Biosynthetic Studies of the α-amylase inhibitor Trestatin in 
*Streptomyces dimorphogenes*

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The evolution of sweet tooth
Humans evolved to crave sugar
Excess glucose in blood can lead to diabetes and obesity.
Adult diabetes rate in the US (1990 vs 2015)
Trestatins are produced by *Streptomyces dimorphogenes*

Chemical structures of trestatins

- Trestatin A ($n = 2$)
- Trestatin B ($n = 1$)
- Trestatin C ($n = 3$)
Trestatins as useful therapeutic agents for the treatment of diabetes and obesity

- Potent α-amylase inhibitor$^{1,2}$

$^{1}$Golay, Alain, et al.
$^{2}$Eichler, H. G., et al.
Importance of α-amylase inhibitor
Investigating the function of individual genes within the trestatin gene cluster by gene inactivation
Checking for the production of trestatin in *Streptomyces dimorphogenes*
Confirmation of trestatin B production
Identification genes within the trestatine biosynthetic gene cluster

Genome sequence data

BLAST
Some major genes include trsA3, trsB4 and trsB6
2-epi-5-epi-valiolone synthase (EEVS) catalyzes the cyclization of sedoheptulose 7-phosphate to 2-epi-5-epi-valiolone.

![Chemical structures showing the cyclization process.](image-url)
C$_7$N-aminocyclitol-containing natural products (pseudooligosaccharides)

Valienamine

2-epi-5-epi-valiolone
Glycosyltransferases vs Pseudoglycosyltransferases

α-D-galactopyranose

Pseudo-α-D-galactopyranose

Trestatin A (n = 2)
Trestatin B (n = 1)
Trestatin C (n = 3)

trehalose unit

Glycosyltransferases

Pseudoglycosyltransferases
trsB4 or trsB6 might code for pseudoglycosyltransferase.

Glycosyltransferases

2-epi-5-epi-valiolone synthase (EEVS)

Trestatin A (n = 2)
Trestatin B (n = 1)
Trestatin C (n = 3)
pTMAD003 and pTMAD004
Construction of pTMAD003

Polymerase chain reaction (PCR)

DNA assembly

trsA1 → trsB4 → trsA2
DNA assembly overview

DNA assembly diagram

Assembly master mix action
Fragments from trsA1 and trsA2 were successfully cloned into pTMN002 plasmid, giving pTMAD003.
Construction of pTMAD004

DNA assembly

Polymerase chain reaction (PCR)
Fragments from trsB5 and trsB7 were successfully cloned into pTMN002 plasmid, giving pTMAD004.

pTMAD004

+XbaI, +HindIII and +EcoRI
Conjugation between *E. coli* and *Streptomyces dimorphogenes* was not successful.

Plasmid pTMAD003

E. coli ET12567/pUZ8002

Conjugation between *E. coli ET12567/pUZ8002/pTMAD003* and *Streptomyces dimorphogenes*
Conjugation between *E. coli* and *Streptomyces dimorphogenes* was not successful

Plasmid pTMAD004

*E. coli* ET12567/pUZ8002

Conjugation between *E. coli* ET12567/pUZ8002/pTMAD003 and *Streptomyces dimorphogenes*
Future work: Triparental Mating

**Step 1:** Conjugation initiated by self-mobilizable “helper plasmid” (red); helper plasmid is transconjugated into donor strain that contains plasmid with desired functions (green).

**Step 2:** Green donor plasmid is mobilized *in trans* by helper, transfers desired donor plasmid into recipient cell.
Once the plasmid enters *Streptomyces dimorphogenes* homologous recombination will take place.

Comparison of metabolic profiles of wild-type and mutant strains of *S. dimorphogenes*. Compare the metabolic profiles of the wild-type and the mutant strains of *S. dimorphogenes*.
Summary

- Rate of heart disease, diabetes and obesity is on the rise

- Trestatins can be developed as a useful therapeutic for the treatment of diabetes and obesity

- Only little information available on mode of formation in nature
Summary

• Trestatin biosynthetic gene cluster is thought to contain one glycosyltransferases and one pseudoglycosyltransferase

• pTMAD003 and pTMAD004 were successfully constructed

• Future work focus on alternative transformation systems


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Thank You!
Questions?