

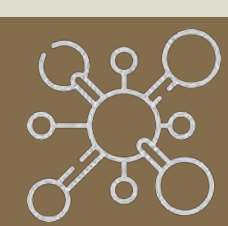
Analysis of NaOCl-Sensing Transcriptional Repressor NieR in *Agrobacterium tumefaciens* C58

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Introduction

Hypochlorous acid (HOCl) is naturally produced by neutrophils in the innate immune system and is commonly used as the active ingredient of household bleach (**sodium hypochlorite; NaOCl**) for disinfection of various microorganisms.

***Agrobacterium tumefaciens* C58**, a plant pathogen causing crown gall disease, was used as a model to study the hypochlorite stress response in bacteria.

NieR is a transcriptional regulator of the *nieAB-sdh* operon. The NieA and NieB belong to the HlyD family (membrane fusion protein) and the AcrB/AcrD/AcrF family (inner membrane transporter), respectively, and may form an efflux pump. The *sdh* encodes a short-chain dehydrogenase.



Highlight



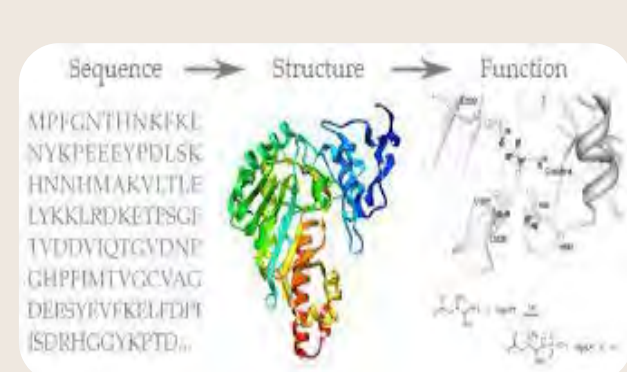
This study emphasized on the role of **the novel NaOCl-inducible transcriptional repressor NieR** that controls an expression of genes encoding efflux pump components in *A. tumefaciens*. Understanding of NaOCl-sensing mechanisms in bacteria may be useful for developing new treatments to combat infectious diseases.

Objectives

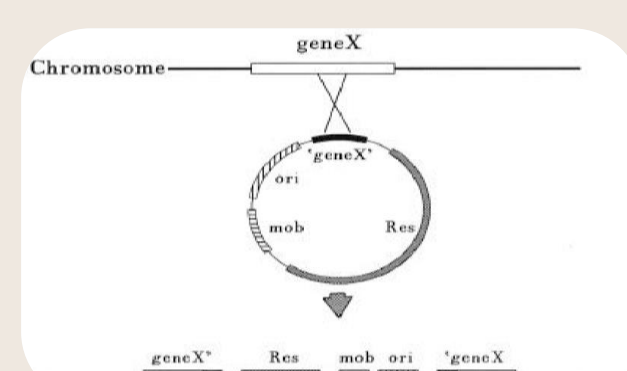


This study aims to investigate and characterize *A. tumefaciens* NieR, a NaOCl-sensing transcriptional regulator, under normal and NaOCl-mediated stress conditions.

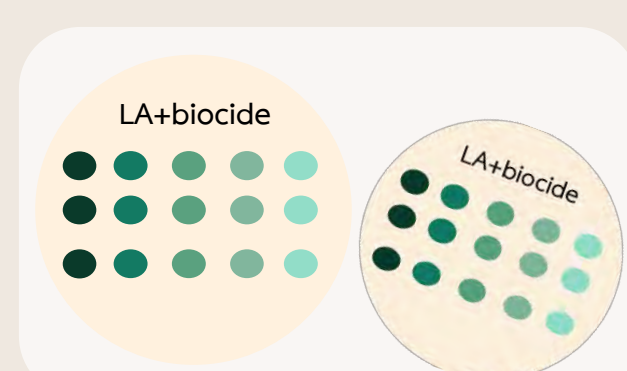
Methodology



Protein prediction (Bioinformatics)



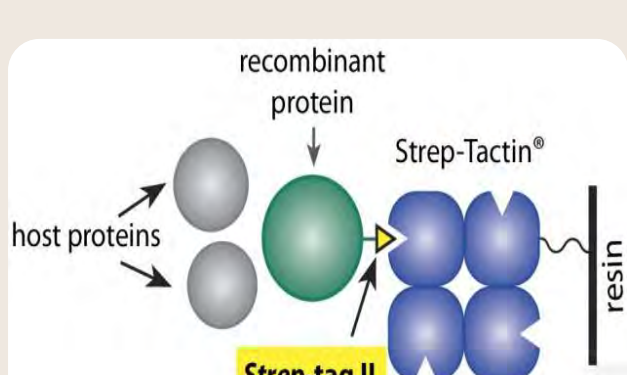
Strains construction



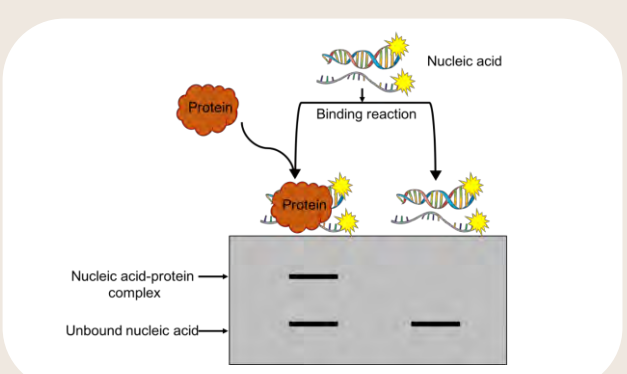
Sensitivity tests



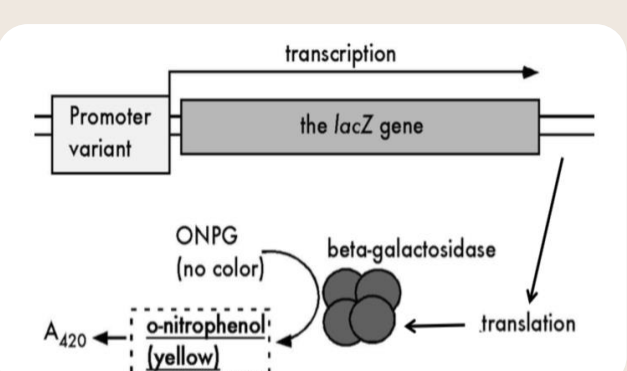
Gene expression analysis



Protein expression and purification



Identification of DNA-binding motifs



Promoter activity analysis



Statistical data analysis

Results and discussion

Bioinformatics

NaOCl sensing residues via sulfonamide formation

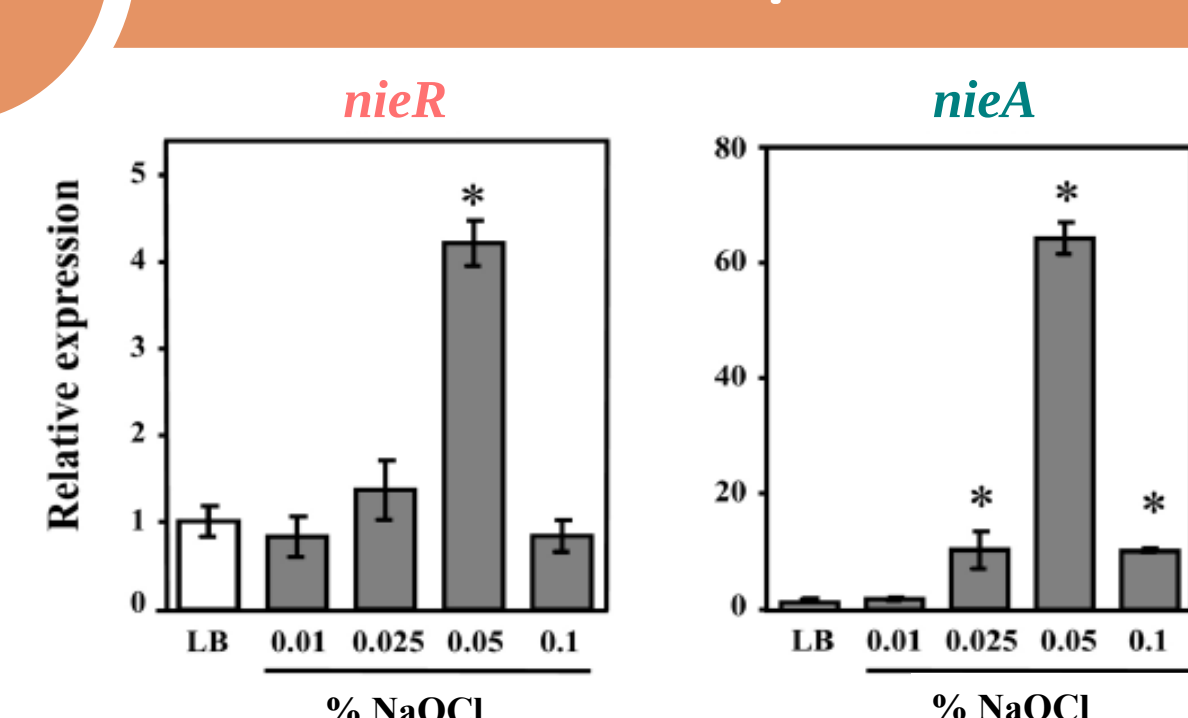
Protein alignment [CLUSTALW]

<i>A. tumefaciens</i> NieR	MRVSRQAQENRRVITVASRLFREHGFDTGLKDLMKGAGLTGGFYKQFASKDDLAL	60
<i>E. coli</i> NemR	--MNKHTEHDTREHLLATGEQLCLRQFTGMGLSELLKTAEVKPGSFYHFRSKEAFGVA	58
<i>A. tumefaciens</i> NieR	ASRRALASATRRWSDAAAD-TSDPLEATMAFYLS---KDHGEKAEGPLVALGSDAARQ	116
<i>E. coli</i> NemR	MLERHYAAYHQRLLTELLQSGEGNYRDRILAYYQQLNQFCQHGTSGLTVKLSAEVCDL	118
<i>A. tumefaciens</i> NieR	SAEVRPFEDGIRGHFEVLDELGHADSS-----GPSEKAMATLLSMVGAVTLSTLTIE	169
<i>E. coli</i> NemR	SEDMRSAMDKGARGVIALLSQALENGRENHCLTFCGEPLQQAQVLYALWLGANLQAISR	178
<i>A. tumefaciens</i> NieR	DERLSSEVLDAAAGEVKRIARSDGDD	195
<i>E. coli</i> NemR	SFE---PLENALAHVKNIITPAV-	199

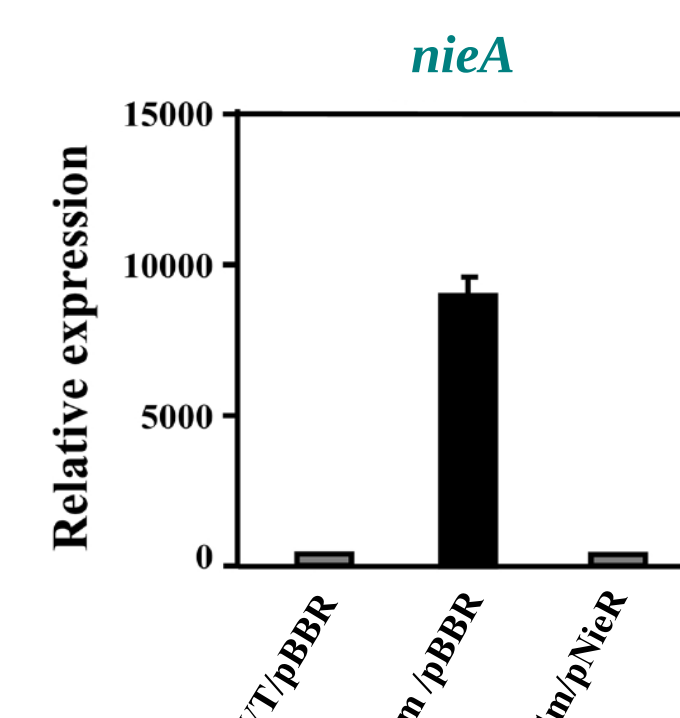


Gene expression

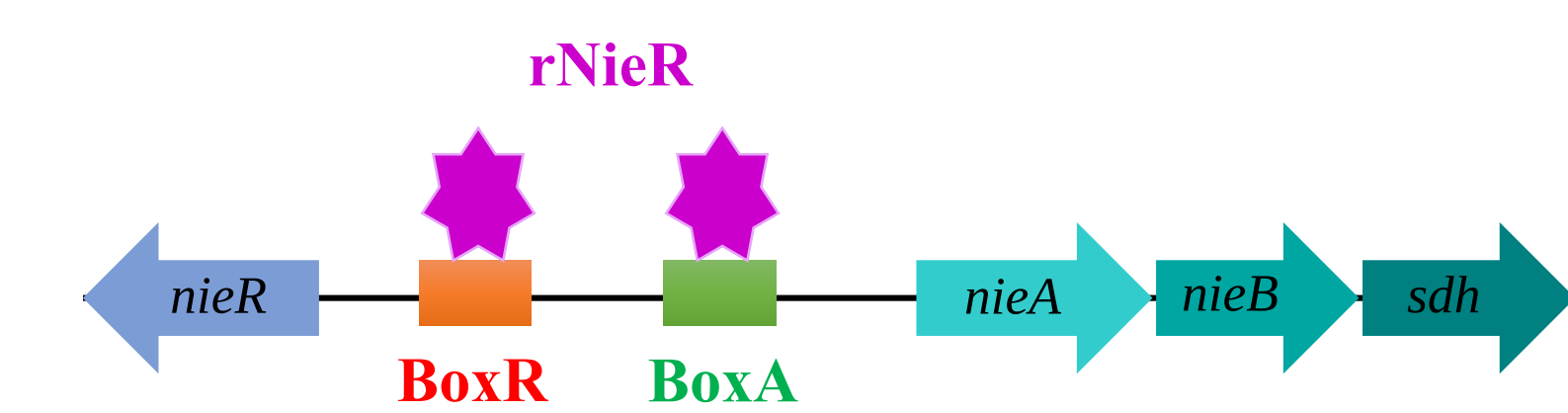
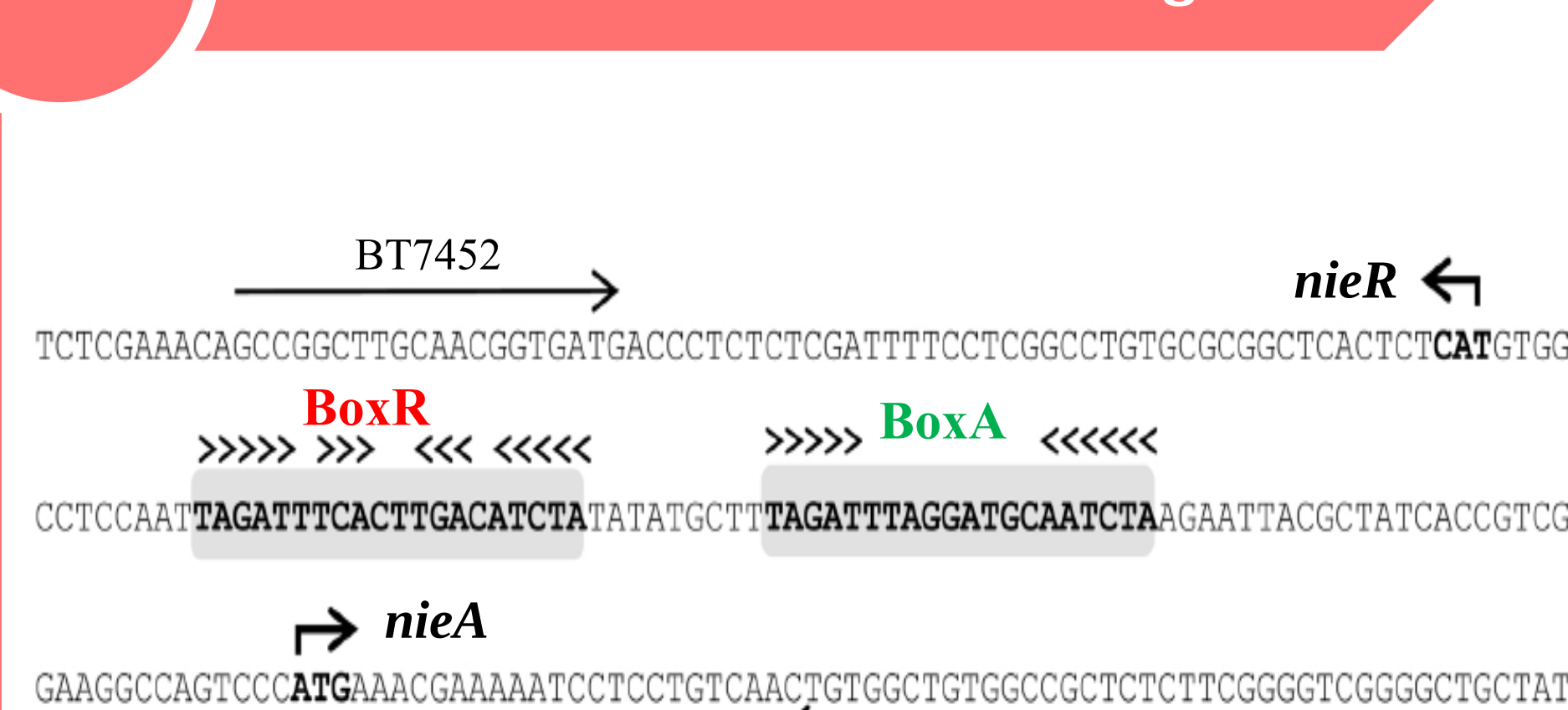
NieR is a repressor of *nieA* promoter



The *nieR* and *nieA* expression were particularly inducible by NaOCl.



Identification of NieR binding sites



The position of NieR binding sites can be found as the protected regions that covered two imperfect inverted repeats [BoxA & BoxR].

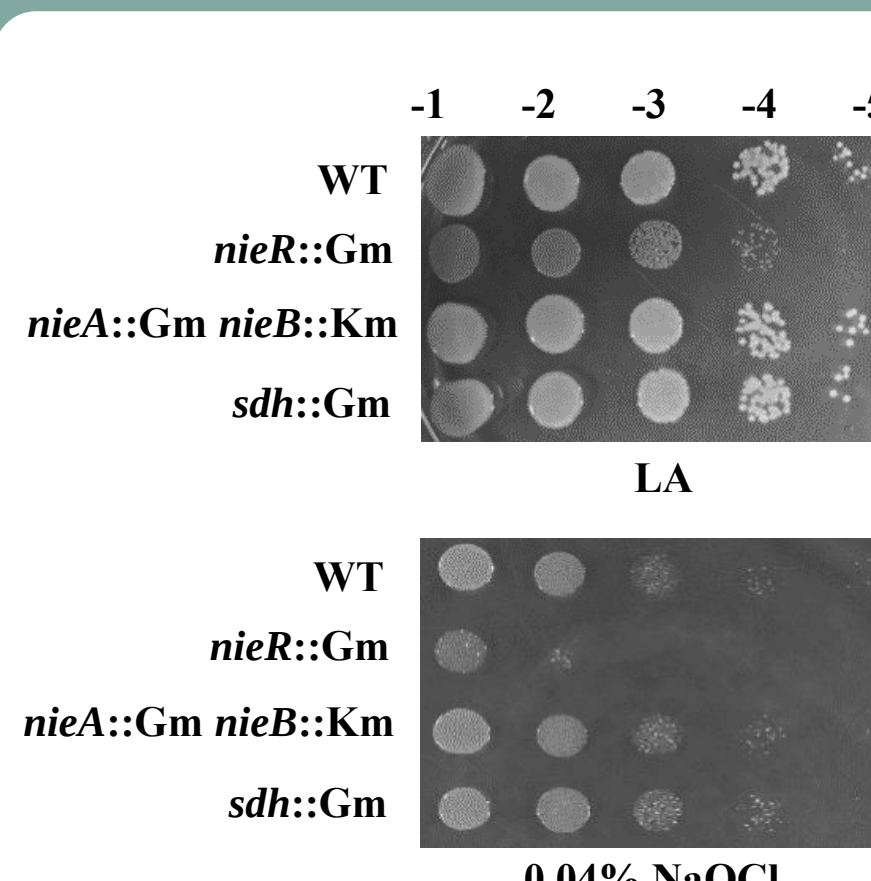
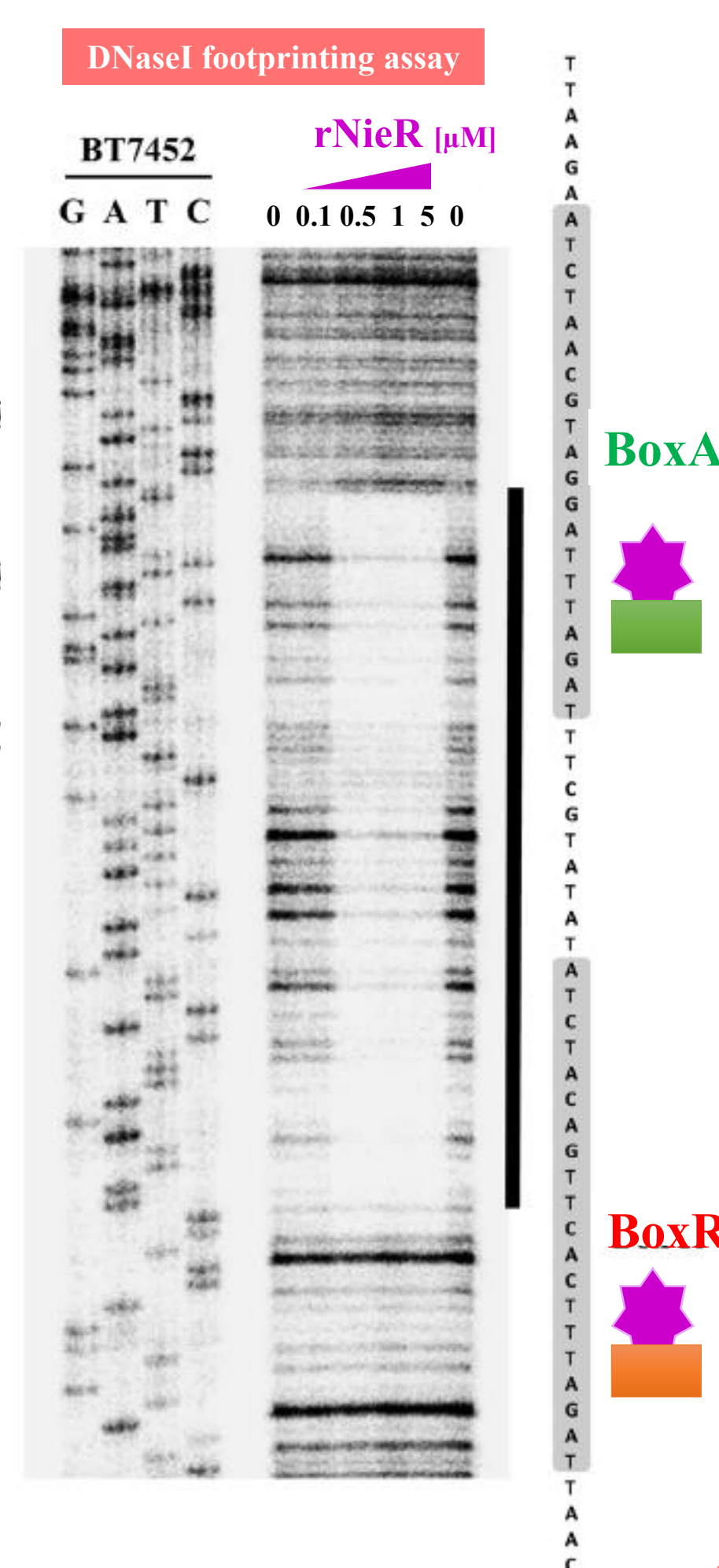


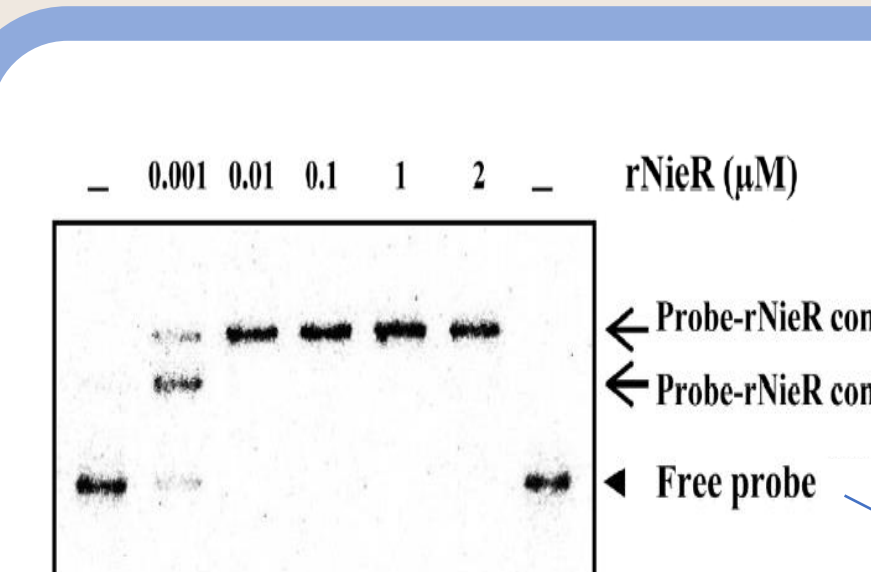
Plate sensitivity assay

MICs of different antibiotics for *A. tumefaciens*

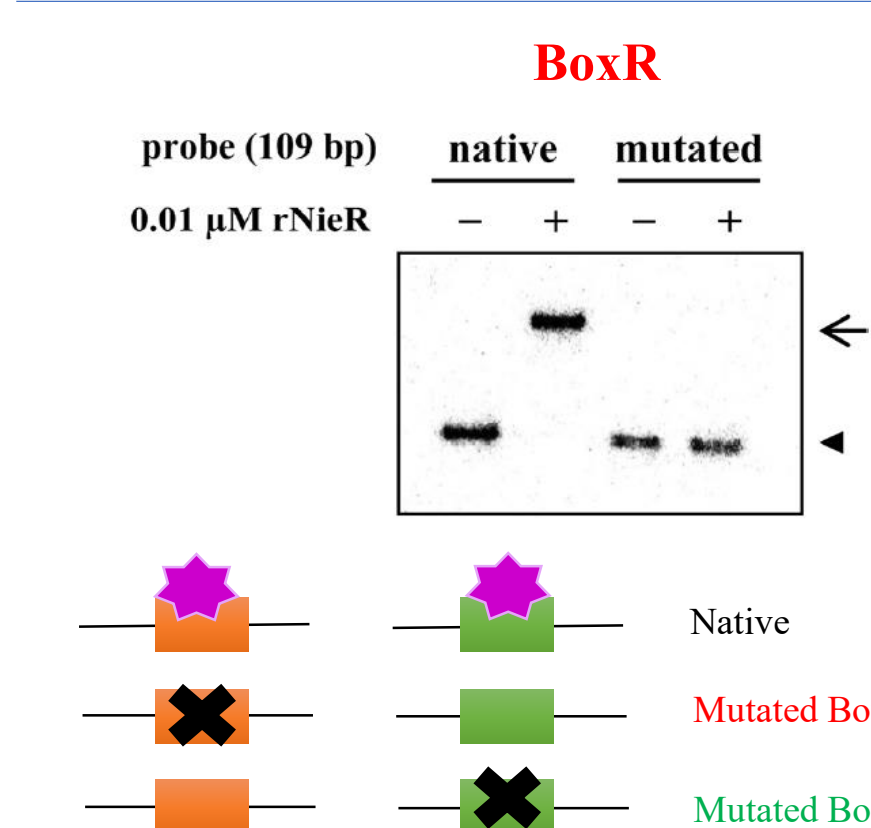
Antibiotic	WT	<i>nieR::Gm</i>
Nalidixic acid (μg/ml)	4.5	2.5
Novobiocin (μg/ml)	3.5	2.5
Ciprofloxacin (ng/ml)	24	10
Tetracycline (μg/ml)	0.9	0.5

The *nieR* mutant exhibited a small colony phenotype and hypersensitivity to NaOCl and antibiotics, including ciprofloxacin, nalidixic acid, novobiocin, and tetracycline.

Physiological study

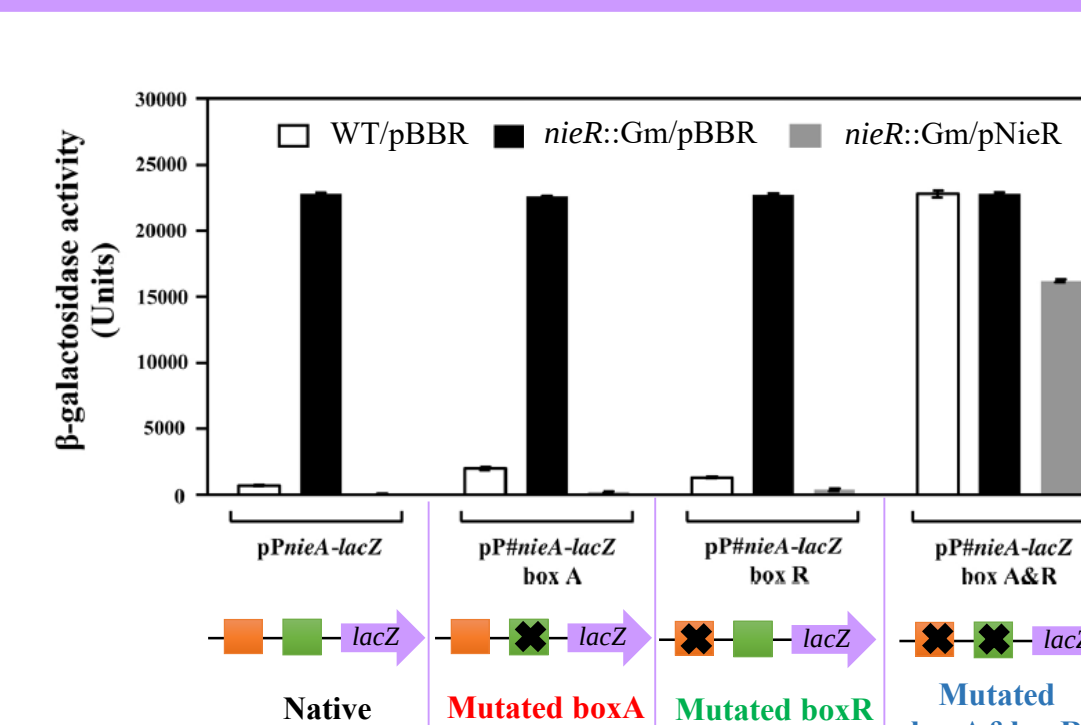
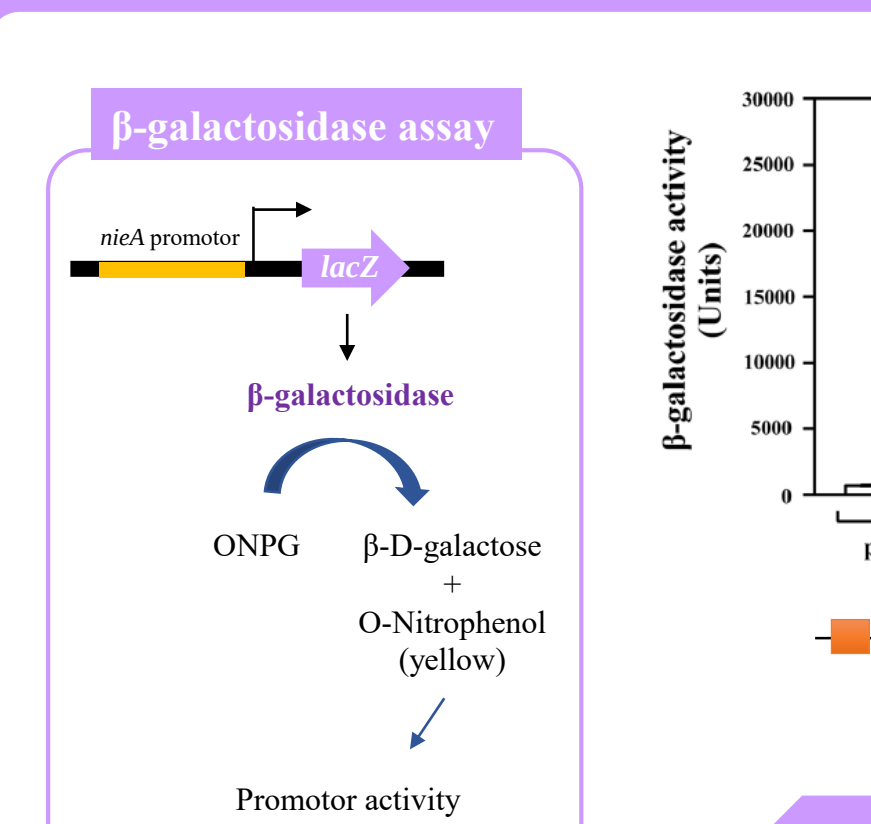


rNieR specifically bound to boxA and boxR to form a complex.



EMSA

Characterization of NieR binding sites



nieA promoter-lacZ fusions

The promoter-lacZ fusions and mutagenesis of either boxA or boxR further confirmed a crucial role of these boxes for NieR repression.

Promoter activity measurement

Reference



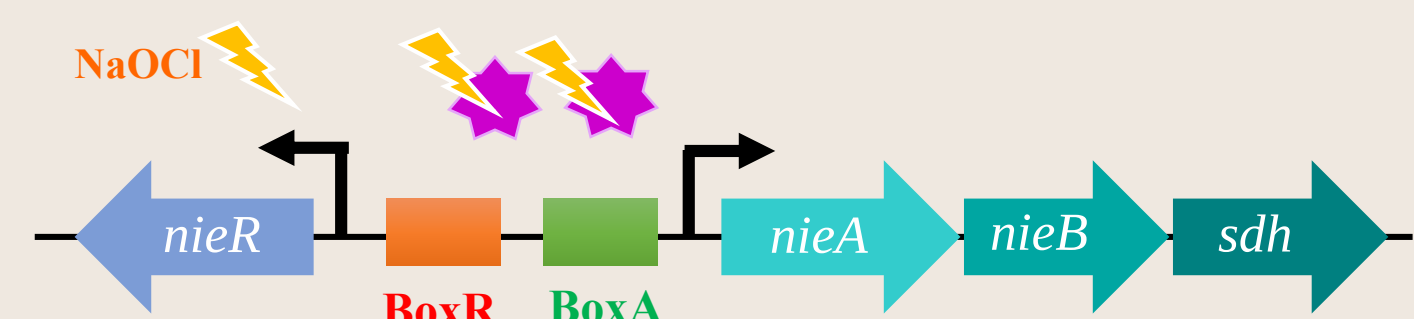
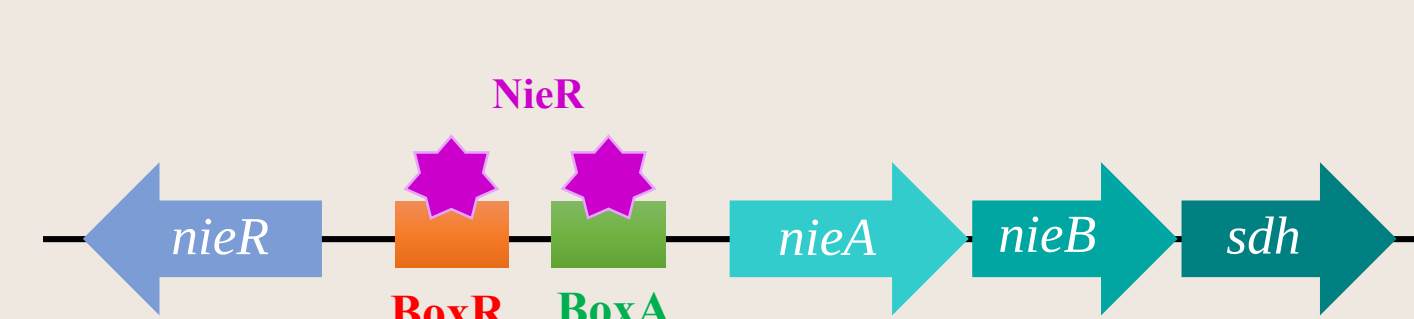
Duang-nkern J, Nontaleerak B, Udomkanarat T, Saninjuk K, Sukchawalit R, Mongkolsuk S. 2021. NieR is the repressor of a NaOCl-inducible efflux system in *Agrobacterium tumefaciens* C58. Microbiol Res 251:126816.

Acknowledgement

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Conclusion

- The *A. tumefaciens* NieR is the NaOCl-sensing repressor that negatively controlled the expression of the NaOCl-inducible efflux genes *nieAB* and its own expression.
- The NieR directly bound to two binding sites, NieR boxes A and R, in the intergenic region of the divergent *nieAB-sdh* operon and *nieR*.
- NieR dissociated from the NieR boxes (A and R) under NaOCl exposure, thereby allowing the transcription of the *nieAB-sdh* operon and *nieR*.
- The *A. tumefaciens* *nieR* was essential for normal growth, survival under NaOCl stress and antibiotic resistance.



Future plan