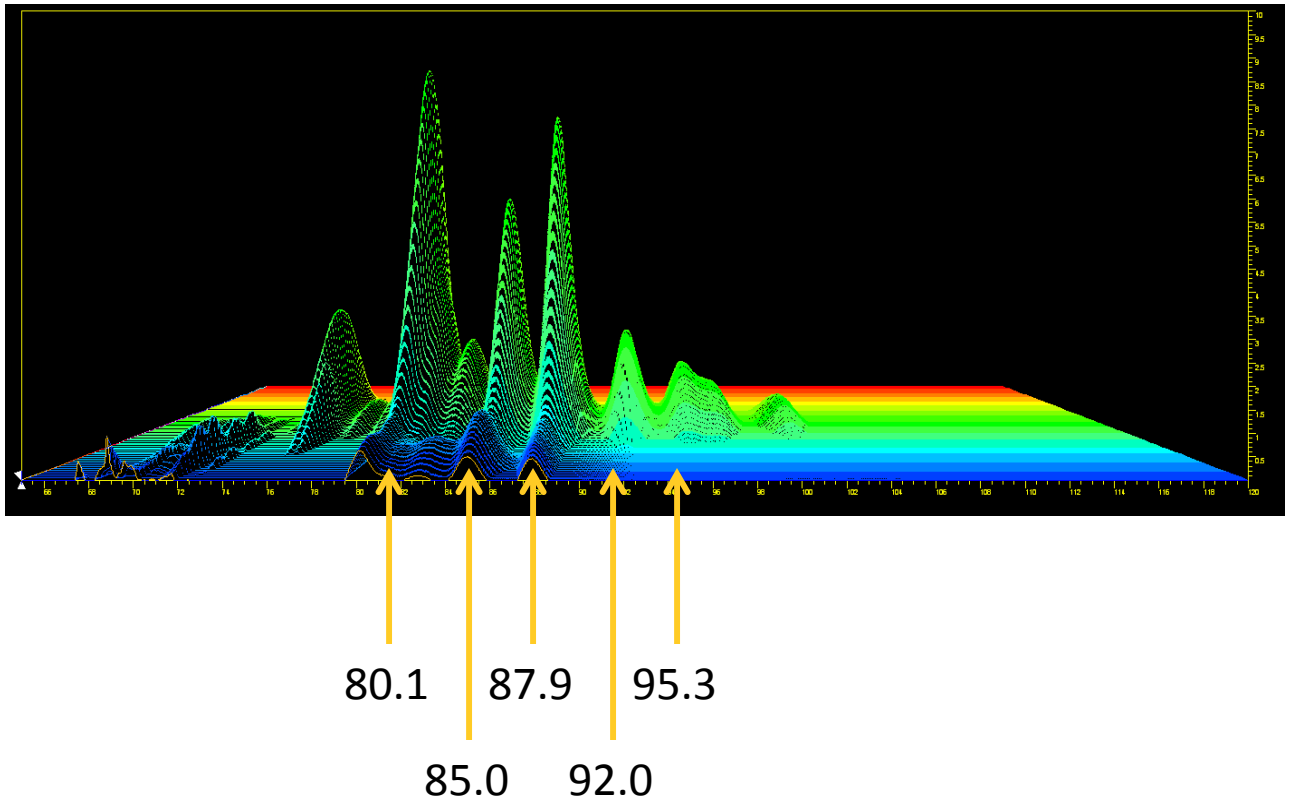

TSA for 36 hrs @ 30°C
c = Chryseobacterium angstadii IM
h = Chryseobacterium aquaticum KCTC
r = Chryseobacterium greenlandense
y = Chryseobacterium jejuense DSM
s = Chryseobacterium joostelI DSM
e = Chryseobacterium luteum DSM
o = Chryseobacterium oranimense DSM
h = Chryseobacterium piperi CTM
a = Chryseobacterium shigense DSM
c = Chryseobacterium soldanellicola DSM
t = Chryseobacterium soli DSM
e = Chryseobacterium vrystaatense LMG


79.6
87.3
84.6

C. vrystaatense

TSA for 36 hrs @ 30°C

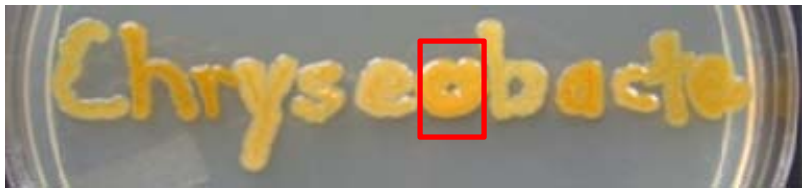
c = *Chryseobacterium angstadii* KM^T
h = *Chryseobacterium aquaticum* KCTC 12483^T
r = *Chryseobacterium greenlandense* UM534^T
y = *Chryseobacterium jejuense* DSM 19999^T
s = *Chryseobacterium joostelii* DSM 18212^T
e = *Chryseobacterium luteum* DSM 18605^T
o = *Chryseobacterium oranimense* DSM 24030^T
b = *Chryseobacterium piperi* CTM^T
a = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17071^T
t = *Chryseobacterium soli* DSM 19298^T
e = *Chryseobacterium vrystaatense* LMG 22846^T



Future Work

- Optimize:
  - Analyze structuresof

    C. oranimense preparative fractions
- Choose other species to purify
- Publish structures



TSA for 36 hrs @ 30°C

References

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