



Study of the flexibility of the CLP assembly line in *Pseudomonas* bacteria

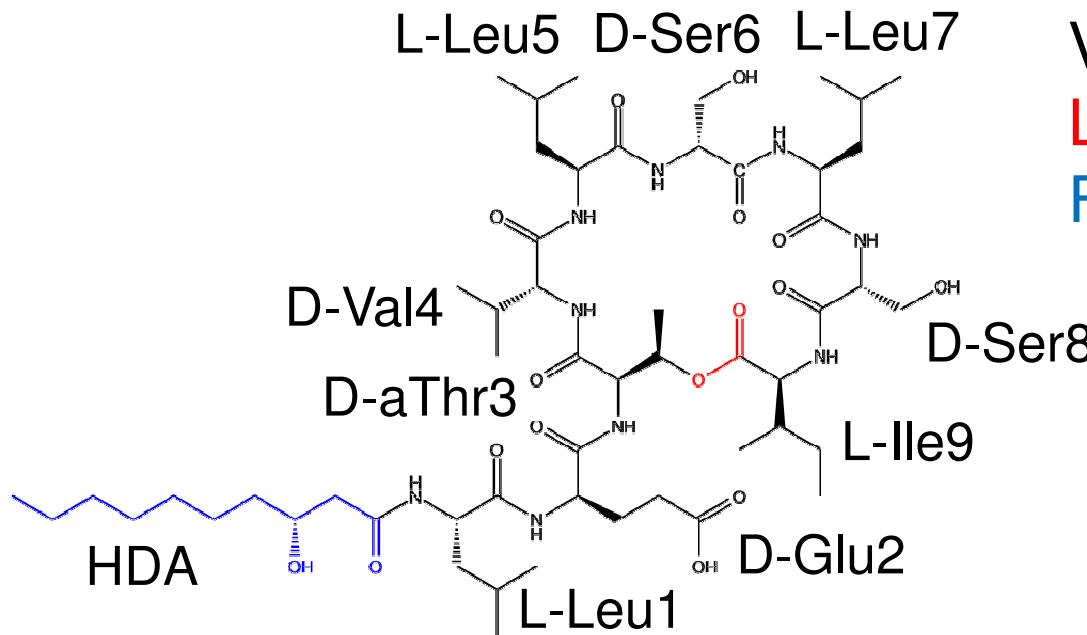


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Outline

- Cyclolipodepsipeptides
- Extraction and isolation
- Quantification of CLPs → LC-MS
- Structural analysis → **Solution NMR Spectroscopy**
 - NMR assignment of viscosin
 - Structure elucidation of minor 5
- Conclusion
- Safety issues

Cyclolipodepsipeptides (CLPs)



Viscosin
Lactone/depsi bond
Fatty acid

Changes in growth stage and conditions

- Variances in relative quantities of produced CLPs
- Identification of new CLP variants

Extraction and Isolation Procedure

3 days growth
 $\frac{1}{4}$ KB

3 days growth
 $\frac{1}{2}$ KB

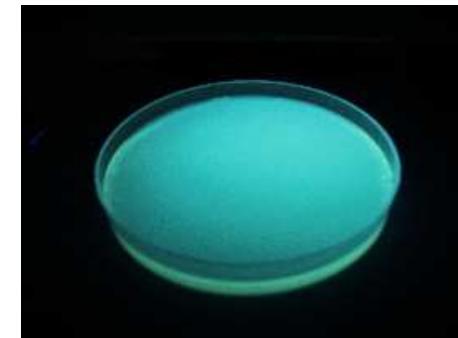
3 days growth
1 KB

3 days growth
1 KB

4 days growth
1 KB

7 days growth
1 KB

- Cultures are scraped
- 3 to 4 times diluted with H_2O
- Acidified to pH 2
- Alkalized to pH 8
- Acidified to pH 2



Extraction and Isolation Procedure

3 days growth
1/4 KB

3 days growth
1/2 KB

3 days growth
1 KB

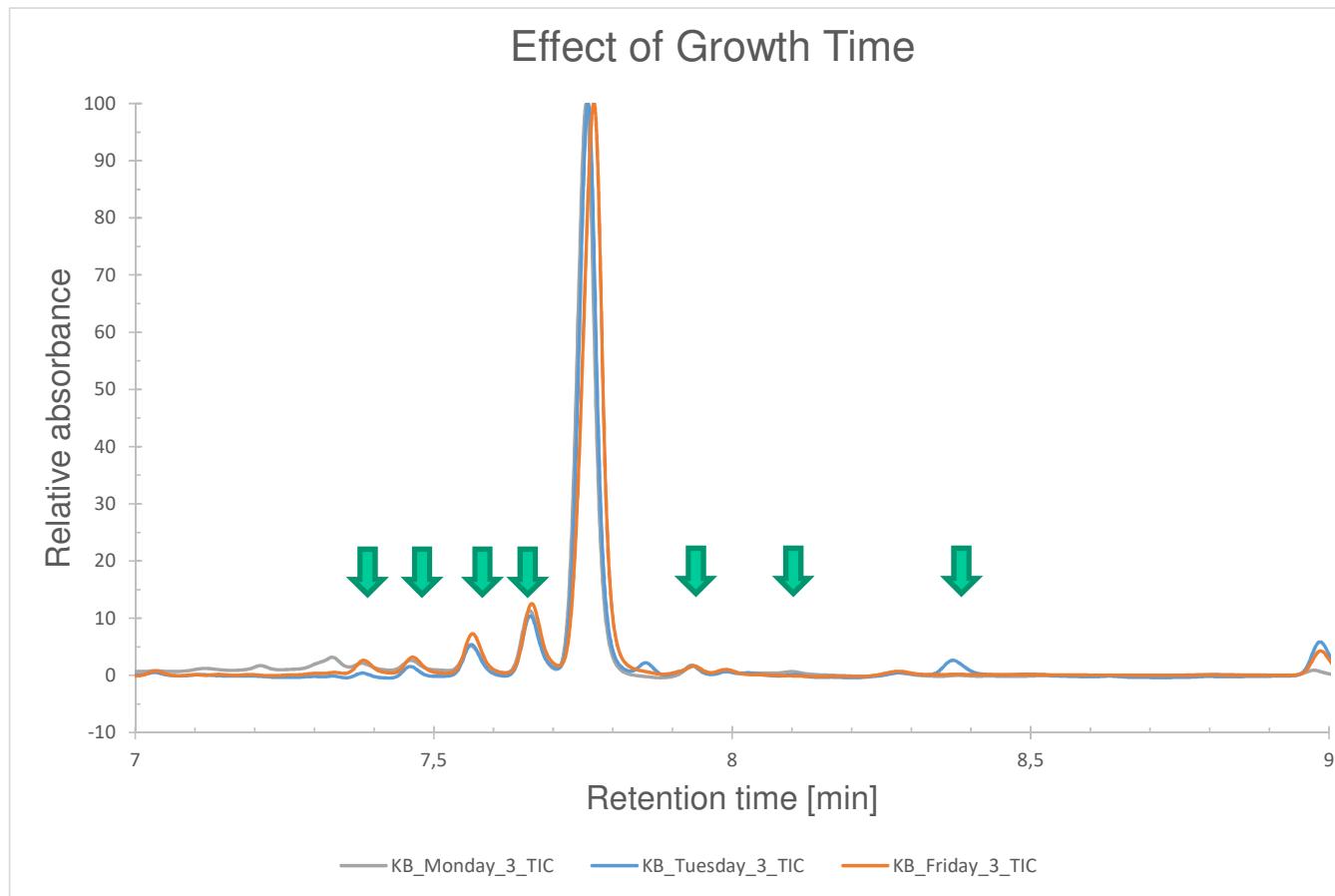
3 days growth
1 KB

4 days growth
1 KB

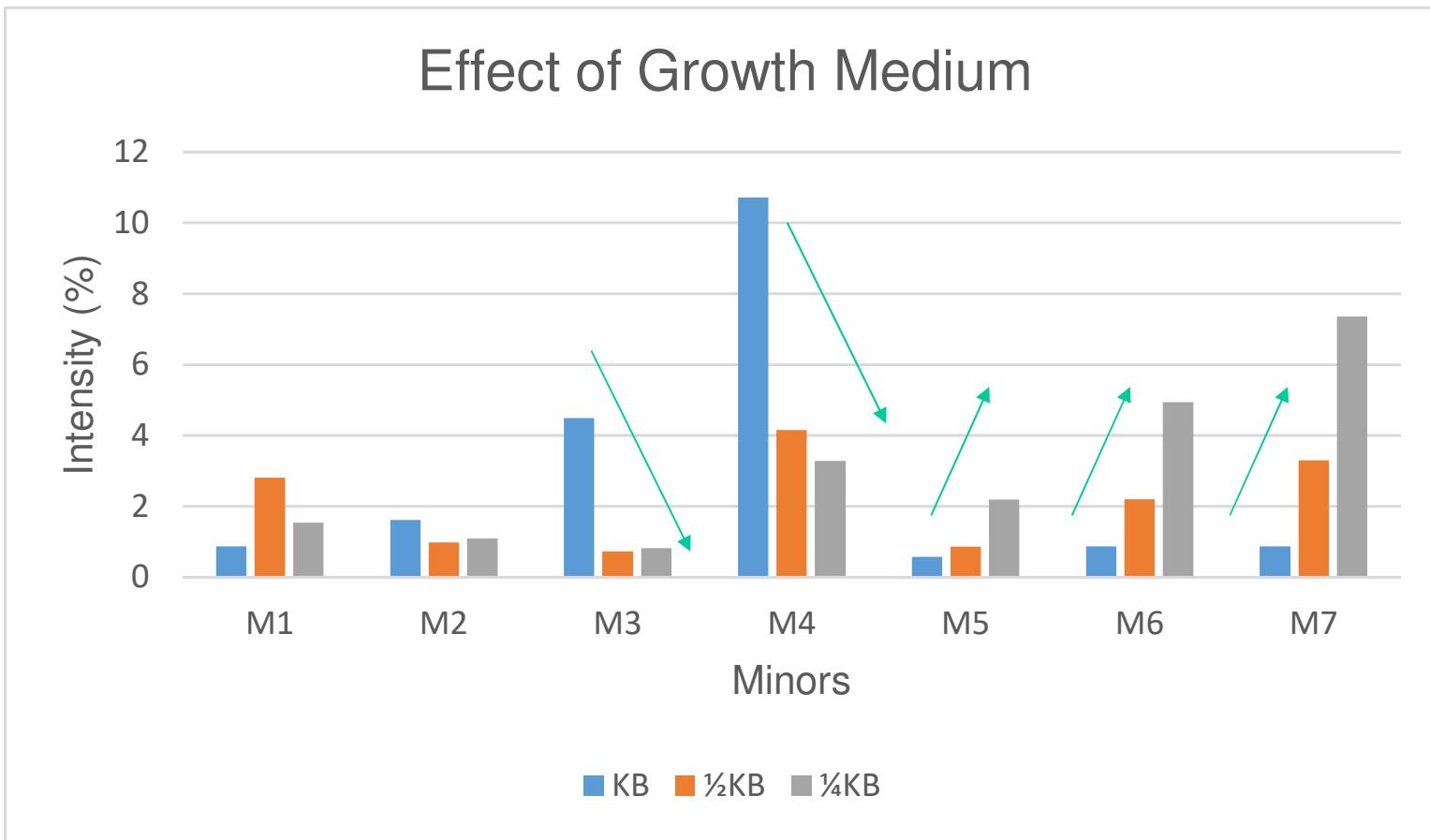
7 days growth
1 KB

Growth Time (days)	Growth Medium	# Agar plates	mass (mg)	mass/plate (mg)
3	1/4 KB	53	74	1.4
3	1/2 KB	51	147.4	2.9
3	1 KB	34	343.8	10.1
4	1 KB	22	97.5	4.4
7	1 KB	20	137.6	6.9

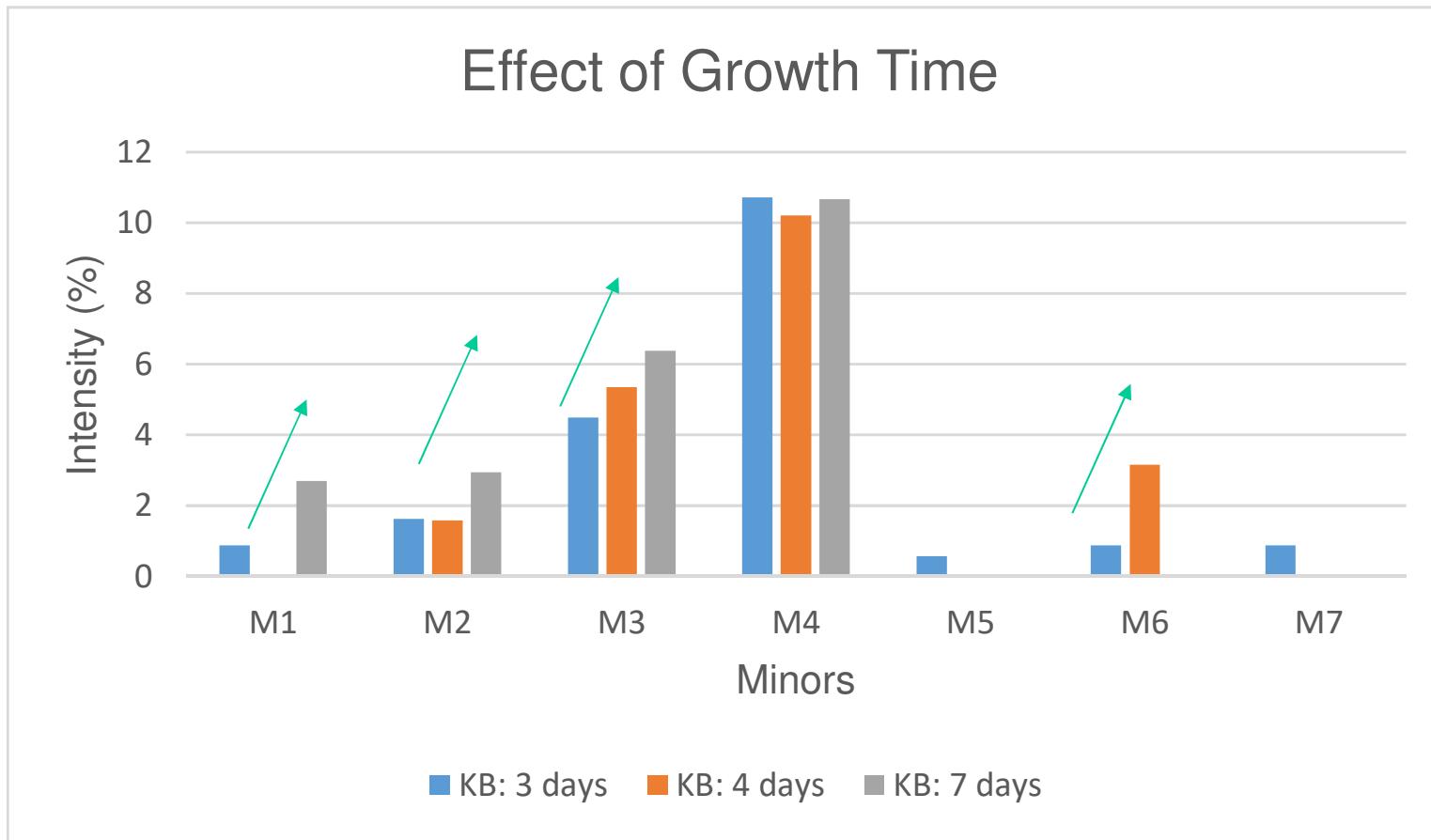
LC-MS



LC-MS

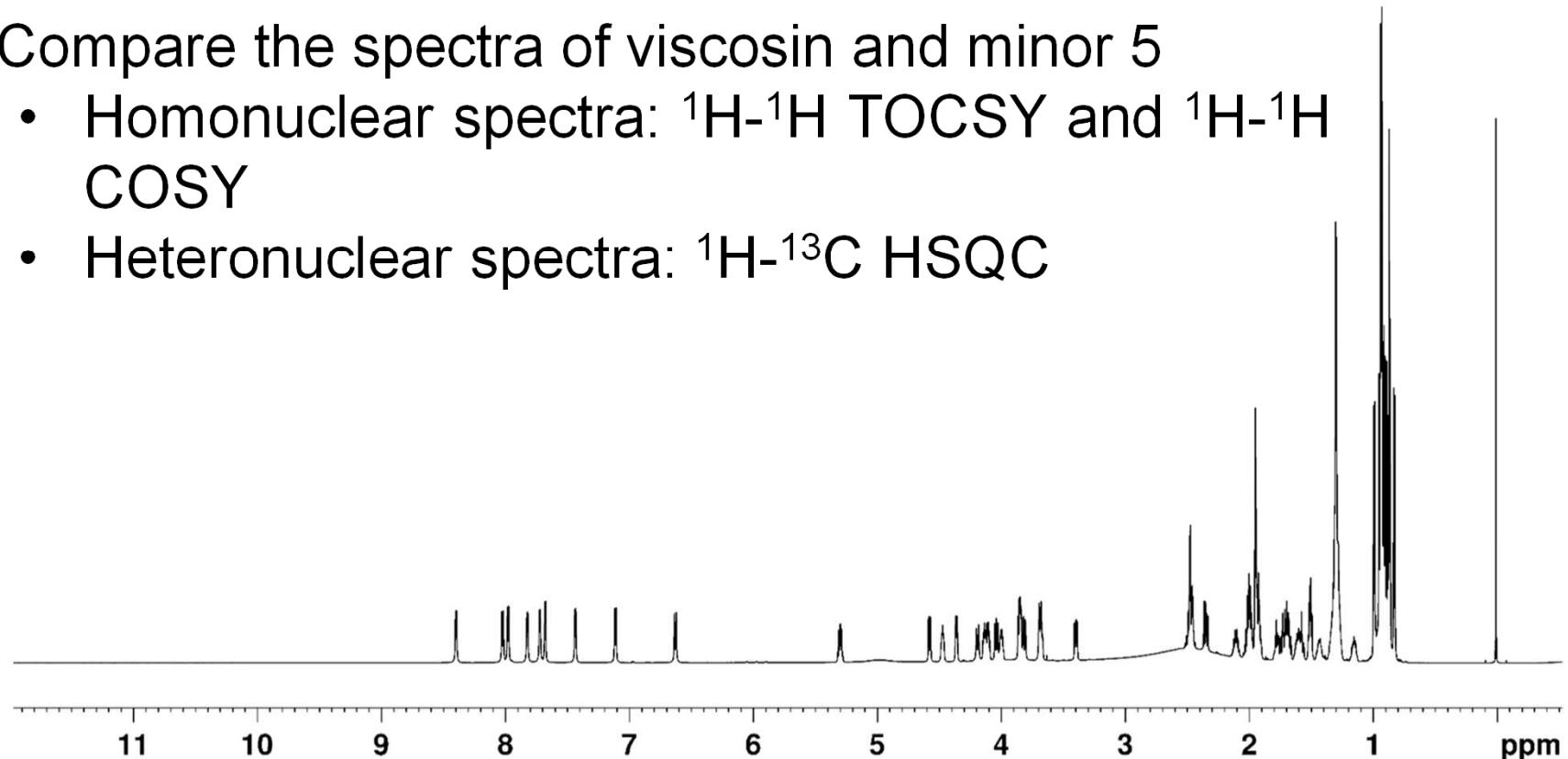


LC-MS



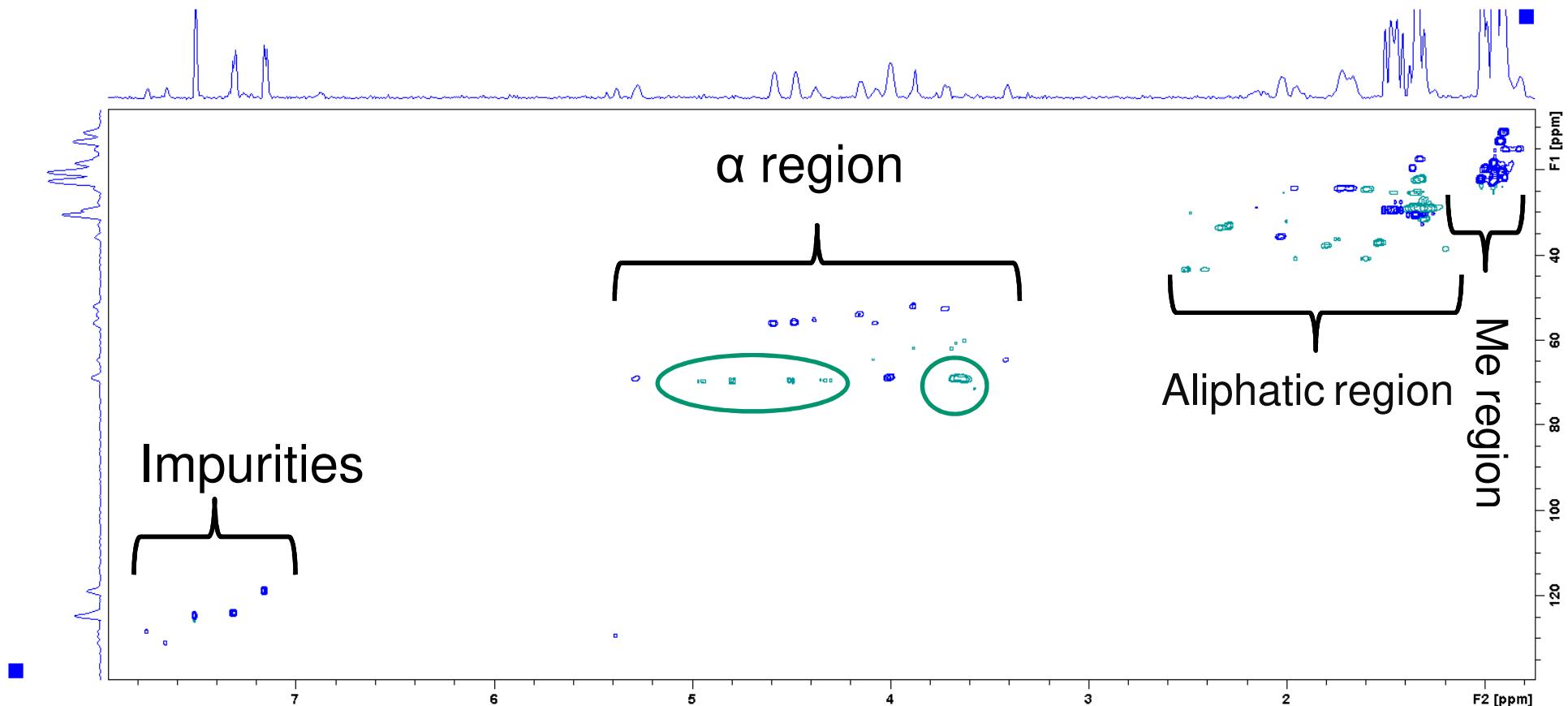
Partial structure elucidation of minor 5

- Compare the spectra of viscosin and minor 5
 - Homonuclear spectra: ^1H - ^1H TOCSY and ^1H - ^1H COSY
 - Heteronuclear spectra: ^1H - ^{13}C HSQC

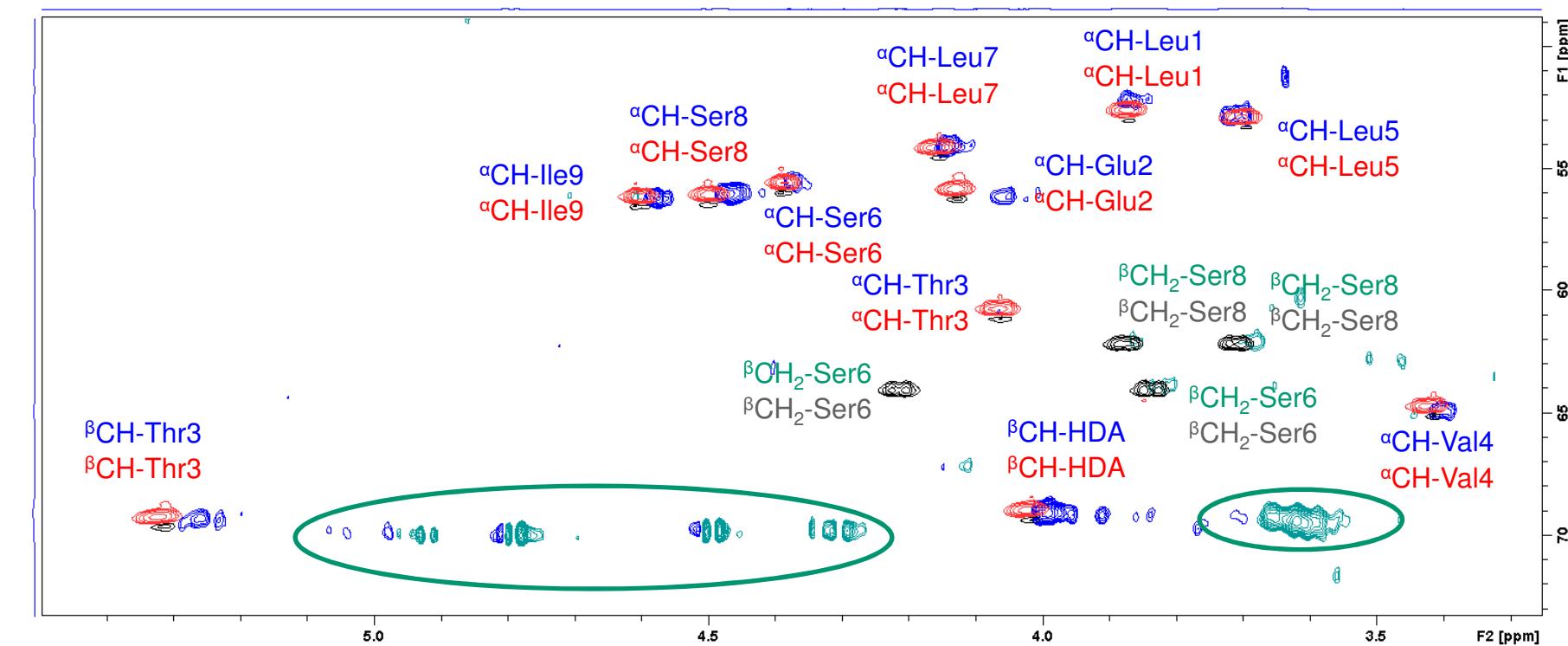




^1H - ^{13}C HSQC minor 5 and viscosin



^1H - ^{13}C HSQC α region zoom; Overlay of minor 5 Me/CH; viscosin Me/CH and minor 5 CH_2 ; viscosin CH_2



Conclusions

- Effect of decreasing medium concentration
 - Increase in relative minor quantities of minor 5-7
 - Decrease in relative minor quantities of minor 3-4
- Effect of growth time
 - Increase in relative minor quantities of minor 1-3 and 6
- Structural elucidation of minor 5
 - The peptide fragment is identical to viscosin

Safety issues

NMR spectrometers

- Large magnetic force
→ Inner Zone: $B \geq 1 \text{ mT}$
- Outer Zone: $B \geq 0.3 \text{ mT}$
- Cryogenic liquids

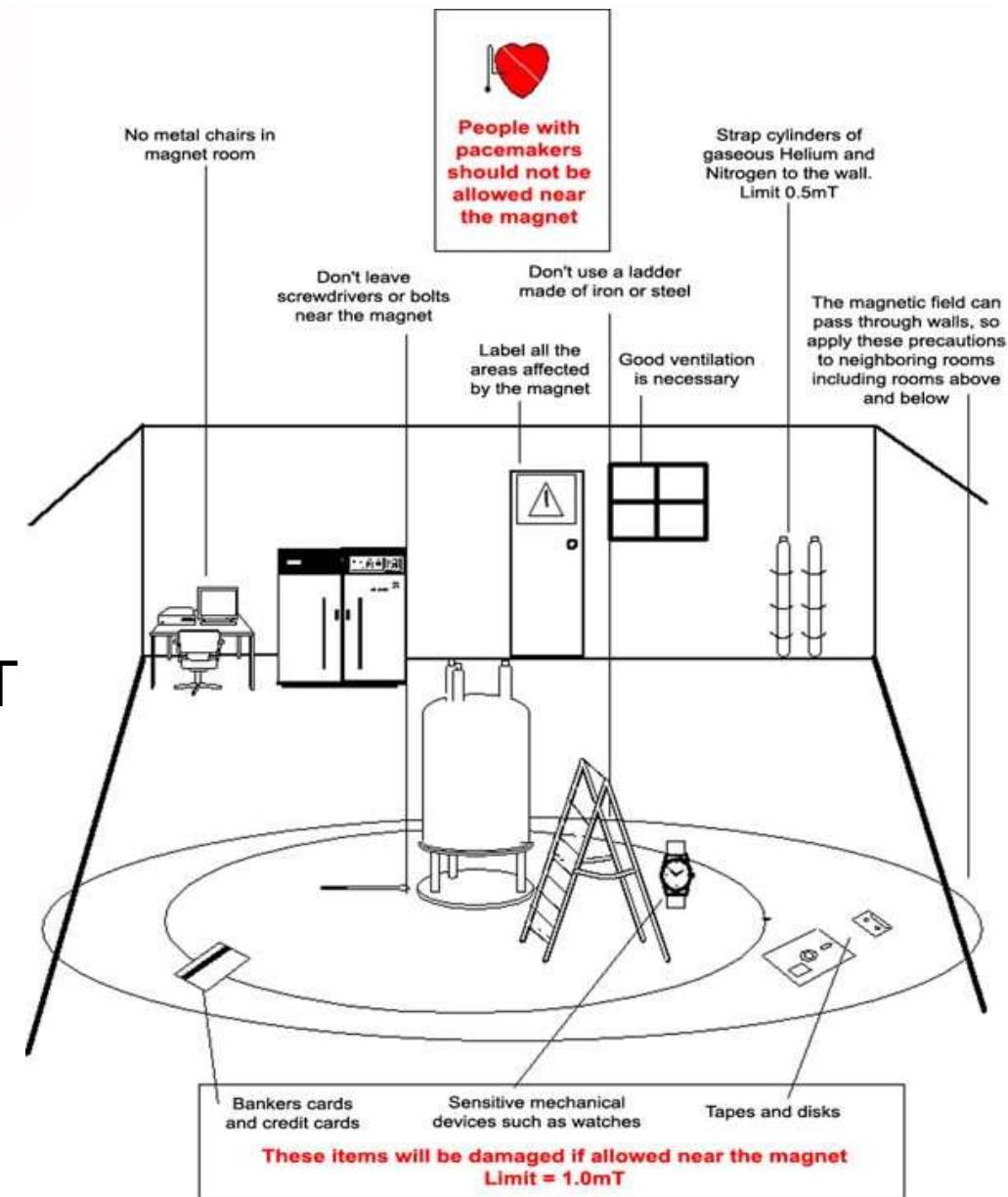




Safety issues

NMR spectrometers

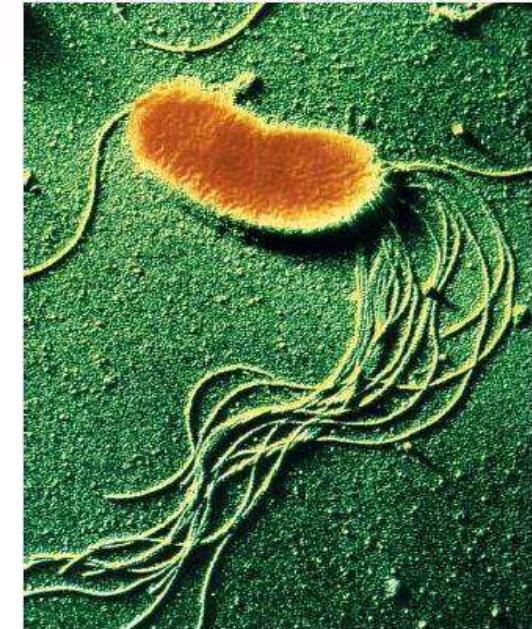
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- Cryogenic liquids



Safety issues

Microorganisms

- Sterilize equipment and material
- Disinfect work areas after use
- Biohazard autoclave bag



Pseudomonas Fluorescens

Working with organic solvents

- Acetonitrile
- Acetonitrile-d3
- Methanol

