Sveučilište u Zagrebu
PRIRODOSLOVNO-MATEMATIČKI FAKULTET
Kemijski odsjek

Robert Pendelić

Reporterski sustav za karakterizaciju utišanih biosintetskih genskih klastera

Kemijski seminar I
Poslijediplomski sveučilišni studij Kemija (smjer Biokemija)

Izrađen prema:

Liu X. i sur. (2022) A visualization reporter system for characterizing antibiotic biosynthetic gene clusters expression with high-sensitivity. *Communications Biology* **5**, 901

Sadržaj

Sekundarni metaboliti

Kvorum signalizacija

Korištene metode

Testni reporterski sojevi

Karakterizacija *ovm* genskog klastera

Karakterizacija *ang* genskog klastera

Karakterizacija *oxa* genskog klastera

Zaključak

Sekundarni metaboliti

Male, biološki aktivne molekule

- Antibiotici
- Biopolimeri
- Alkaloidi
- Pigmenti

Ekološki značaj

- Primarni su za odgovorni rast i razvoj

Sekundarni metabolit	Komercijalna/medicinska upotreba	Organizam
Kofein	Kava	Rod *Coffea*
Nikotin	Cigarete	*Nicotiana tabacum*
Morfin	Analgetik	*Papaver somniferum*
Penicilin	Antibiotik	Rod *Penicillium*
Lovastatin	Statin	*Aspergillus terreus*
Ciklosporin A	Imunosupresiv	*Tolypocladium inflatum*
Doksorubicin	Antitumorski agens	*Streptomyces peucetius*
Tetraciklin	Antibiotik	*S. aureofaciens*
Amfotericin B	Fungicid	*S. nodosus*
Rapamicin	Imunosupresiv	*S. hygroscopicus*
Bt Toksin	Kontrola štetnih kukaca	*Bacillus thuringiensis*
Streptomicin	Kontrola štetnih bakterija	*S. griseus*

Sekundarni metaboliti mikroorganizama

Duga povijest istraživanja

- 1940. – 1970. "Zlatno razdoblje otkrića **antibiotika**"

Streptomicete

- Biotvornice sekundarnih metabolita
- 60% danas poznatih antibiotika[1]
- Više od 60 spojeva u medicini, agrikulturi i/ili industriji[2]
- Veliki genomi[3]

Tihi genski klasteri

Sekundarni metabolit	Komercijalna/medicinska upotreba	Organizam
Kofein	Kava	Rod *Coffea*
Nikotin	Cigarete	*Nicotiana tabacum*
Morfin	Analgetik	*Papaver somniferum*
Penicilin	Antibiotik	Rod *Penicillium*
Lovastatin	Statin	*Aspergillus terreus*
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Bt Toksin	Kontrola štetnih kukaca	*Bacillus thuringiensis*
Streptomicin	Kontrola štetnih bakterija	*S. griseus*

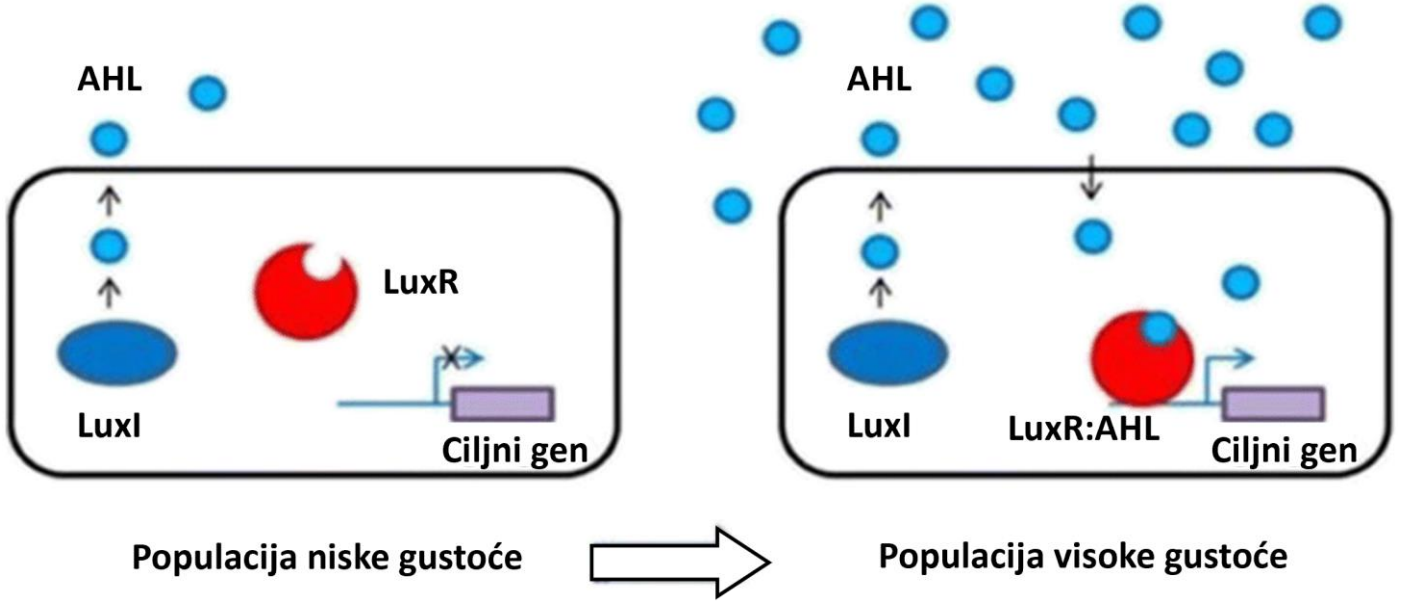
1) Bérdy J (2012) *J Antibiot* **65**, 385-395.
2) Katz L i Baltz RH (2016) *J Ind Microbiol Biotechnol.* **43**, 155-176.
3) Otani H, Udwary DW i Mouncey NJ (2022) *Sci Rep.* **12**, 18909.

Kvorum signalizacija (QS)

Detekcija **gustoće** populacije
- "Kvorum bakterija"
- Signalne molekule

Gram-negativne bakterije
- N-acil homoserin laktoni (AHL)

Komunikacija bakterija[1]
- Biofilm
- Virulencija
- Motilitet
- Bioluminiscencija
- Sporulacija



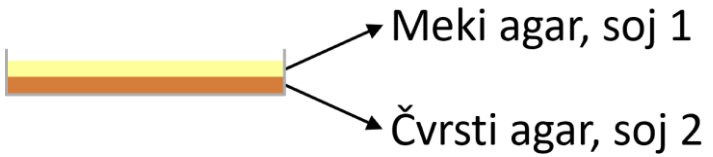
Preuzeto i prilagođeno prema Kareb, O i Aïder, M (2020) *Probiotics & Antimicro. Prot.* **12**, 5–17.

1) Miller MB i Bassler BL (2001) *Annu Rev Microbiol.* **55**, 165-199.

Metode

Genetičko inženjerstvo

Nasađivanje bakterija u dva sloja
- *Double-layer plate method*



Agar-čep metoda
- *Agar plug method*

Oxford cup assay
- Tekući gornji medij

Kokultivacija – lokalni paralelni uzgoj
- Tekući/kruti medij

HPLC

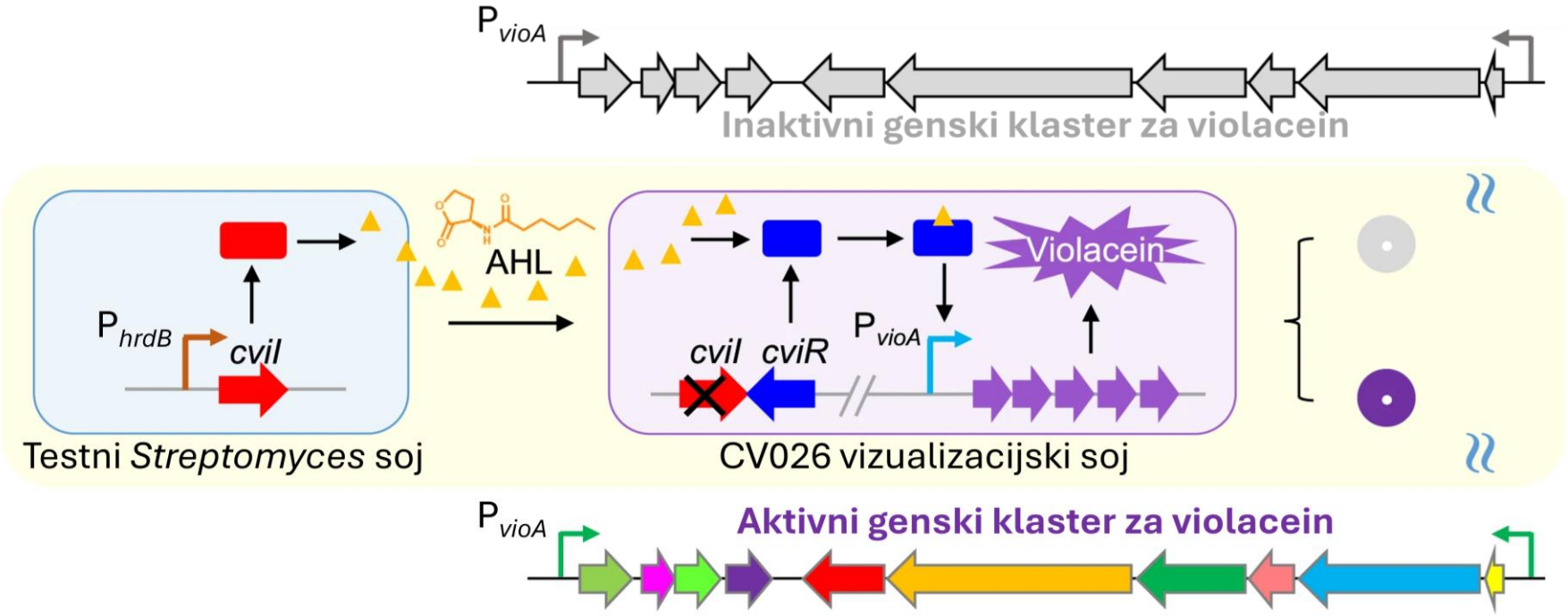
Testni reporterski sojevi

Gram-pozitivne streptomicete

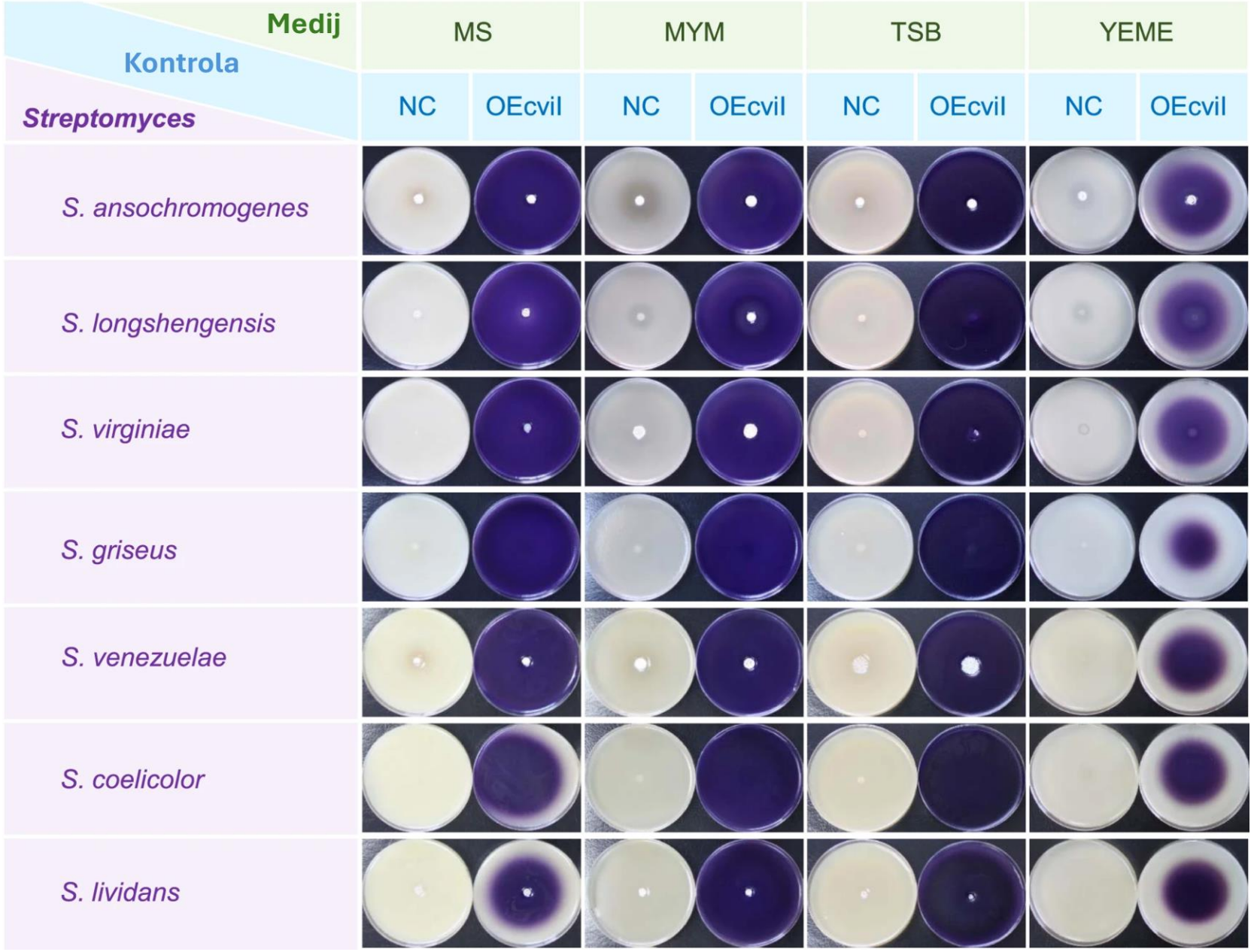
- CviI = AHL sintaza
  - Iz **sveprisutnih** SAM i acil-ACP[1]

Gram-negativna *Chromobacterium violaceum*

- QS sinteza violaceina – CviR i AHL (C6-HSL)

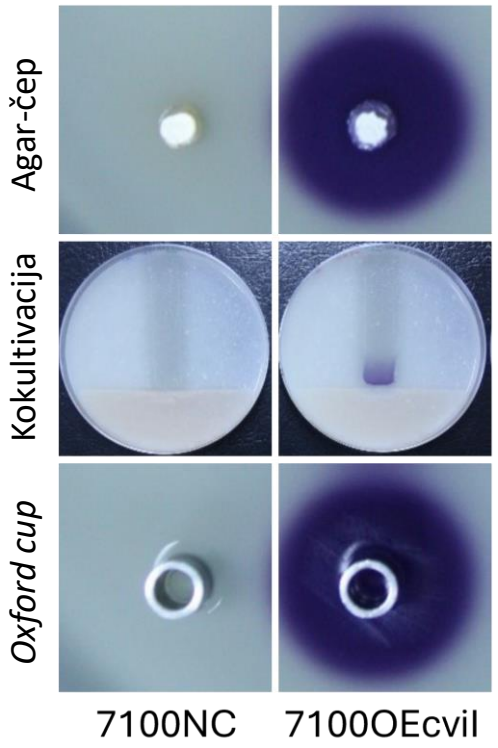


1) Parsek MR i sur. (1999) *Proc. Natl Acad. Sci. USA* **96**, 4360-4365.



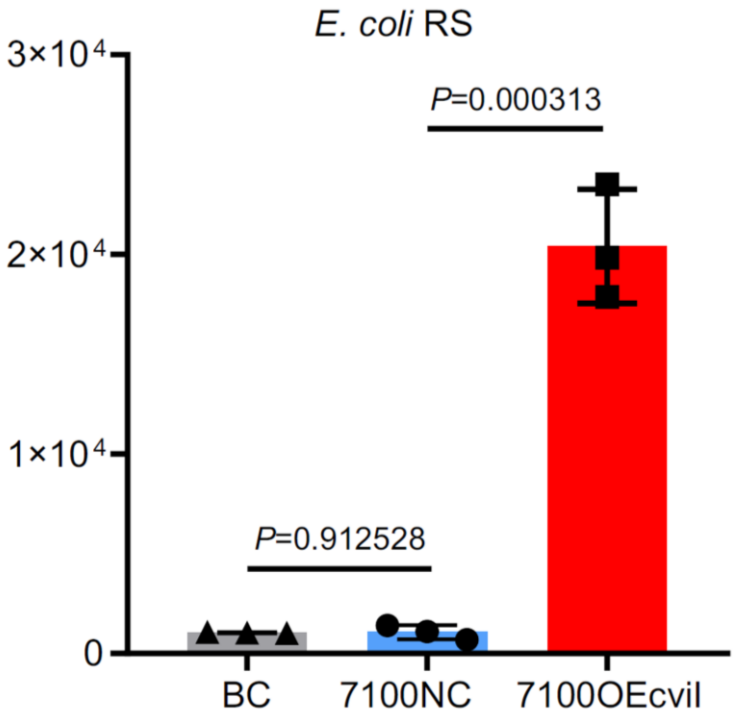
Evaluacija testnih sojeva

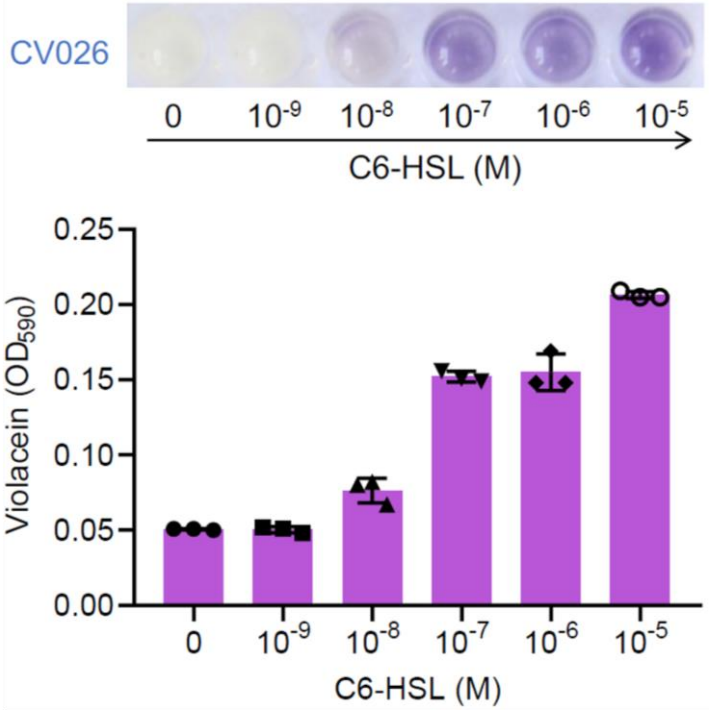
Evaluacija testnih sojeva



Potvrda rezultata pomoću *E. coli* reporterskog soja[1]

Osjetljivi sustav – 10 nM C6-HSL

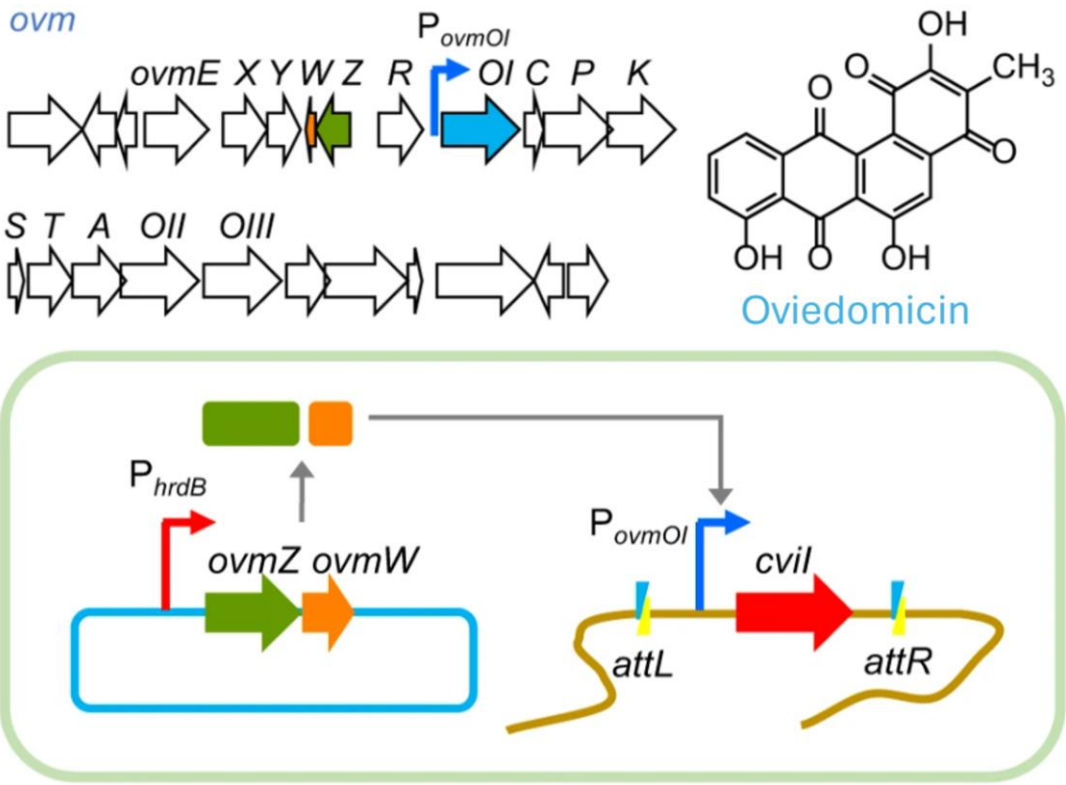


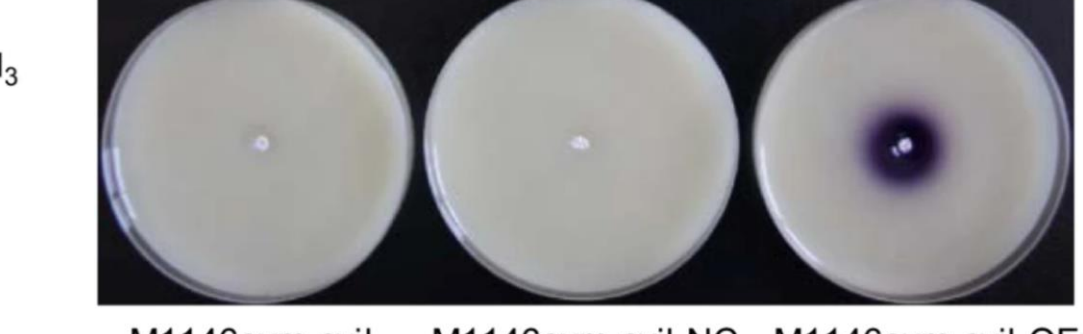


1) Liu X i sur. (2021) *Sci. China Life Sci.* **64**, 1575-1589.

Karakterizacija *ovm* genskog klastera

Regulatorni proteini OvmZ i OvmW[1] – aktivacija P_{ovmOI} (ključni gen)





Oviedomicin

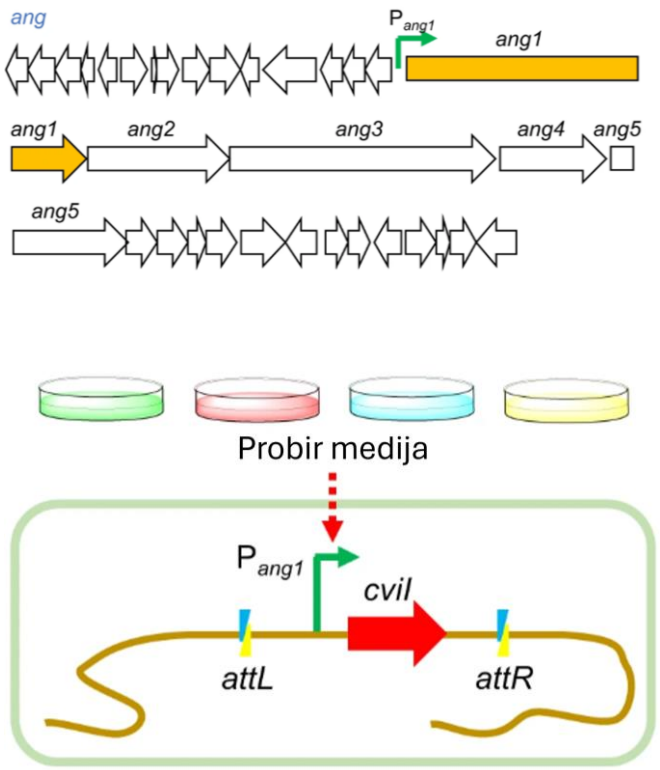
M1146ovm-cvil M1146ovm-cvil-NC M1146ovm-cvil-OEzw

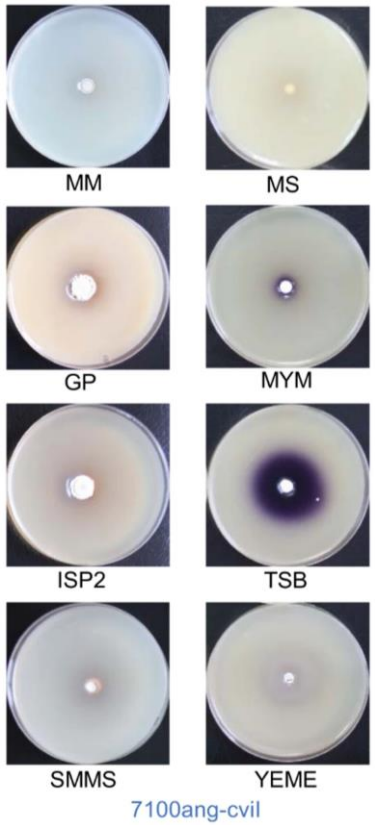
7100ovm-cvil 7100ovm-cvil-NC 7100ovm-cvil-OEzw

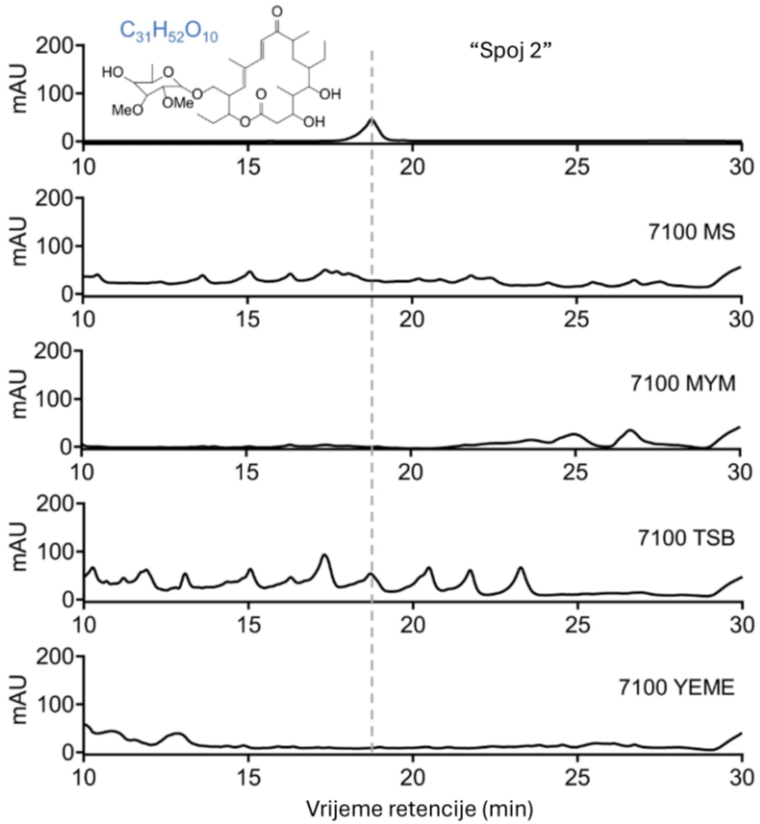
1) Xu J i sur. (2017) *J. Biol. Chem.* **292**, 19708-19720.

Karakterizacija *ang* genskog klastera

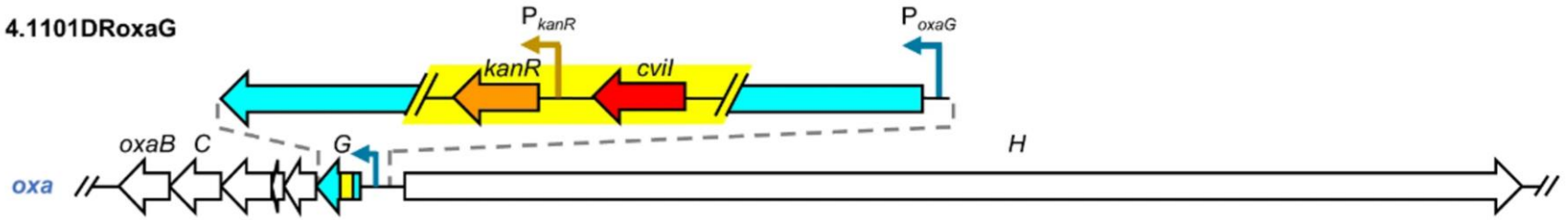
Ključni gen *ang1*[1] i *ang*-specifični produkt "Spoj 2"[2]

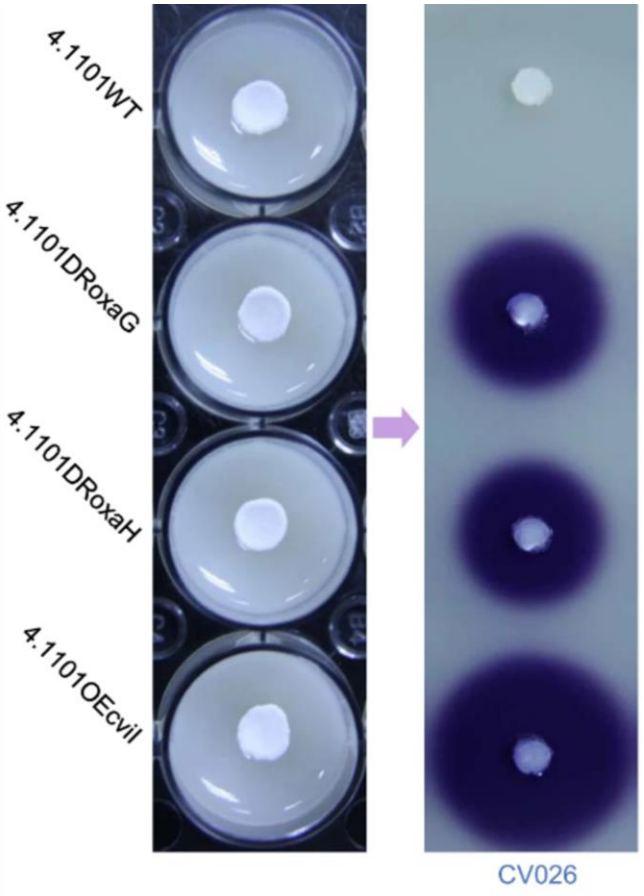


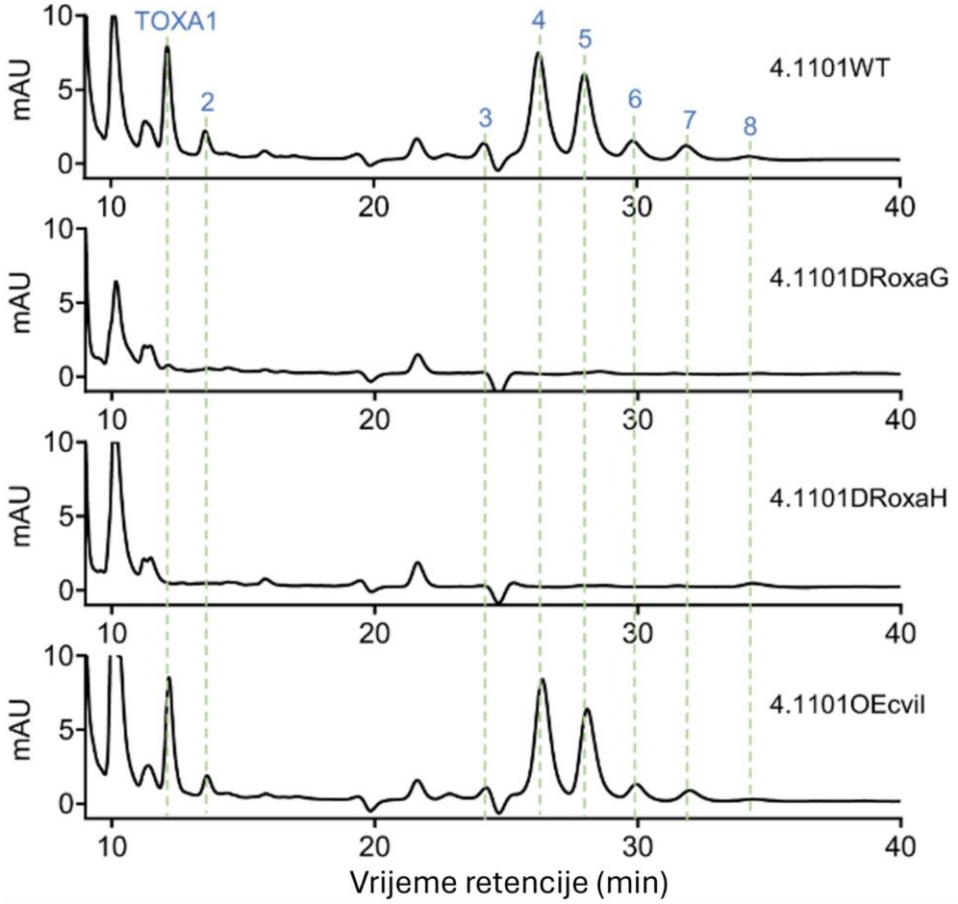




1) Li Y i sur. (2021) *Antibiotics (Basel)* **10**, 34572679.
2) Lu C i sur. (2015) *Microb. Cell Fact.* **14**, 173.







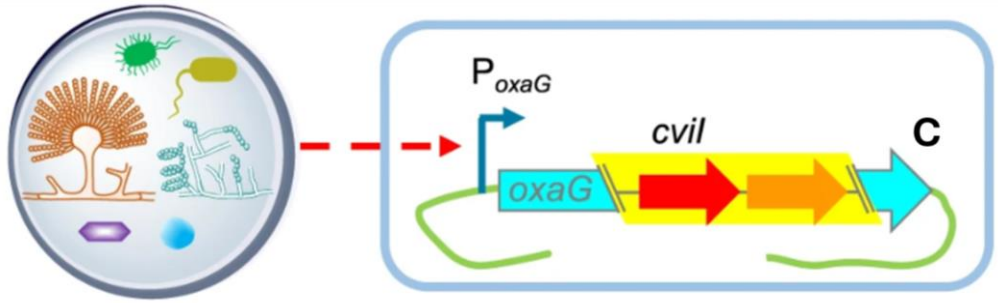
Karakterizacija *oxa* genskog klastera

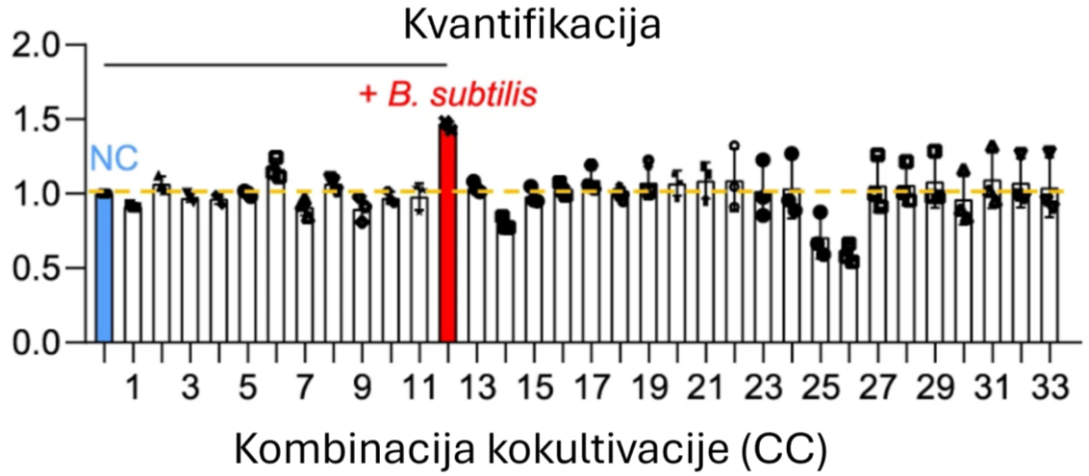
Sličnost s *ozm* genskim klasterom[1]

- Ključni geni *oxaG* i *oxaH*

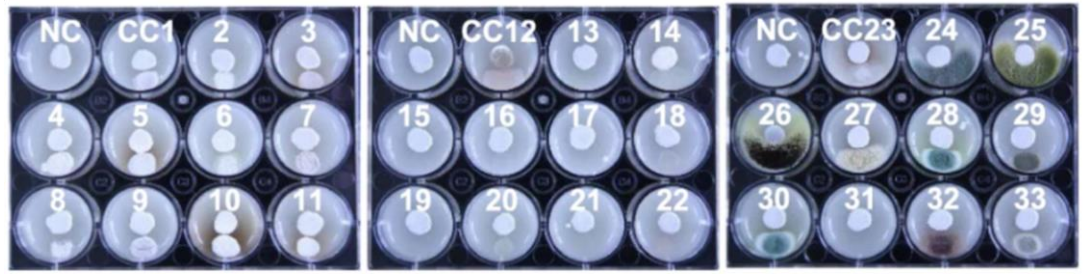
1) Zhao C i sur. (2010) *J. Biol. Chem.* **285**, 20097-20108.

Optimizacija *oxa* ekspresije

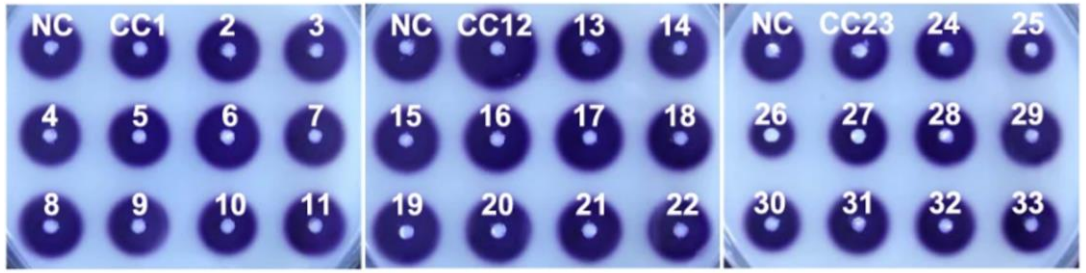




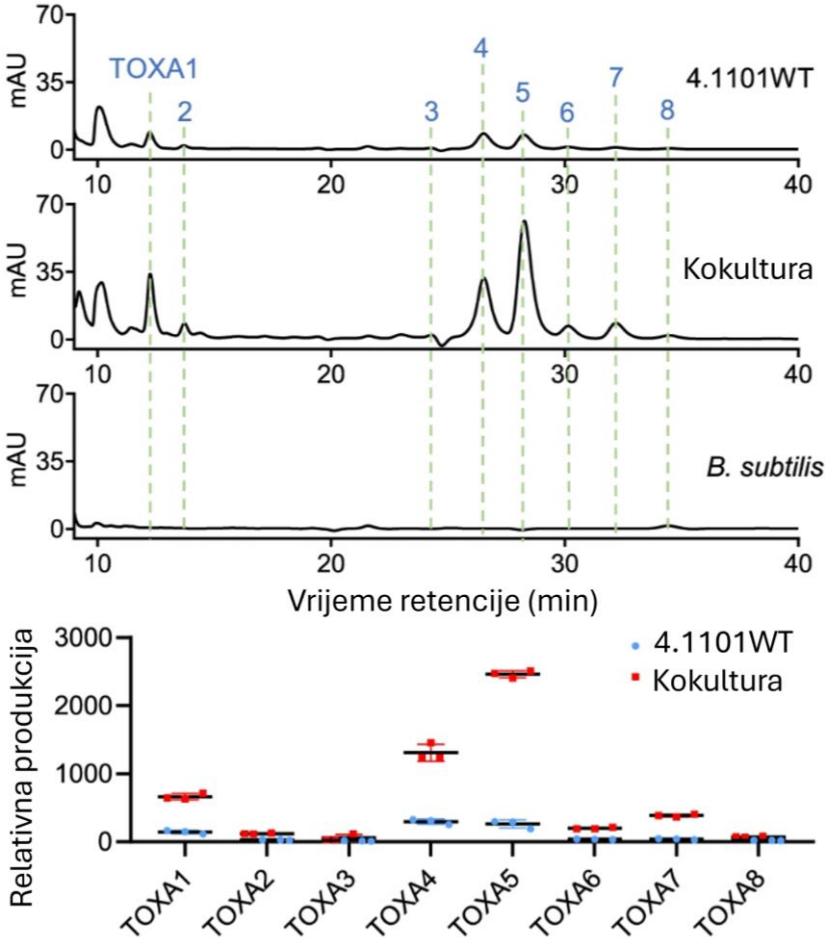
Kokultivacija

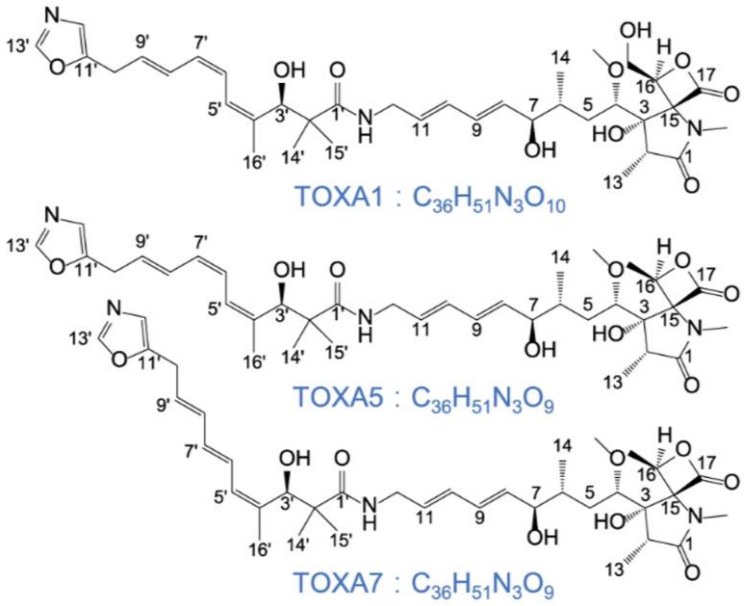


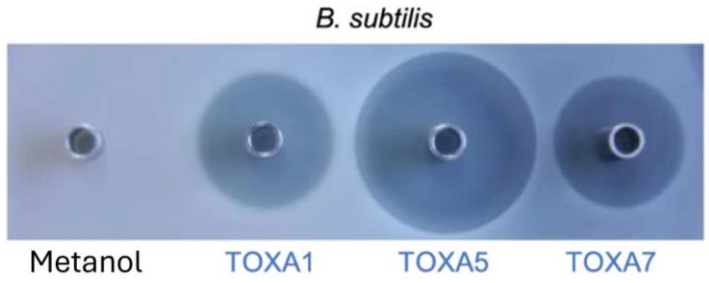
VRS-bAHL detekcija



Identifikacija TOXA spojeva







Zaključak

Dizajniran je efikasan sustav za analizu utišanih biosintetskih genskih klastera

Prednosti

- Izvrsna osjetljivost
- Minimalna pozadinska interferencija
- Široka kompatibilnost
- Mogućnost kvantifikacije

Mane

- Kompleksnost i vrijeme
- Potrebni specifični uvjeti
- Prethodno poznavanje genskog klastera

Hvala na pozornosti!