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Genetic basis of β-lactam resistance in *Corynebacterium auriscanis* and association with otitis externa in dogs and cats

Inaugural-Dissertation

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submitted by

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Abstract

Vetsuisse Faculty University of Bern 2025

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Genetic basis of β-lactam resistance in *Corynebacterium auriscanis* and association with otitis externa in dogs and cats

Corynebacterium (C.) auriscanis is an opportunistic pathogen isolated from canine otitis externa. We found a surprisingly high prevalence of β-lactam resistant isolates (47%). To determine the genetic base of this phenotype a selection of isolates of *C. auriscanis* with high and low minimal inhibitory concentration values were subjected to whole genome sequencing. Comparative analysis revealed a gene cassette containing three genes (*hdfR* encoding a LysR-family transcriptional regulator, *blaB* encoding a β-lactamase related protein and *pbp2c* encoding a D,D-transpeptidase) as the likely resistance-encoding determinant. This locus had previously been described in *C. jeikeium* as well as *C. diphtheriae*. In our six *C. auriscanis* isolates the *pbp2c* locus was always associated with the same IS*3* family transposase, an association also found on *C. diphtheriae* plasmid CP091096, indicating horizontal gene transfer between species. To elucidate the function of the three genes in the *pbp2c* locus we constructed plasmids with different combinations of these genes, transformed β-lactam sensitive isolates with the plasmids and tested resistance in the mutants phenotypically. We confirmed Pbp2c to be the primary factor conferring β-lactam resistance and HdfR and BlaB being important for expression and regulation. Interestingly, resistance to all β-lactams including carbapenems was mostly constitutive in *C. auriscanis* while an induction effect was visible for *C. glutamicum* and *C. rouxii*.

otitis, corynebacteria, carbapenem, transpeptidase, β-lactamase

Zusammenfassung

Vetsuisse Faculty University of Bern 2025

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Genetische Grundlage der β-Laktam Resistenz von *Corynebacterium auriscanis* und deren Assoziation mit Otitis Externa in Hunden und Katzen

Corynebacterium (*C.*) *auriscanis* ist ein opportunistischer Erreger isoliert aus Hunden mit Otitis Externa. Die Isolate zeigten eine hohe Resistenzrate (47%) gegenüber β-Laktam Antibiotika. Bei Sequenzierung des Genoms einer Auswahl von *C. auriscans* Isolaten mit hohen und niedrigen minimalen Hemmkonzentrationswerten wurde eine Genkassette mit drei Genen gefunden (*hdfR*, das für einen Transkriptionsregulator der LysR-Familie kodiert, *blaB*, das für ein β-Laktamase-verwandtes Protein kodiert, und *pbp2c*, das für eine D,D-Transpeptidase kodiert), welche wahrscheinlich für den beobachteten Phänotyp verantwortlich ist. Die gleichen Gene wurden zuvor bereits bei *C. jeikeium* und *C. diphtheriae* nachgewiesen. In den sechs *C. aursicanis* Isolaten war der *pbp2c*-Lokus immer mit einer Transposase der IS*3*-Familie assoziiert, was so auch auf dem *C. diphtheriae*-Plasmid CP091096 gefunden wurde, was auf einen horizontalen Gentransfer hindeutet. Um die Funktion dieser drei Gene zu klären, wurden Plasmide mit verschiedenen Kombinationen der Gene konstruiert und β-Laktam-empfindliche Isolate damit transformiert. Die Resistenz der Mutanten wurde phänotypisch getestet. Wir bestätigten, dass Pbp2c der primäre Faktor ist, der zur β-Laktam-Resistenz führt, wobei HdfR und BlaB für die Expression und Regulation wichtig sind. Die Resistenz gegen β-Laktame, einschließlich Carbapeneme, bei *C. auriscanis* war mehrheitlich konstitutiv, während bei *C. glutamicum* und *C. rouxii* ein Induktionseffekt zu beobachten war.

Otitis, Corynebacteria, Carbapeneme, Transpeptidase, β-Laktamase

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Genetic basis of β-lactam resistance in *Corynebacterium auriscanis* and association with otitis externa in dogs and cats

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ABSTRACT

Corynebacterium (*C.*) *auriscanis* is an opportunistic pathogen regularly isolated from canine otitis externa, an important condition often hard to treat. We found a surprisingly high prevalence of β-lactam resistant isolates of *C. auriscanis* (47 %), even though β-lactams are not routinely used for otitis externa treatment in Switzerland. To determine the genetic base of this phenotype, a selection of isolates of *C. auriscanis* with high and low minimal inhibitory concentration values were subjected to whole genome sequencing. Comparative analysis revealed a gene cassette containing three genes (*hdfR* encoding a LysR-family transcriptional regulator, *blaB* encoding a β-lactamase related protein and *pbp2c* encoding a D,D-transpeptidase) as the likely resistance-encoding determinant in the isolates from otitis externa. This locus had previously been described in *C. jeikeium* as well as *C. diphtheriae* and was associated with mobile genetic elements. In our six *C. auriscanis* isolates the *pbp2c* locus was always associated with the same IS*3* family transposase, an association also found on *C. diphtheriae* plasmid CP091096, indicating horizontal gene transfer between species. To elucidate the function of the three genes in the *pbp2c* locus, we constructed plasmids with different combinations of these genes, transformed β-lactam sensitive isolates with the plasmids and tested resistance in the mutants phenotypically. By doing so we confirmed Pbp2c to be the primary factor conferring β-lactam resistance and HdfR and BlaB being important for expression and regulation. Interestingly, resistance to all β-lactams including carbapenems was constitutive in one *C. auriscanis* transformant while an induction effect was visible for the other transformed *C. auriscanis* strain, *C. glutamicum* and *C. rouxii* as previously described for *C. jeikeium*. Therefore, testing of β-lactam resistance should be done in combination including induction in *Corynebacterium* spp.

1. Introduction

Corynebacteria are gram positive, small, club-shaped, non-spore-forming rods belonging to the family of the *Corynebacteriaceae* (Markey, 2013). The genus *Corynebacterium* (*C.*) contains many species with diverse ecological niches. Currently there are 167 recognized species (https://lpsn.dsmz.de/genus/corynebacterium, accessed 11.11.2024) that are found as commensals on the skin, mucous membranes (nasopharynx), and intestinal tracts of a wide variety of host species. Some of them are also found in the environment. Due to their wide distribution corynebacteria are often considered incidental findings in bacteriological diagnostics. However, the importance of corynebacteria as opportunistic pathogens has come more into focus in the last decades (Bernard, 2012; Funke et al., 1997). This is also the case in veterinary medicine where corynebacteria, especially *C. auriscanis,* are increasingly recognized as cause of otitis externa in dogs (Aalbaek et al., 2010; Henneveld et al., 2012; Vinhal et al., 2024). However, due to the frequent simultaneous isolation of staphylococci, the clinical relevance of the corynebacteria is not always clear (Aalbaek et al., 2010). In addition to the opportunistic pathogens, the genus *Corynebacterium* contains established pathogens such as *C. pseudotuberculosis* causing caseous lymphadenitis (pseudotuberculosis) in goats and sheep, *C. kutscheri* causing pseudotuberculosis in mice, rats and guinea pigs and the *C. renale* group *(C. renale, cystitidis* and *pilosum*) causing cystitis and pyelonephritis in cattle (Funke et al., 1997). *C. bovis* is a commensal on the skin of cattle but can also cause subclinical mastitis (Markey, 2013).

Otitis externa, an inflammatory condition affecting the external ear canal, is often diagnosed in small-animal practice most commonly in canine patients (Murphy, 2001). Generally, it is a complex disease influenced by predisposing factors. As there is a diverse microbial

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Table 1
Corynebacterium auriscanis isolates subjected to whole genome sequencing.

Sample number	Year of isolation	Source	Host	MIC Amp (mg/L)
20KM1532	2020	Ear	Dog	0.5
22KM2839	2022	Ear	Dog	0.5
23KM1744	2023	Ear	Dog	1
23KM0279	2023	Ear	Dog	1
21KM2728	2021	Eye	Dog	1
23KM1528	2023	Surgical wound	Dog	> 32
23KM1762	2023	Ear	Dog	> 32
20KM1400	2020	Ear	Dog	> 32
21KM1388	2021	Nasal discharge	Cat	> 32
22KM0841	2022	Ear	Dog	> 32
23KM1552	2023	Urine	Cat	> 32

Table 2
Description of plasmids constructed in this work.

Plasmid name [genes]	Description
pmrdA_1 [*pbp2c*]	*pbp2c* with natural promoter, without *hdfR* and *blaB*
pmrdA_2 [*hdfR, blaB, pbp2c*]	Entire *pbp2c* locus (*hdfR*, *blaB* and *pbp2c*)
pmrdA_3 [*hdfR, blaB*]	Putative regulatory genes only (*hdfR* and *blaB*)
pmrdA_4 [*hdfR, pbp2c*]	*pbp2c* locus without *blaB* gene (only *hdfR* and *pbp2c*)
pmrdA_5 [*pbp2c*]	*pbp2c* with alternative promoter, without *hdfR* and *blaB*
pmrdA_7 [*hdfR, blaB, aph*]	*pbp2c* replaced with *aph, tet(L)* as selector

Natural promoter = promoter found in 23KM1762, presumptively regulated by HdfR and BlaB, alternative promoter= *aph* gene promoter taken from pPBEx2, not regulated

population that resides on the skin there are multiple opportunistic pathogens that can be involved in a secondary infection of the ear canal. Chronic inflammation can also lead to progressive pathologic changes of the ear canal, which, together with the involved pathogens, can lead to treatment failure (Murphy, 2001; Bajwa, 2019; Rosser, 2004). Different bacterial species as well as fungi have been found to be associated with otitis externa. The most frequently identified bacteria are *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Enterococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp. while *Malassezia* spp. dominate among the fungi (Saengchoowong et al., 2023; Saridomichelakis et al., 2007; Tesin et al., 2023). *Corynebacterium* spp. were found in a study from 2023 from Saengchoowong *et al.* to be both part of the normal microbiota and opportunistically dominate in otitis externa cases. *C. auriscanis* is one of the corynebacteria isolated from cases of otitis externa (Vinhal et al., 2024).

Data on antimicrobial resistance for *C. auriscanis* are largely absent, although Henneveld *et al.* reported high resistance rates against several antimicrobials including ampicillin for 40 % of *Corynebacterium* spp. isolated from canine or feline otitis cases (Henneveld et al., 2012). Multiple resistance determinants have been described for corynebacteria, however for β-lactams data are scarce and resistance mechanisms remain unclear. In *C. striatum* and *C. urealyticum* resistance has been attributed to genes being annotated as β-lactamases (Federal Office of Public Health and Federal Food Safety and Veterinary Office, 2024; Lavollay et al., 2009) however without experimental confirmation. A recent study on the resistance situation in non-diphtheriae corynebacteria from urinary tract infections revealed poor sensitivity to penicillin and cefotaxime (Hennart et al., 2020). Two thirds of the analyzed isolates were found to be resistant to penicillin and 53 % to cefotaxime, but the genes responsible for the resistance could not be conclusively identified. The authors detected *bla* which codes for a putative class A β-lactamase and *ampC* which codes for a class C β-lactamase. However, the resistances could not always be traced back to one of the two genes, and the frequency of occurrence varied greatly depending on the species. Recently Lavollay *et al.* showed that inducible β-lactam resistance

Table 3
Primers and PCR conditions for amplification of overlapping plasmid parts. Plasmids pmrdA_1-5 used the same backbone.

Plasmid pmrdA_1	
Primer name	**Sequence (5′ -> 3′)**
mrdA_gb_F	CTA ATC AAA GCT GGG GAC AAG AAT TTC TGC CAC TTG AAT ACA CAC
mrdA_gb_R	TCT GAG AAT AGT GTA TGC GGA ACC CGT GCT AGA GGT TAT GAC
vec_5808_R	TTG TCC CCA GCT TTG ATT AG
vec_875_F	CCG CAT ACA CTA TTC TCA GA
PCR for insert	30 s 98°C [10 s 98°C, 10 s 69°C, 1 min 72°C]x35 7 min 72°C
PCR for backbone	30 s 98°C [10 s 98°C, 10 s 49°C, 2.5 min 72°C]x35 7 min 72°C
Plasmid pmrdA_2	
Primer name	**Sequence (5′ -> 3′)**
mrdA_cassette_R	TCT GAG AAT AGT GTA TGC GGG GCA TCC AAA AAG GCT GGA TC
mrdA_cassette_F	CTA ATC AAA GCT GGG GAC AAT GCA CAA TGA GCG GTC CG
PCR for insert	30 s 98°C [10 s 98°C, 10 s 69°C, 2 min 72°C]x35 7 min 72°C
Plasmid pmrdA_3	
Primer name	**Sequence (5′ -> 3′)**
pmrdA_3_blaB_R	TCT GAG AAT AGT GTA TGC GGC ATT TCA TTC ACA TAT AGG CGA TGG T
pmrdA_3_blaB_F	CTA ATC AAA GCT GGG GAC AAT GCA CAA TGA GCG GTC CG
PCR for insert	30 s 98°C [10 s 98°C, 10 s 69°C, 1.5 min 72°C]x35 7 min 72°C
Plasmid pmrdA_4	
Primer name	**Sequence (5′ -> 3′)**
pmrdA_4_hdfR_R	GGC AGA AAT TTC ATT GGT TAA TCC TTC GAC A
pmrdA_4_hdfR_F	CTA ATC AAA GCT GGG GAC AAT GCA CAA TGA GCG GTC CG
pmrdA_4_mrdA_R	TCT GAG AAT AGT GTA TGC GGC CGC AAC ACG CCC GGA AAA G
pmrdA_4_mrdA_F	TAA CCA ATG AAA TTT CTG CCA CTT GAA TAC ACA CT
PCR for insert1	30 s 98°C [10 s 98°C, 10 s 60°C, 1 min 72°C]x35 7 min 72°C
PCR for insert2	30 s 98°C [10 s 98°C, 10 s 60°C, 2 min 72°C]x35 7 min 72°C
Plasmid pmrdA_5	
Primer name	**Sequence (5′ -> 3′)**
aph_pro_F	CTA ATC AAA GCT GGG GAC AAG CCA CGT TGT GTC TCA AAA TCT
aph_pro_R	TAG TCA TCA TAA CAC CCC TTG TAT TAC TGT
mrdA_cdc_R	AAG GGG TGT TAT GAT GAC TAA GCA CAA TCG TTT CC
mrdA_cdc_F	TCT GAG AAT AGT GTA TGC GGT TAT TGA ATT CCA GAG AAT TTC TGA ACA
PCR for insert1	30 s 98°C [10 s 98°C, 10 s 56°C, 15 s 72°C]x35 7 min 72°C
PCR for insert2	30 s 98°C [10 s 98°C, 10 s 62°C, 2 min 72°C]x35 7 min 72°C
Plasmid pmrdA_7	
Primer name	**Sequence (5′ -> 3′)**
backbone1_R	ATG TAT TCA CAA CAC CCC TTG TAT TAC TGT
backbone1_F	GTT TTT CTA ACC GCA TAC ACT ATT CTC AGA ATG AC
tetL_R	CCA ATT CTG ATT AGA AAT CCC TTT GAG AAT GT
tetL_F	AAG GGG TGT TGT GAA TAC ATC CTA TTC ACA ATC GA
backbone2_R	GAC ACA CAG CTT GTC CCC AGC TTT GAT TAG GA
backbone2_F	GGA TTT CTA ATC AGA ATT GGT TAA TTG GTT GT
mrdA_reg_R	TAT GGC TCA TGG GAG TGA CGT TAA GTG AGA CG
mrdA_reg_F	CTG GGG ACA AGC TGT GTG TCA CTT CCT TTT TGT
Kan_R	GTG TAT GCG GTT AGA AAA ACT CAT CGA GCA TCA
Kan_F	CGT CAC TCC CAT GAG CCA TAT TCA ACG GGA
PCR backbone1	30 s 98°C [10 s 98°C, 10 s 57°C, 1 min 72°C]x35 7 min 72°C
PCR *tet(L)*	30 s 98°C [10 s 98°C, 10 s 57°C, 1 min 72°C]x35 7 min 72°C
PCR backbone2	30 s 98°C [10 s 98°C, 10 s 57°C, 2 min 72°C]x35 7 min 72°C
PCR insert1 (mrdA_reg)	30 s 98°C [10 s 98°C, 10 s 57°C, 2 min 72°C]x35 7 min 72°C
PCR insert2 (kanR)	30 s 98°C [10 s 98°C, 10 s 57°C, 1 min 72°C]x35 7 min 72°C

in *C. jeikeium* is due to the penicillin binding protein 2c (*pbp2c*) locus (Pitcher et al., 1989). Pbp2c is an alternative transpeptidase associated with two upstream genes encoding putative regulator proteins. One is a transcriptional regulator of the LysR family and the other a protein of the class A β-lactamase family, which does however not hydrolyze β-lactams but is instead involved in regulation of *pbp2c* expression (Pitcher et al., 1989).

In this study we show that the high prevalence of β-lactam resistance

Table 4
Corynebacterial isolates and the plasmids they were transformed with.

Species	Isolate	Source	GenBank	Characteristics	Plasmids introduced
Corynebacterium auriscanis	23KM1744	this study (isolated from dog ear)	CP168250	Amp wildtype, no acquired resistance genes	pmrdA_1, pmrdA_2, pmrdA_3, pmrdA_4, pmrdA_5
Corynebacterium auriscanis	20KM1532	This study (isolated from dog ear)	CP168258	Amp wildtype, no acquired resistance genes	pmrdA_2
Corynebacterium glutamicum	DSM 20300 ^{T}	DSMZ	NC_006958.1	Type isolate	pmrdA_1, pmrdA_2, pmrdA_3, pmrdA_4, pmrdA_5, pmrdA_7
Corynebacterium rouxii	23KM0776	this study (isolated from dog ear)	CP168248	Low virulence member of the *C. diphtheriae* species complex	pmrdA_2, pmrdA_5

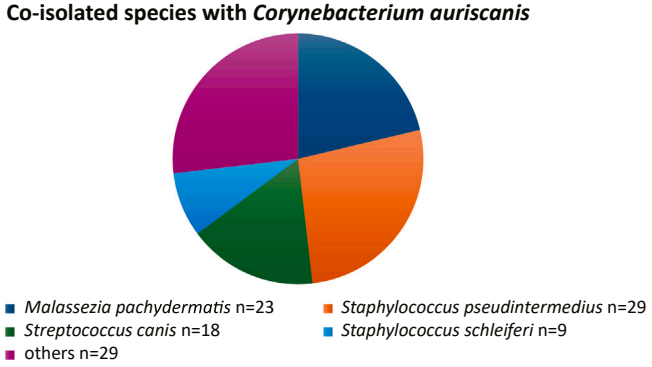


Fig. 1. Opportunistic pathogens isolated together with *Corynebacterium auriscanis* in canine otitis cases.

Table 5
Genome sequence information of the *Corynebacterium auriscanis isolates* used in this study.

Sample number	GenBank accession number	Genome size in bp	%GC	Completeness (assembly could be circularized)	Plasmids
20KM1400	CP168259	2500381	58.55	complete	none
20KM1532	CP168258	2491436	58.51	complete	none
21KM1388	CP168257	2490213	58.56	complete	none
21KM2728	CP168256	2536714	58.49	complete	none
22KM0841	CP168255	2560284	58.54	complete	none
22KM2839	CP168927	2617319	58.51	incomplete	none
23KM0279	CP168253	2522030	58.45	complete	CP168254
23KM1528	CP168252	2548693	58.58	complete	none
23KM1552	CP168251	2590824	58.53	complete	none
23KM1744	CP168250	2482661	58.59	complete	none
23KM1762	CP168249	2579168	58.55	complete	none

in *C. auriscanis* isolated from companion animals is associated with mobile elements carrying the *pbp2c* locus. We also show that expressing *pbp2c* in wild-type *C. auriscanis* confers high level β-lactam resistance and that the two upstream genes regulate the expression of *pbp2c*.

2. Materials and methods

2.1. Prevalence of C. auriscanis

We queried our laboratory database for bacterial species isolated from ear swabs of dogs and cats submitted between 2020 and 2023. In addition, we searched for *C. auriscanis* isolated from other sources. Summary statistics of co- isolated species were produced with Microsoft® Excel® for Microsoft 365 MSO (Version 2411 Build 16.0.18227.20082). The confidence interval for proportion was calculated with the function prop.test() in R version 4.4.1.

2.2. Isolates

C. auriscanis isolates of this study were isolated in our veterinary diagnostic laboratory between 2020 and 2023 from clinical samples submitted by veterinary clinics and private practitioners. We investigated isolates that had been subjected to routine antimicrobial susceptibility testing by broth microdilution (n = 38). Twenty isolates had a minimal inhibitory concentration (MIC) for ampicillin of $\leq$ 2 mg/L and 18 isolates (47 %) had an MIC of $>$ 32 mg/L. Subsequently five isolates of the low MIC group and six isolates of the high MIC group were randomly selected for further analysis (Table 1).

2.3. DNA extraction and sequencing

The isolates were grown on sheep blood agar (BD Difco™ Trypticase Soy Agar II catalog number 254087) and genomic DNA was isolated as described by Pitcher et al. (Pitcher et al., 1989) with a modified suspension buffer containing 20 mM Tris [pH8.0], 2 mM EDTA, 50 U/ml mutanolysin (Sigma-Aldrich), 1.2 % Triton X-100 (Sigma-Aldrich), 20 mg/ml lysozyme (Sigma-Aldrich) and 50 mM glucose (Merck). The genomic DNA was then isolated using the DNeasy PowerClean Cleanup Kit (Qiagen) and submitted to the Next Generation Sequencing platform of the University of Bern (Switzerland) for PacBio HiFi sequencing. There Multiplexed SMRTbell libraries were prepared for sequencing on the Revio instrument using the HiFi plex prep kit 96 according to the PacBio guideline for preparing multiplexed whole genome and amplicon libraries with one exception: gDNA was sheared using a Covaris g-TUBE (Covaris; 10145) following the PacBio technical note for Covaris g-TUBE DNA shearing for SMRTbell prep kit 3.0. The sheared gDNA was then concentrated and cleaned using 1 x SMRTbell clean-up beads. Samples and afterwards the library pool were quantified and qualified using a Qubit 4.0 fluorometer (Qubit dsDNA HS Assay kit; Q32851, Thermo Fisher Scientific) and an Advanced Analytical FEMTO Pulse instrument (Genomic DNA 165 kb Kit; FP-1002–0275, Agilent), respectively. Instructions in SMRT Link Sample Setup were followed to prepare the SMRTbell library for sequencing (PacBio SMRT Link v13.1). SMRT sequencing was performed on the Revio controlled by instrument software 13.0.0.212033 and with a 24 h movie time.

2.4. Bioinformatic analyses

PacBio HiFi reads were converted from bam to fastq format using bam2fastq v3.1.1. followed by filtering with Filtlong v0.2.1 and assembly with Flye v2.9.2. Assembly quality was assessed using Quast 5.2.0 and assemblies were submitted to GenBank for annotation with NCBI Prokaryotic Genome AnnotationPipeline (PGAP). For comparison, assemblies were also annotated with prokka v1.14.6 (Seemann, 2014). Genomes were visualized and aligned using Geneious Prime® 2024.0.7 and the Mauve plugin. Similar sequences in GenBank were discovered using BLAST (BLAST: Basic Local Alignment Search Tool (nih.gov)). The detected insertions were visualized and compared using clinker v0.0.27. Core genome single nucleotide polymorphism (SNP) based phylogenetic clustering was performed using snippy v4.6.0, and a neighbor joining tree was calculated using MEGA 11 (Tamura et al., 2021).

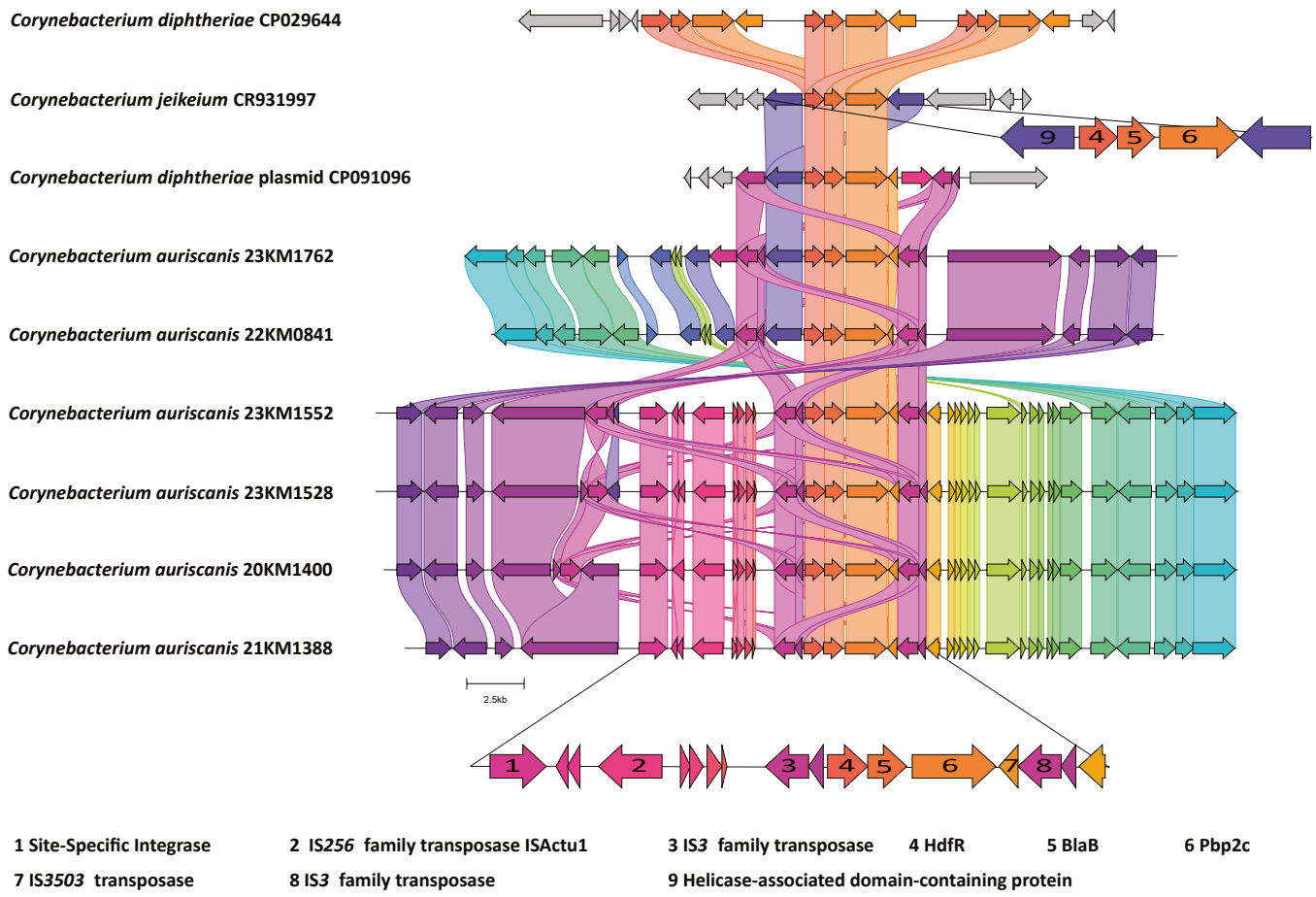


Fig. 2. Comparison of the region containing the gene cluster with *hdfR*, *blaB* and *pbp2c* of *Corynebacterium auriscanis* and other corynebacteria that exhibit a resistance using clinker v0.0.27 a tool for visualization of groups of homologous biosynthetic gene clusters. The *pbp2c* locus is consistently associated with an IS*3* family transposase (3) in the analyzed *Corynebacterium auriscanis* isolates, which is also present in *Corynebacterium diphtheriae* plasmid CP091096.

2.5. Cloning and transformation experiments

2.5.1. Construction of plasmids

To test the function of the three genes (*hdfR* encoding a LysR-family transcriptional regulator, *blaB* encoding a β-lactamase related protein and *pbp2c* encoding a D,D-transpeptidase) constituting the pbp2c locus of *C. auriscanis* a total of six plasmids (Table 2, Supplementary Figure 1) were constructed by Gibson assembly (Gibson, 2011). The backbone encoding *E. coli* origin of replication, replicase Orf-1 from *C. glutamicum* pBL1 and kanamycin resistance gene *aph* (amino-glycoside-3′-phosphotransferase) was derived from pPBEx2 (Bakkes et al., 2020). The *pbp2c* locus was amplified from isolate 23KM1762. *tet* (L) used as alternative selector in pmrdA_7 was taken from *C. auriscanis* 23KM1528. Primers were designed using Geneious Prime® 2024.0.7. DNA fragments of the vector and the inserts were amplified performing PCR using Phusion Hot Start II High Fidelity DNA polymerase (Thermo Scientific) following the Thermo Fisher protocol (Table 3). Fragment assembly was performed using NEBuilder® HiFi DNA Assembly Master Mix 2x (E5520S) (New England Biolabs®) according to manufacturer's instructions.

2.5.2. Transformation procedure

Plasmids were inserted by electroporation into *Escherichia coli* DH5α for plasmid amplification and maintenance as well as into four *Corynebacterium* isolates to test resistance conferring properties of the different plasmids phenotypically (Table 4). Preparation of competent corynebacterial cells and electroporation was performed according to the protocol described by Ruan et al. (2015) with small adaptations. Briefly, 2 µl plasmid DNA (about 200 ng/µl) was added to 80 µl of competent cells, chilled on ice and then transferred to a 1 mm electroporation cuvette. Electroporation was performed with parameters set at 1.8 kV and 5 ms using the Gene Pulser Xcell™ electroporator (Bio Rad). After electroporation 1 ml of brain heart infusion (BHI) broth (BD Difco™) was added to the suspension and was incubated for 6 min at 46°C in a water bath (this step was omitted for *E. coli*). Cells were afterwards incubated for 2 h at 30°C on a shaker at 550 rpm (1 h for *E. coli*). Subsequently, the cells were plated on selective BHI (CM1136B, Thermo Scientific™) plates containing 25 mg/L kanamycin and for pmrdA_7 also plates containing 10 mg/L tetracycline. *C. glutamicum* was incubated at 30°C the other corynebacteria and *E. coli* at 37°C.

2.6. Antimicrobial resistance testing

Minimal inhibitory concentrations (MIC) of antimicrobials were determined using Mueller-Hinton fastidious (MH-F) broth (Sensititre™ Cation Adjusted Mueller-Hinton Broth w/ TES (Thermo Scientific™) with 5 % horse blood SR0048C (Thermo Scientific Oxoid) and 20 mg/L β-NAD (Sigma-Aldrich)) following the EUCAST guidelines for corynebacteria (Berger et al., 2024; The European Committee on Antimicrobial Susceptibility Testing, 2024). *Streptococcus pneumoniae* ATCC 49616 was used as quality control strain. For screening purposes commercial Sensititre™ plates (Thermo Fisher Scientific) COMPAN1F and EUVSEC2 were used. Experiments were performed at least twice. If more than one dilution step MIC difference was observed, MIC testing was performed a third time, and the median is presented otherwise the first measurement is presented. To account for the different temperature

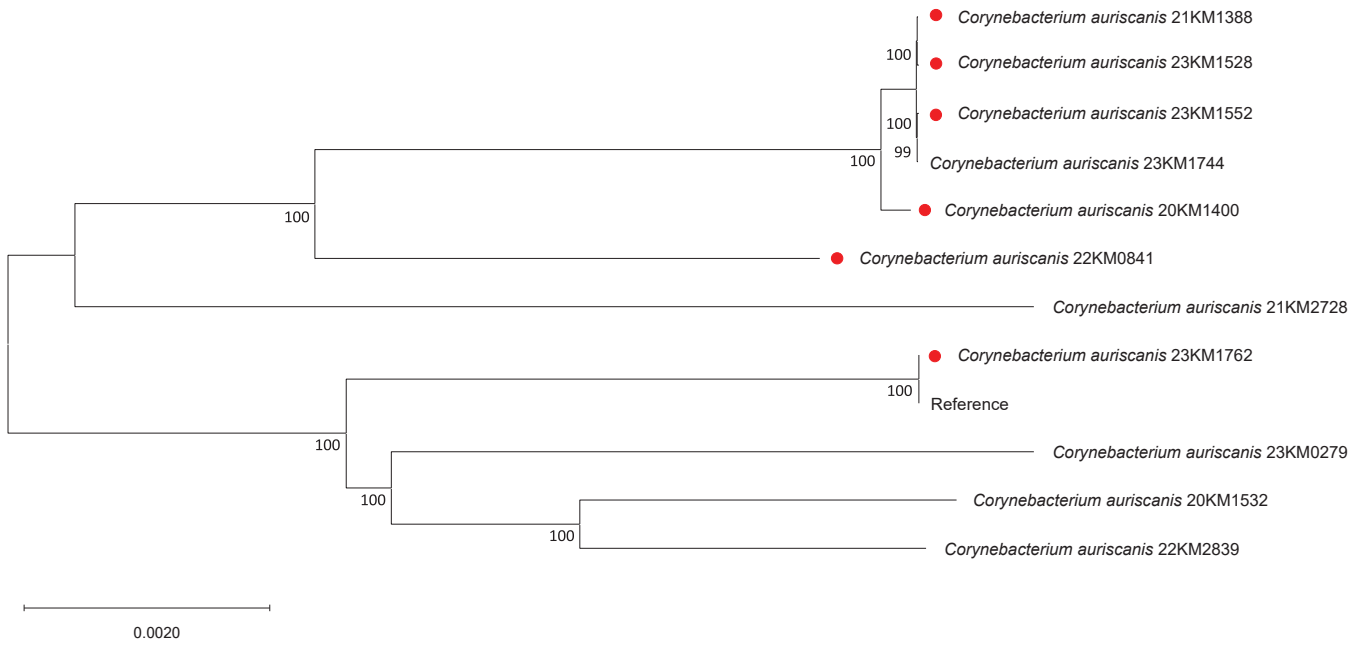


Fig. 3. Core genome SNP based phylogenetic tree of the 11 *Corynebacterium auriscanis* isolates used in the study. The alignment was calculated using snippy v4.6.0 with 23KM1762 set as reference. A neighbor-joining tree was created using Mega 11. Bootstrap values (100 replicates) are shown below the branches. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). There were a total of 2108450 positions in the final dataset. Isolates carrying the *pbp2c* locus are marked in red. They belong to different clusters indicating that the distribution of the *pbp2c* locus is rather due to horizontal gene transfer then clonal expansion.

optimum of *C. glutamicum* the test was also performed at 30°C for this species.

In order to test the inducibility of the *pbp2c* promoter, *C. glutamicum* containing pmrdA_7 (*pbp2c* replaced by *aph,* a kanamycin resistance gene) were grown on sheep blood agar (BD Difco™ Trypticase Soy Agar II catalog number 254087) for 24 h and then tested on serial dilutions of kanamycin with the highest concentration of 256 mg/L. In one dilution series 8 mg/L sulbactam was added to the bacterial suspension to induce the expression of *aph,* which is under control of the *pbp2c* regulation system (*hdfR, blaB,* and *pbp2c* promoter) in pmrdA_7. The assay was performed at 30°C and 35°C and evaluated after 44–48 h.

Additionally, the inducibility of *pbp2c* in *C. glutamicum*, *C. auriscanis* and *C. rouxii* harboring pmrdA_2 (containing the full locus: *hdfR, blaB,* and *pbp2c*) was also tested using a disk diffusion method. Sheep blood agar plates (BD Difco™ Trypticase Soy Agar II catalog number 254087) were prepared with an inoculum of 0.5 McFarland. Two disks were applied: a 1.5 units penicillin disk (Thermo Fisher Scientific™ Oxoid™ catalog Number CT0042B) and a 10 µg meropenem disk (Thermo Fisher Scientific™ BD BBL™ Catalog Number BD 231704) at a distance of 15 mm. Plates were incubated at 37°C for *C. auriscanis* and *C. rouxii* and at 30°C for *C. glutamicum*. The plates were assessed after 44–48 h of incubation.

3. Results

3.1. Prevalence of C. auriscanis

From 2020–2023 we received 535 ear swabs from dogs with otitis externa, and we were able to isolate *C. auriscanis* from 40 swabs (7.5 % CI95: 5.5 %-10.1 %). In one case *C. auriscanis* occurred in pure culture in all others it was associated with other opportunistic pathogens, the most prevalent being *Staphylococcus pseudintermedius*, *Malassezia pachydermatis* and *Streptococcus canis* (Fig. 1).

In the same period, we received 275 ear-specimens from cats from which *C. auriscanis* was not cultured. But *C. auriscanis* was isolated from one cat with chronic cystitis and one cat with nasal discharge. The urine sample was obtained by cystocentesis and contained a pure culture of more than 10^5 CFU/ml of *C. auriscanis* while only a small quantity of *C. auriscanis* was found in the nasal discharge (also in pure culture). In dogs *C. auriscanis* was isolated 13 times from body sites other than the ears, including skin, wounds, eyes and urine, always in mixed culture.

3.2. Sequence analyses

The chromosomes of all 11 isolates were assembled and resulted in a single contig for all but one isolate (Table 5). All isolates had a similar genome size and GC-content, while only one isolate harbored a plasmid.

Genome comparison with mauve revealed that all six isolates with high ampicillin MIC (>32 mg/L) harbored a *pbp2c* locus as previously described in *C. jeikeium* (Lavollay et al., 2024) and *C. diphtheriae* (Forde et al., 2021) (Fig. 2). The *pbp2c* locus had a distinctly lower GC-content of 50.7 % compared to the *C. auriscanis* genomes (58.5 %-58.6 %). *C. jeikeium* also has a high GC-content of 61.6 % while it is lower in *C. diphtheriae* with 53.5 % (Nouioui et al., 2018). The *pbp2c* locus was associated with different mobile elements but always associated with the same IS*3* family transposase. Isolates harboring the *pbp2c* locus belonged to different phylogenetic clusters, indicating mobility as opposed to clonal spread (Fig. 3).

The amino acid sequence of Pbp2c of the analyzed *C. auriscanis* isolates is identical to the one in *C. diphtheriae* CP029644 but shows three amino acid differences to *C. diphtheriae* CP091096 (K79T, Q87K and I292T in CP091096) as well as two amino acid differences to *C. jeikeium* (I292N and A361V in CR931997) (Supplementary Figure 2). The *pbp2c* locus is associated with an IS*3* family transposase in all analyzed *C. auriscanis* isolates as well as in *C. diphtheriae* plasmid CP091096. On the other hand, the same helicase-associated domain-containing protein was detected in two *C. auriscanis* isolates, the *C. diphtheriae* plasmid CP091096 and *C. jeikeium* indicating possible genetic exchange.

Table 6
Minimal inhibitory concentration (MIC) values of the *Corynebacterium auriscanis* isolates analyzed in this study.

MIC* mg/L
Corynebacterium auriscanis

		Isolates without *pbp2c* locus					Isolates with *pbp2c* locus					
Antimicrobials	**Range mg/L**	**20KM1532**	**22KM2839**	**23KM0279**	**21KM2728**	**23KM1744**	**20KM1400**	**21KM1388**	**23KM1528**	**23KM1552**	**22KM0841**	**23KM1762**
Penicillins												
Penicillin	0.06–8	0.5	0.5	1	1	2	> 8	> 8	> 8	> 8	> 8	> 8
Ampicillin	0.25–16	0.5	0.5	1	1	1	> 16	> 16	> 16	> 16	> 16	> 16
Amoxicillin / clavulanic acid 2:1	4/2–32/16	≤ 4/2	≤ 4/2	≤ 4/2	≤ 4/2	≤ 4/2	> 32/16	> 32/16	> 32/16	> 32/16	> 32/16	> 32/16
First-generation cephalosporins												
Cefazolin	4–16	≤ 4	≤ 4	≤ 4	≤ 4	8	> 16	> 16	> 16	> 16	> 16	> 16
Second-generation cephalosporins												
Cefoxitin	0.5–64	8	8	8	16	16	32	64	64	> 64	> 64	> 64
Third-generation cephalosporins												
Cefpodoxime	2–16	≤ 2	≤ 2	4	4	8	> 16	> 16	> 16	> 16	> 16	> 16
Cefotaxime	0.25–64	1	1	2	2	4	> 64	> 64	> 64	> 64	> 64	> 64
Fourth-generation cephalosporin												
Cefepime	0.06–32	0.5	0.5	1	2	2	> 32	> 32	> 32	> 32	> 32	> 32
Carbapenems												
Imipenem	0.12–16	≤ 0.12	≤ 0.12	≤ 0.12	0.25	0.25	> 16	> 16	> 16	> 16	> 16	> 16
Meropenem	0.03–16	0.25	0.5	0.5	0.5	0.5	> 16	> 16	> 16	> 16	> 16	> 16

*Minimal inhibitory concentrations of antimicrobials were determined using Mueller-Hinton fastidious broth following EUCAST guidelines. Plates were read after 44–48 h of incubation.

Table 7
Minimal inhibitory concentrations (MIC) values from *Corynebacterium auriscanis*, *Corynebacterium glutamicum* and *Corynebacterium rouxii* with plasmids.

MIC* mg/L

		Corynebacterium auriscanis					*Corynebacterium glutamicum*						*Corynebacterium rouxii*		
Antimicrobials	**Range mg/L**	**23KM1744**	**23KM1744 pmrdA_2**	**23KM1744 pmrdA_5**	**20KM1532**	**20KM1532 pmrdA_2**	**DSM20300^T 35°C**	**DSM20300^T 30°C**	**DSM20300^T pmrdA_2 35°C**	**DSM20300^T pmrdA_2 30°C**	**DSM20300^T pmrdA_5 35°C**	**DSM20300^T pmrdA_5 30°C**	**23KM0776**	**23KM0776 pmrdA_2**	**23KM0776 pmrdA_5**
Penicillins															
Penicillin	0.06–8	2	> 8	8	0.5	> 8	0.5	0.25	4	4	2	1	0.5	> 8	4
Ampicillin	0.25–16	1	> 16	8	0.5	> 16	0.5	≤ 0.25	4	16	2	2	0.5	> 16	8
Amoxicillin / clavulanic acid 2:1	4/2–32/16	≤ 4/2	> 32/16	8/4	≤ 4/2	> 32/16	≤ 4/2	≤ 4/2	≤ 4/2	8/4	≤ 4/2	≤ 4/2	≤ 4/2	> 32/16	≤ 4/2
First-generation cephalosporins															
Cefazolin	4–16	8	> 16	16	≤ 4	> 16	≤ 4	≤ 4	≤ 4	8	≤ 4	≤ 4	≤ 4	> 16	8
Second-generation cephalosporins															
Cefoxitin	0.5–64	16	> 64	64	8	32	1	≤ 0.5	8	8	8	8	1	64	64
Third-generation cephalosporins															
Cefpodoxime	2–16	8	> 16	> 16	≤ 2	> 16	≤ 2	≤ 2	16	> 16	8	16	4	> 16	> 16
Cefotaxime	0.25–64	4	> 64	32	1	> 64	2	1	16	32	8	8	2	16	8
Fourth-generation cephalosporin															
Cefepime	0.06–32	2	> 32	32	0.5	> 32	1	1	16	32	8	4	1	> 32	4
Carbapenems															
Imipenem	0.12–16	0.25	> 16	4	≤ 0.12	> 16	≤ 0.12	≤ 0.12	0.5	1	0.25	≤ 0.12	≤ 0.12	> 16	1
Meropenem	0.03–16	0.5	> 16	8	0.25	4	0.12	0.12	1	2	0.5	0.5	0.25	> 16	4

* Minimal inhibitory concentrations of antimicrobials were determined using Mueller-Hinton fastidious broth following EUCAST guidelines. Plates were read after 44–48 h of incubation.

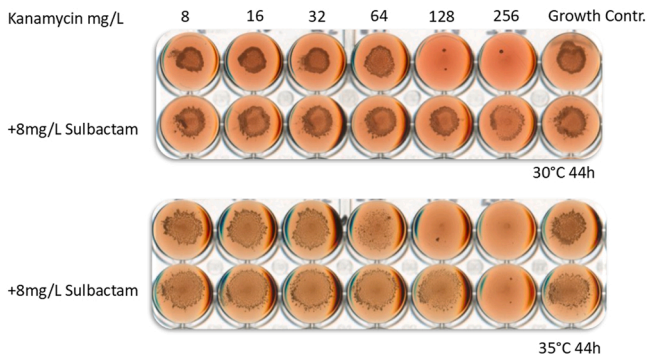


Fig. 4. Kanamycin MIC as determined by broth dilution after 44 h incubation of *Corynebacterium glutamicum* harnboring pmrdA_7 (*pbp2c* replaced with with kanamycin resistance gene *aph*) in medium with or without added sulbactam. The experiment was performed at 30°C and 35°C showing a more pronounced effect of the sulbactam at 30°C. Exposure to sulbactam likely leads to an upregultion of the expression of the gene controlled by HdfR and BlaB which normally is *pbp2c* but in this construct (pmrdA_7) is the kanamycin resistance gene *aph*.

3.3. MIC testing of field isolates and transformed corynebacteria

MIC values for penicillin in isolates lacking the *pbp2c* locus ranged between 0.5 and 2 mg/L and for ampicillin between 0.5 and 1 mg/L (Table 6). However, for the isolates harboring the *pbp2c* locus these MIC values were elevated to > 8 mg/L and > 16 mg/L for penicillin and ampicillin, respectively. For these isolates a high MIC was observed for all β-lactam antibiotics including the carbapenems (Table 6).

The construction of the six plasmids went smoothly and the plasmid sequences were confirmed by sequence analysis (data not shown). The introduction of *pbp2c* with a functional promotor (pmrdA_2 with natural promotor and regulator genes and pmrdA_5 with *aph* gene promotor needing no regulators) in a susceptible *C. auriscanis* isolate led to an increase in the MIC values of the β-lactam antibiotics, including carbapenems (Table 7). The MIC values increased four-fold or higher for penicillin and eight-fold or higher for ampicillin. For the cephalosporins, the increase was in a similar range or higher, up to sixteen-fold for cefepime, although it was not as marked for cefazolin. A similar effect was observed in *C. rouxii* with a penicillin and ampicillin MIC increase of eightfold and higher and a 32-fold increase for cefepime. For *C. glutamicum* with plasmid pmrdA_2 the effect was less marked with an eight-fold MIC increase for the penicillins as well as cefoxitine and 16-fold for cefepime at 35°C. A tendency to higher MIC values was observed at 30°C (Table 7). Concerning the carbapenems, an over 32-fold increase was observed for both imipenem and meropenem in *C. auriscanis* 23KM1744 as well as *C. rouxii* with pmrdA_2 while it was only 16-fold for meropenem in *C. auriscanis* 20KM1532. The increase was less marked for *C. glutamicum* where the MIC values for imipenem and meropenem only increased eight-fold with pmrdA_2. Overall, the MIC increase was higher with pmrdA_2 (full *pbp2c* locus) than with pmrdA_5 (*pbp2c* with alternative promoter) which might be due to a lower expression level in this system.

For all the other constructs lacking *pbp2c* with a functional promoter (pmrdA_1 (*pbp2c* without regulator genes *hdfR* and *blaB*), pmrdA_3 (*hdfR* and *blaB* without *pbp2c*) and pmrdA_4 (*hdfR* and *pbp2c* without *blaB*)) no significant MIC increase was observed in neither the heterologous *C. glutamicum* model nor in *C. auriscanis* (Supplementary Table 1).

The regulation system consisting of *hdfR* and *blaB* with the *pbp2c* promoter was tested by replacing *pbp2c* with kanamycin resistance gene *aph* in plasmid pmrdA_7 (*hdfR*, *blaB*, *aph*). A kanamycin MIC increase from 128 mg/L to > 256 mg/L under exposure to sulbactam was observed for *C. glutamicum* harboring pmrdA_7 after 44 h at 30°C and from 128 mg/L to 256 mg/L at 35°C (Fig. 4). This indicates that exposure to sulbactam leads to an upregultion of the expression of the gene controlled by HdfR and BlaB which normally is *pbp2c* but in this construct is the kanamycin resistance gene *aph*.

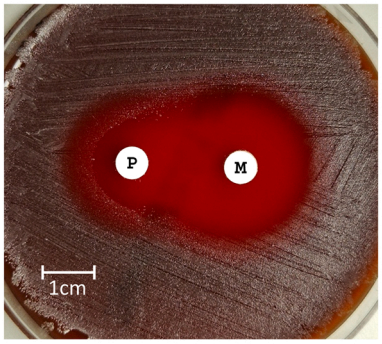
The disk diffusion assay showed an induction of meropenem resistance by penicillin leading to a "D" shape blunting of the inhibition zone for *C. auriscanis* 20KM1532, *C. glutamicum* and *C. rouxii* when harboring pmrdA_2 (full *pbp2c* locus) (Fig. 5). This indicates that meropenem resistance increases under penicillin exposure. No inhibition zone was observed for *C. auriscanis* (23KM1744) pmrdA_2, which is consistent with high MIC values for meropenem in broth microdilution tests (Fig. 5, Table 7).

4. Discussion

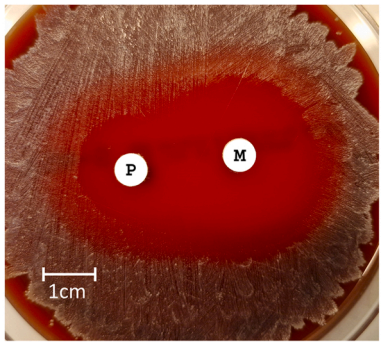
C. auriscanis was detected in 7.5 % of ear swabs from clinical cases of otitis externa analyzed in our diagnostic laboratory not ruling out a potential involvement of the bacteria in the disease. However, co-isolation with other pathogens suggests a rather subordinate role in pathogenicity. Nevertheless, it has been shown that corynebacteria are rarely isolated from healthy ears (Aalbaek et al., 2010), meaning that *C. auriscanis* should not simply be considered a commensal. Of particular note was the high prevalence (47 %) of isolates with high MICs for β-lactam antibiotics, including carbapenems. This is consistent with the already mentioned generally high resistance rates against antimicrobials of corynebacteria isolated from canine and feline otitis cases reported by Henneveld *et al.* (Henneveld et al., 2012). The guidelines for prudent use of antibiotics in dogs and cats of Switzerland (https://www.vetpharm.uzh.ch/Cms/Antibioticscout/index.html, accessed 16.12.2024) recommend a combination of glucocorticoids, antimycotics and antibiotics for topical use for the treatment of otitis externa. In Switzerland, authorized products contain mostly polymyxin B, gentamicin or florfenicol (https://www.vetpharm.uzh.ch/). Products containing marbofloxacin or enrofloxacin should only be used as second line (https://www.vetpharm.uzh.ch/Cms/Antibioticscout/index.html, accessed 16.12.2024). This fact does not explain the high rate of β-lactam resistance in *C. auriscanis* isolated from clinical samples. The selection could be a result of a general high use of β-lactam antibiotics as first line treatment for other indications than ear infections. In 2023 about 80 % of the antibiotics given to dogs in Switzerland were β-lactams (Federal Office of Public Health and Federal Food Safety and Veterinary Office, 2024). This might lead to unintended selection of resistant skin commensals, which may then act as opportunistic pathogens.

The analysis of the sequence data of randomly selected isolates revealed that the common feature of the resistant isolates was a three gene locus that had already been detected in *C. diphtheriae* and in *C. jeikeium* (Lavollay et al., 2009; Hennart et al., 2020). The *pbp2c* locus was consistently associated with an IS*3* family transposase in *C. auriscanis*. Interestingly, the same transposase was also present in *C. diphtheriae* plasmid CP091096 described by Xiaoli et al. (2023). This plasmid was found in a non-toxigenic biovar gravis isolate and also harbored the erythromycin resistance gene *erm*(X) (Xiaoli et al., 2023). This indicates that transposable elements carrying *pbp2c* can be integrated into multi-resistance plasmids and can also probably migrate between different *Corynebacterium* species.

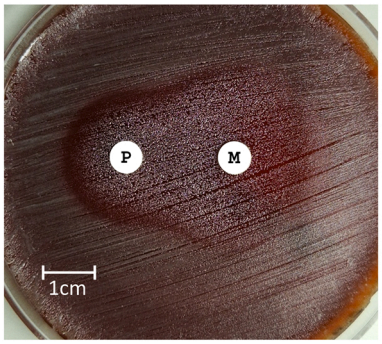
Our transformation experiments show that Pbp2c from *C. auriscanis* confers β-lactam resistance in different corynebacteria, however the strongest phenotype was shown in *C. auriscanis.* This species showed the highest MIC values and one of the two tested transformants (23KM1744) also required no induction by penicillin for meropenem resistance as opposed to the second *C. auriscanis* (20KM1532), *C. glutamicum* and *C. rouxii* (Fig. 5). Lavollay *et al.* also showed a similar induction of meropenem resistance in *C. jeikeium* by clavulanate and ampicillin (Lavollay et al., 2024), an indication that the regulation of expression might be species or even isolate dependent, posing a challenge for standardized resistance testing. Interestingly, induction in *C. glutamicum* was more pronounced at lower temperatures possibly due to the lower growth optimum of this species.



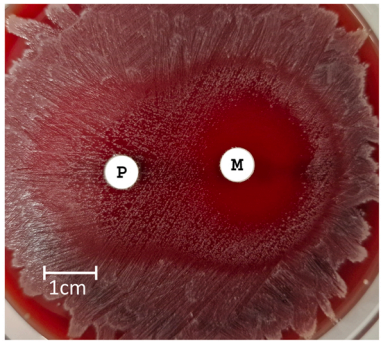
Corynebacterium auriscanis 23KM1744



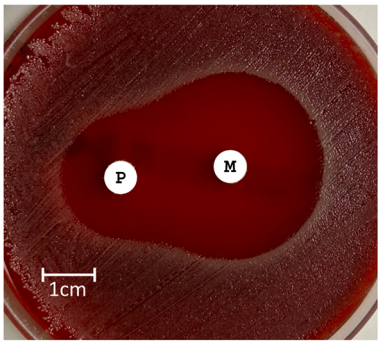
Corynebacterium auriscanis 20KM1532



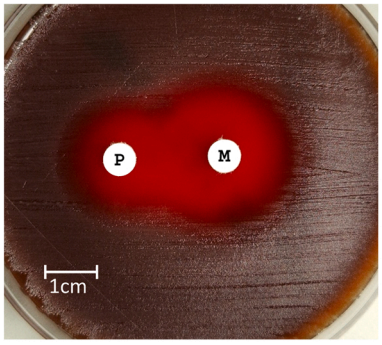
Corynebacterium auriscanis 23KM1744 pmrdA_2



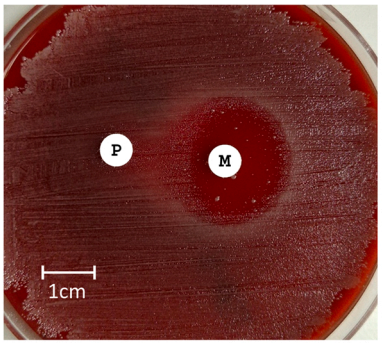
Corynebacterium auriscanis 20KM1532 pmrdA_2



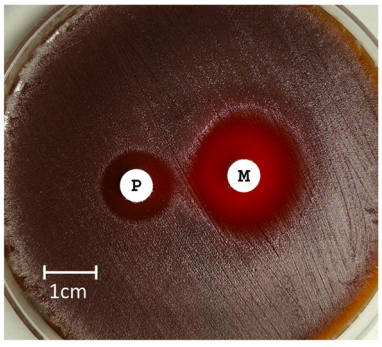
Corynebacterium glutamicum



Corynebacterium rouxii



Corynebacterium glutamicum pmrdA_2



Corynebacterium rouxii pmrdA_2

(caption on next page)

Fig. 5. Disk diffusion assay for *Corynebacterium auriscanis*, *Corynebacterium glutamicum* and *Corynebacterium rouxii* with and without the plasmid pmrdA_2. Plates were incubated for 48 h at 37°C for *Corynebacterium auriscanis* and *Corynebacterium rouxii* and at 30°C for *Corynebacterium glutamicum*. A blunting of the meropenem inhibition zone towards the penicillin disc can be observed for transformats harboring pmrdA_2 (*hdfR*, *blaB*, *pbp2c*) which indicates a synergism consistent with induction of *pbp2c* expression by penicillin. The exception is *Corynebacterium auriscanis* 23KM1744 which has no inhibition zone for meropenem due to high baseline resistance. Disk P: penicillin 1.5 Units Disk M: meropenem 10 μg.

The introduction of pmrdA_2 (full *pbp2c* locus) and pmrdA_5 (*pbp2c* with alternative promoter) both resulted in elevated MICs for the β-lactam antibiotics while the other constructs lacking *pbp2c* with a functional promoter had no effect. This confirms the findings of Lavollay *et al.* that the expression of *pbp2c* leads to resistance to β-lactam antibiotics in corynebacteria while the two upstream genes are merely involved in regulation of expression (Lavollay et al., 2024). Our results indicate that they are both necessary for expression of *pbp2c* with its natural promoter. The incorporation of *pbp2c* with its natural promoter but without the putative regulator genes *hdfR* and *blaB* (pmrdA_1) did not result in any change in resistance to β-lactam antibiotics and neither did *hdfR* and *blaB* alone (pmrdA_3). These findings align with those of Lavollay *et al.*, who demonstrated that BlaB is not a functional β-lactamase and therefore does not degrade β-lactams (Lavollay et al., 2024). Removing *blaB* (pmrdA_4) also led to loss of the resistant phenotype indicating that both genes are necessary for *pbp2c* to be expressed in sufficient quantity. That the induction of the *pbp2c* promoter does not depend on a feedback mechanism involving Pbp2c could be shown by replacing *pbp2c* with kanamycin resistance gene *aph* (pmrdA_7). In *C. glutamicum* pmrdA_7 kanamycin resistance of the isolate could be induced by exposure to sulbactam. However, the exact mechanism of induction and possible differences between *Corynebacterium* species requires further research.

5. Conclusion

We demonstrated a high (about 50 %) prevalence of β-lactam resistance in *C. auriscanis* isolated from canine otitis externa cases in Switzerland. The resistance is conferred by the alternative transpeptidase Pbp2c also described in other corynebacterial species. The two upstream genes are both necessary for expression, however the exact mechanism of regulation requires further research. Phenotypic detection of the resistance mechanism may require incubation at different temperatures and induction tests, depending on the *Corynebacterium* species in question. The *pbp2c* locus is usually linked with mobile elements in corynebacteria and was also found to be associated with an IS*3* family transposase in our resistant *C. auriscanis* isolates. There is a risk of spread and bystander selection originating from *C. auriscanis* isolates when dogs are treated with β-lactams for other diseases.

CRediT authorship contribution statement

Gross Natascha: Writing – original draft, Visualization, Investigation, Formal analysis. **Brodard Isabelle:** Writing – review & editing, Investigation. **Overesch Gudrun:** Writing – review & editing, Methodology. **Kittl Sonja:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization.

Ethical approval

Field isolates investigated in this study were derived from diagnostic specimens. The latter were routinely processed at the ISO17025 accredited diagnostic laboratory of the Institute of Veterinary Bacteriology, University of Bern, Switzerland. All data were anonymized.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetmic.2025.110526.

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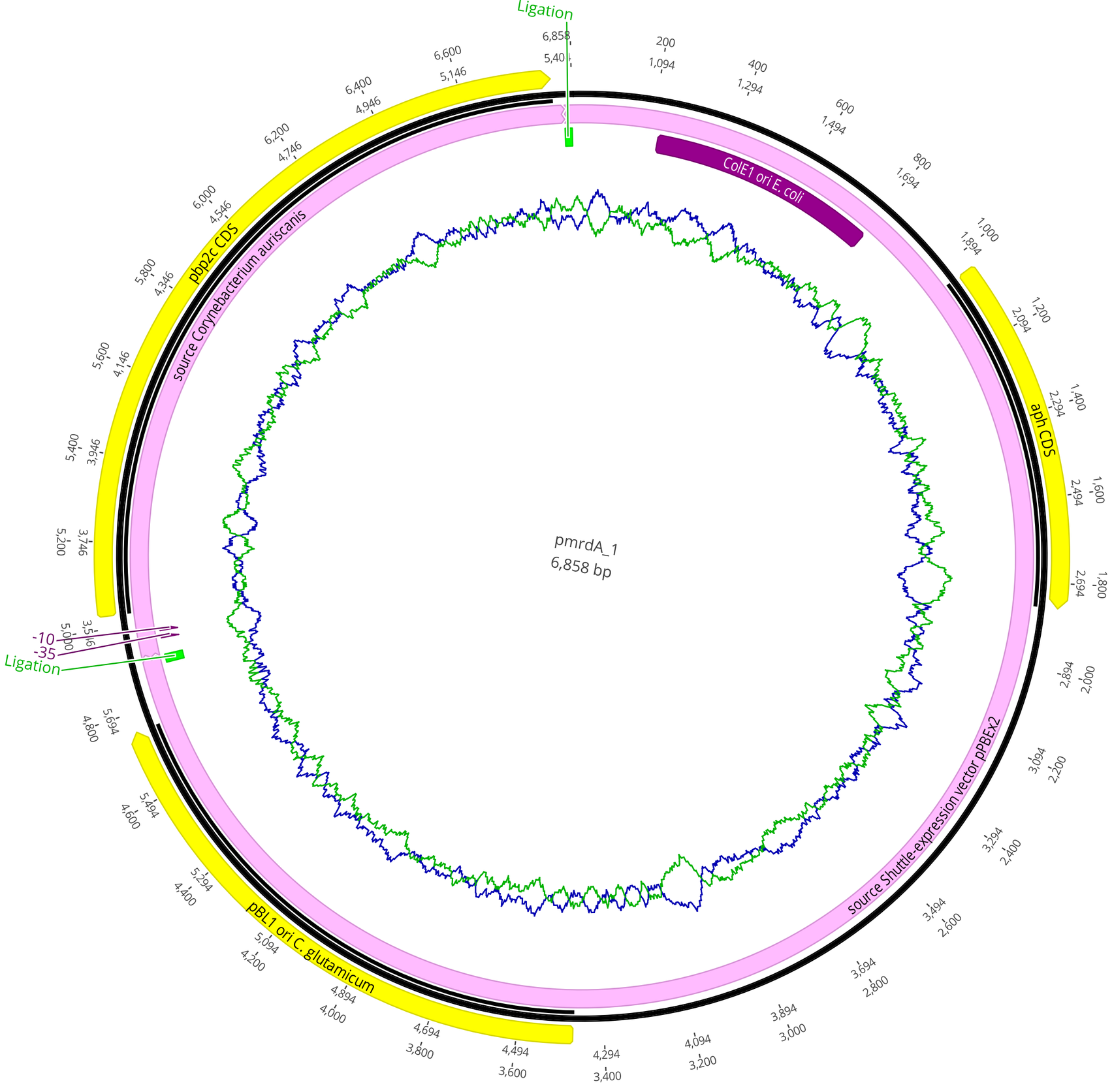
MIC values of antimicrobials after 44-48h	*Corynebacterium glutamicum* DSM20300T	*Corynebacterium glutamicum* DSM20300T_30° C	*Corynebacterium glutamicum* DSM20300T pmrdA_1	*Corynebacterium glutamicum* DSM20300T pmrdA_1_30°C	*Corynebacterium glutamicum* DSM20300T pmrdA_2	*Corynebacterium glutamicum* DSM20300T pmrdA_2_30°C	*Corynebacterium glutamicum* DSM20300T pmrdA_3	*Corynebacterium glutamicum* DSM20300T pmrdA_3_30°C	*Corynebacterium glutamicum* DSM20300T pmrdA_4	*Corynebacterium glutamicum* DSM20300T pmrdA_4_30°C	*Corynebacterium glutamicum* DSM20300T pmrdA_5	*Corynebacterium glutamicum* DSM20300T pmrdA_5_30°C	*Corynebacterium rouxii* 23KM0776	*Corynebacterium rouxii* 23KM0776 pmrdA_2	*Corynebacterium rouxii* 23KM0776 pmrdA_5	*Corynebacterium auriscanis* 23KM1744	*Corynebacterium auriscanis* 23KM1744 pmrdA_1	*Corynebacterium auriscanis* 23KM1744 pmrdA_2	*Corynebacterium auriscanis* 23KM1744 pmrdA_3	*Corynebacterium auriscanis* 23KM1744 pmrdA_4	*Corynebacterium auriscanis* 23KM1744 pmrdA_5	*Corynebacterium auriscanis* 20KM1532	*Corynebacterium auriscanis* 20KM1532 pmrdA_2	*Corynebacterium auriscanis* 22KM2639	*Corynebacterium auriscanis* 23KM0279	*Corynebacterium auriscanis* 21KM2728	*Corynebacterium auriscanis* 20KM1400	*Corynebacterium auriscanis* 21KM1388	*Corynebacteriumauriscanis* 23KM1528	*Corynebacterium auriscanis* 23KM1652	*Corynebacterium auriscanis* 22KM0841	*Corynebacterium auriscanis* 23KM1762
Penicillins																																
PEN (Penicillin)	0.5	0.25	0.5	0.5	4	4	0.5	0.5	0.5	0.5	2	1	0.5	>8	4	2	1	>8	1	1	8	0.5	>8	0.5	1	1	>8	>8	>8	>8	>8	>8
AMP (Ampicillin)	0.5	<=0.25	0.5	<=0.25	4	16	<=0.25	0.5	0.5	<=0.25	2	2	0.5	>16	8	1	1	>16	2	2	8	0.5	>16	0.5	1	1	>16	>16	>16	>16	>16	>16
AUG2 (Amoxicillin / clav 2:1 ratio)	<=4/2	<=4/2	<=4/2	<=4/2	<=4/2	8/4	<=4/2	<=4/2	<=4/2	<=4/2	<=4/2	<=4/2	<=4/2	>32/16	<=4/2	<=4/2	<=4/2	>32/16	<=4/2	<=4/2	8/4	<=4/2	>32/16	<=4/2	<=4/2	<=4/2	>32/16	>32/16	>32/16	>32/16	>32/16	>32/16
TIC (Ticarcillin)	<=8	<=8	<=8	<=8	<=8	64	<=8	<=8	<=8	<=8	16	16	<=8	>64	64	16	<=8	>64	<=8	>64	16	>64	<=8	>64	<=8	<=8	16	>64	>64	>64	>64	>64
TIM2 (Ticarcillin / clav constant 2)	<=8/2	<=8/2	<=8/2	<=8/2	16/2	64/2	<=8/2	<=8/2	<=8/2	<=8/2	<=8/2	<=8/2	<=8/2	>64/2	32/2	<=8/2	<=8/2	>64/2	<=8/2	>64/2	16/2	>64/2	<=8/2	>64/2	<=8/2	<=8/2	<=8/2	>64/2	>64/2	>64/2	>64/2	>64/2	>64/2
Cephalosporin 1st																																
FAZ (Cefazolin)	<=4	<=4	<=4	<=4	<=4	8	<=4	<=4	<=4	<=4	<=4	<=4	<=4	>16	8	8	8	>16	8	8	16	<=4	>16	<=4	<=4	<=4	>16	>16	>16	>16	>16	>16
Cephalosporin 2nd																																
FOX (Cefoxitin)	1	<=0.5	1	<=0.5	8	8	<=0.5	<=0.5	1	1	8	8	1	64	64	16	16	>64	32	32	64	8	32	8	8	16	32	64	64	>64	>64	>64
Cephalosporin 3rd																																
XNL (Ceftiofur)	4	2	4	2	>4	>4	4	4	4	4	>4	>4	4	>4	>4	>4	>4	>4	>4	>4	>4	4	>4	2	>4	>4	>4	>4	>4	>4	>4	>4
FOV (Cefovezin)	2	2	4	2	>8	>8	2	4	4	4	8	8	2	>8	8	8	4	>8	4	8	>8	2	>8	1	2	4	>8	>8	>8	>8	>8	>8
POD (Cefpodoxim)	<=2	<=2	4	<=2	16	>16	<=2	<=2	4	<=2	8	16	4	>16	>16	8	8	>16	8	8	>16	<=2	>16	<=2	4	4	>16	>16	>16	>16	>16	>16
TAZ (Ceftazidim)	32	16	32	16	>128	>128	16	32	32	16	128	128	32	>128	128	64	64	>128	128	128	>128	16	>128	16	32	64	>128	>128	>128	>128	>128	>128
TC (Ceftazidime+Clav)	<=0.12/4	<=0.12/4	<=0.12/4	<=0.12/4	64/4	>128/4	<=0.12/4	<=0.12/4	<=0.12/4	<=0.12/4	128/4	128/4	2/4	>128/4	128/4	32/4	32/4	>128/4	128/4	128/4	>128/4	4/4	>128/4	4/4	8/4	32/4	>128/4	>128/4	>128/4	>128/4	>128/4	>128/4
FOT (Cefotaxime)	2	1	2	1	16	32	1	2	2	1	8	8	2	32	8	4	4	>64	4	4	32	1	>64	1	2	2	>64	>64	>64	>64	>64	>64
FC (Cefotaxime+Clav)	<=0.06/4	<=0.06/4	<=0.06/4	<=0.06/4	32/4	64/4	<=0.06/4	<=0.06/4	<=0.06/4	<=0.06/4	8/4	4/4	0.12/4	64/4	8/4	2/4	2/4	>64/4	2/4	4/4	32/4	0.25/4	>64/4	0.25/4	0.5/4	1/4	>64/4	>64/4	>64/4	>64/4	>64/4	>64/4
Cephalosporin 4th																																
FEP (Cefepime)	1	1	2	1	16	32	1	1	2	1	8	4	1	>32	4	2	2	>32	4	4	32	0.5	>32	0.5	1	2	>32	>32	>32	>32	>32	>32
Carbapenem																																
IMI (Imipenem)	<=0.12	<=0.12	<=0.12	<=0.12	0.5	1	<=0.12	<=0.12	<=0.12	<=0.12	0.25	<=0.12	<=0.12	>16	1	0.25	0.25	>16	0.5	0.5	4	<=0.12	>16	<=0.12	<=0.12	0.25	>16	>16	>16	>16	>16	>16
ETP (Ertapenem)	0.5	0.5	0.5	0.5	2	>2	0.5	0.5	0.5	0.5	2	1	0.5	>2	>2	2	2	>2	>2	>2	>2	1	>2	1	1	2	>2	>2	>2	>2	>2	>2
MERO (Meropenem)	0.12	0.12	0.12	0.12	1	2	0.12	0.12	0.12	0.12	0.5	0.5	0.25	>16	4	0.5	0.5	>16	1	2	8	0.25	4	0.5	0.5	0.5	>16	>16	>16	>16	>16	>16
Macrolides																																
ERY (Erythromycin)	<=0.5	2	<=0.5	2	<=0.5	1	<=0.5	1	<=0.5	1	<=0.5	1	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	>4	<=0.5	>4	<=0.5	<=0.5	>4	<=0.5	<=0.5	>4
Tetracyclines																																
DOX (Doxycycline)	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2
Aminoglycosides																																
AMI (Amikacin)	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4
GEN (Gentamicin)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	>8	<=1	<=1	<=1	<=1	<=1	<=1
Phenicols																																
CHL (Chloramphenicol)	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	8	8	<=4	8	<=4	<=4	<=4	<=4	<=4	>16
Quinolones																																
ENRO (Enrofloxacin)	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	<=0.25	<=0.25	<=0.25	2	2	2	2	2	2	<=0.25	<=0.25	0.5	<=0.25	2	2	2	2	2	2	2
MAR (Marbofloxacin)	1	1	1	1	1	1	1	1	1	1	1	1	<=0.25	<=0.25	<=0.25	>2	>2	>2	>2	>2	>2	0.5	0.5	2	0.5	>2	>2	>2	>2	>2	>2	>2
other																																
RIF (Rifampin)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
SXT (Trimethoprim / Sulfamethoxazole)	<=0.5/9.5	<=0.5/9.5	<=0.5/9.5	<=0.5/9.5	1/19	<=0.5/9.5	<=0.5/9.5	<=0.5/9.5	<=0.5/9.5	<=05/9.5	<=0.5/9.5	<=0.5/9.5	<=0.5/9.5	<=0.5/9.5	<=0.5/9.5	2/38	2/38	2/38	2/38	2/38	2/38	2/38	2/38	1/19	2/38	2/38	>2/38	2/38	2/38	1/19	2/38	2/38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pBL1 ori C. glutamicum
source Corynebacterium auriscanis
pbp2c CDS
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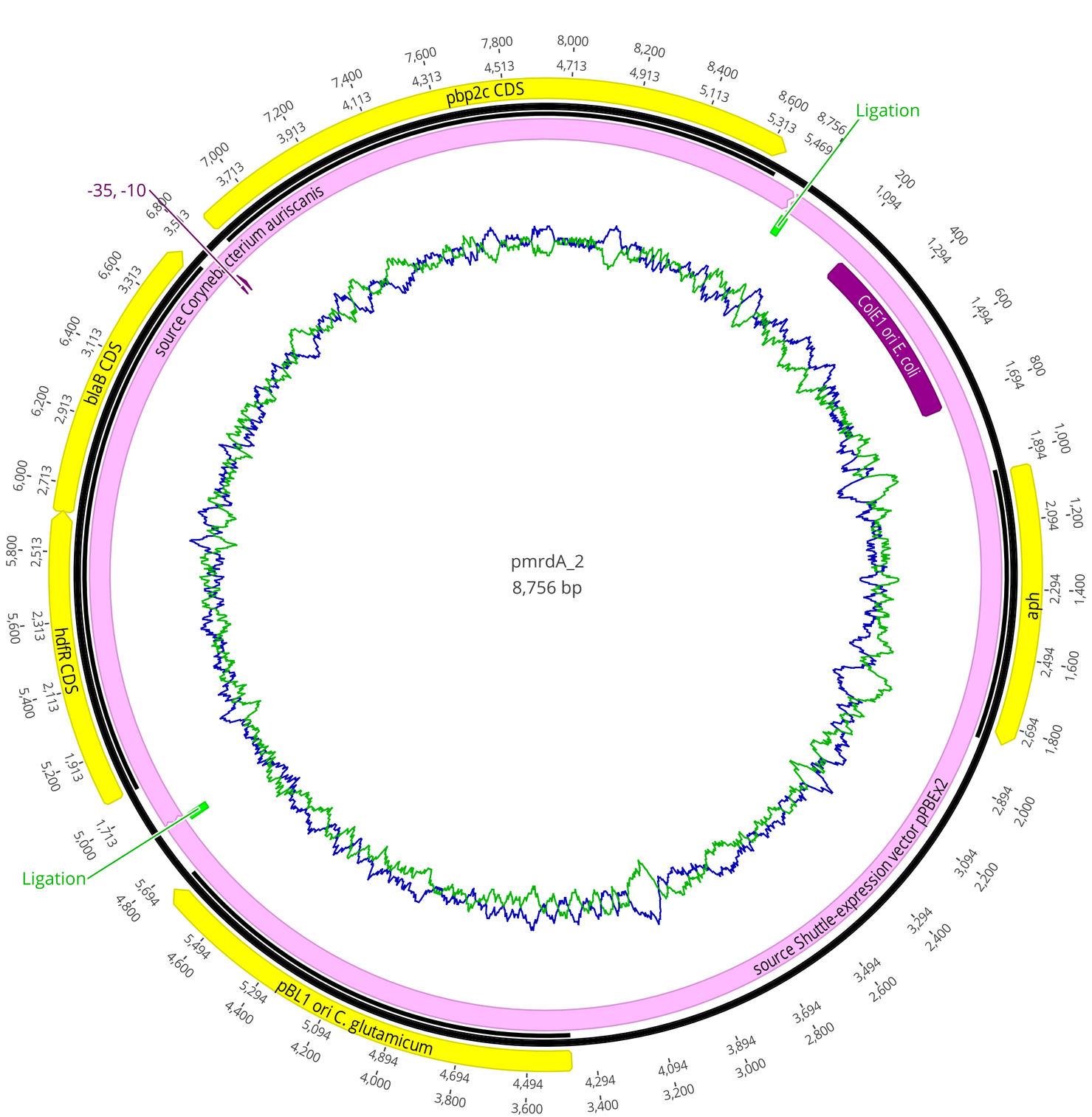
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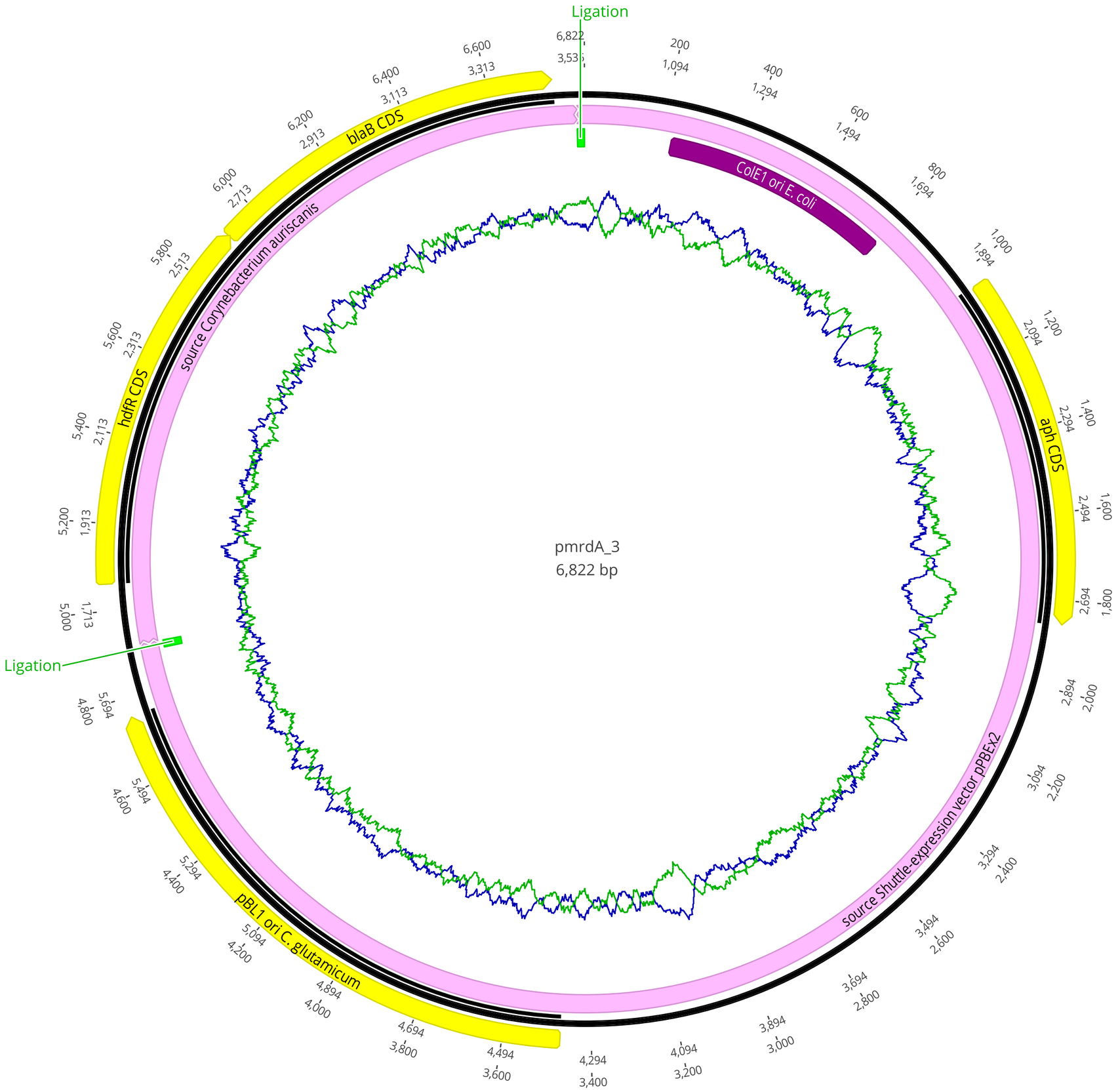
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3,094
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3,294
2,600
3,494
2,800
3,694
3,000
3,894
3,200
4,094
3,400
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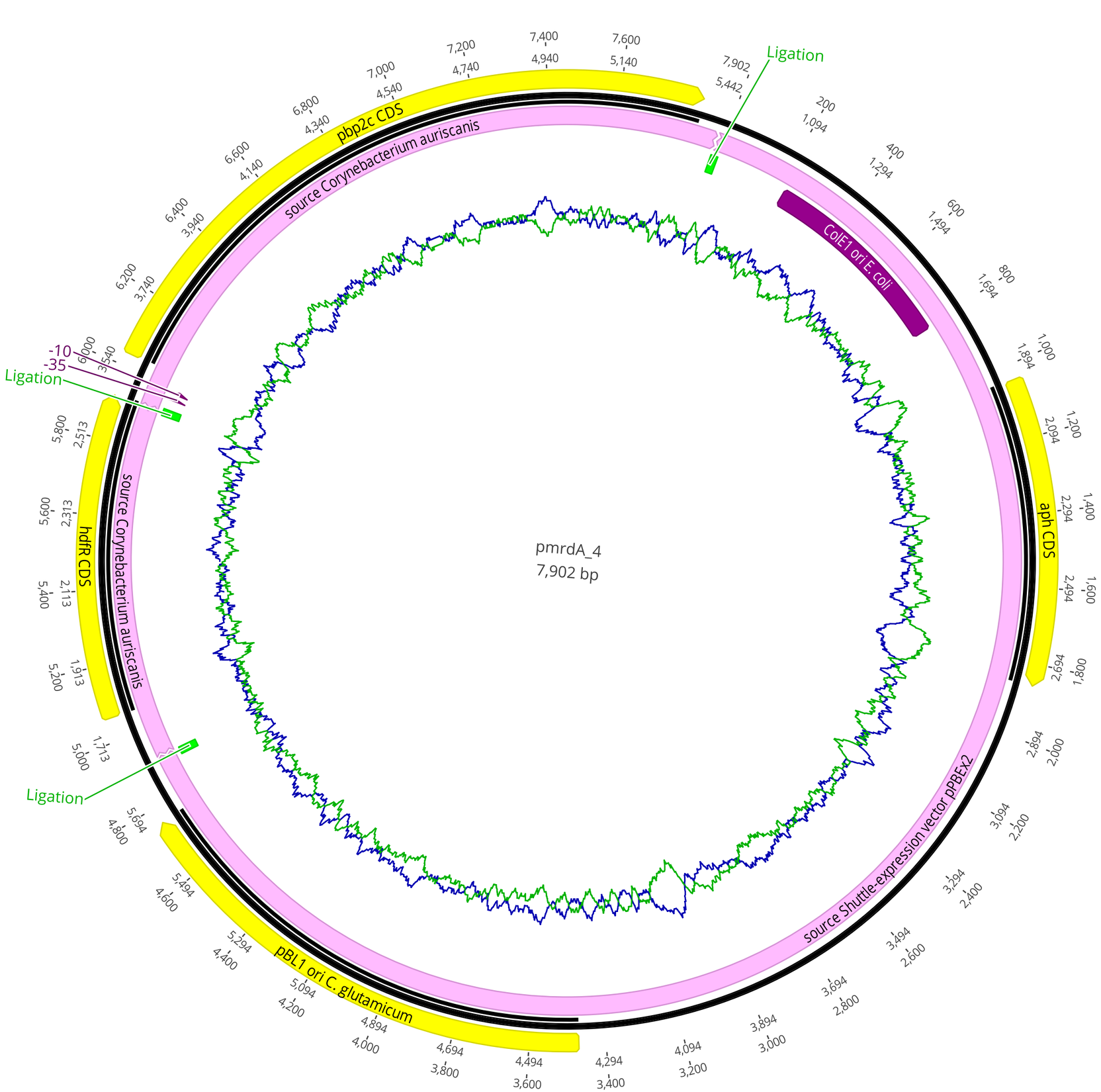
4,694
4,000
4,894
4,200
5,094
4,400
5,294
4,600
5,494
4,800
5,694
5,000
1,713
5,200
1,913
5,400
2,113
5,600
2,313
5,800
2,513
6,000
2,713
6,200
2,913
6,400
3,113
6,600
3,313
blaB CDS
ColE1 ori E. coli
aph CDS
source Shuttle-expression vector pPBEx2
pBL1 ori C. glutamicum
hdfR CDS
source Corynebacterium auriscanis
pmrdA_3
6,822 bp
Ligation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source Corynebacterium auriscanis
ColE1 ori E. coli
aph CDS
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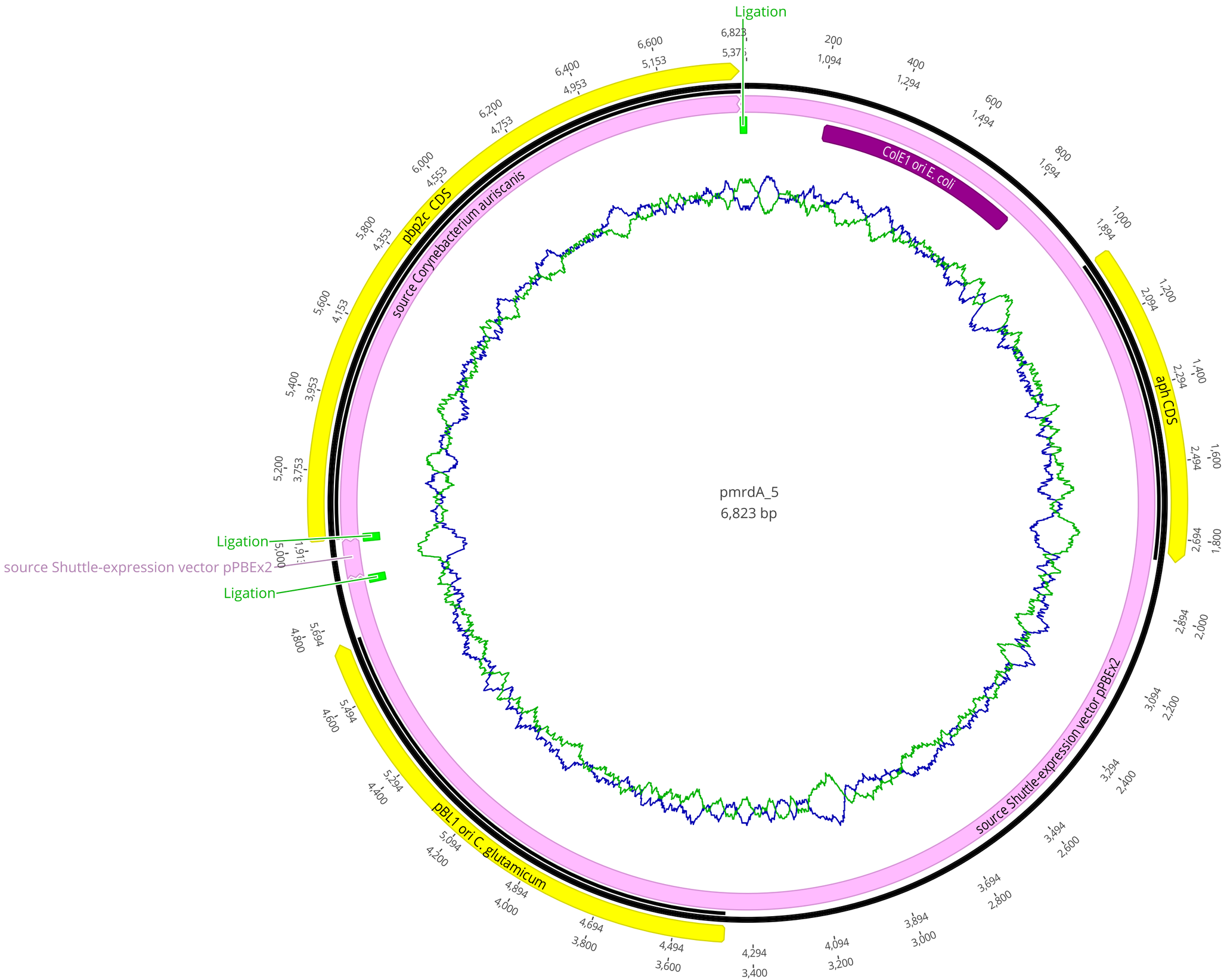
source Corynebacterium auriscanis
hdfR CDS
-10
-35
Ligation
Ligation
Ligation
pmrdA_4
7,902 bp


Ligation
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200
1,094
400
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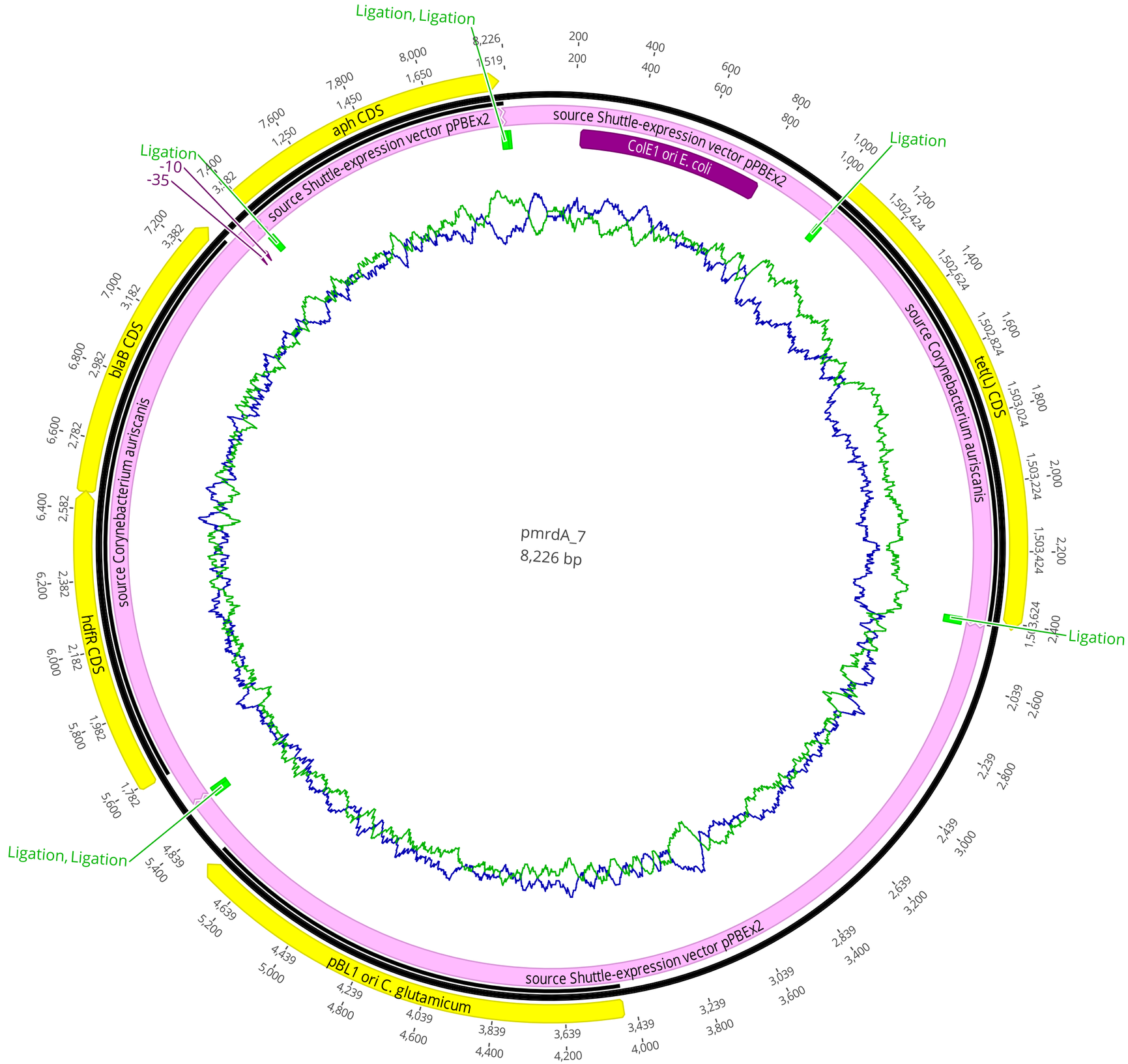
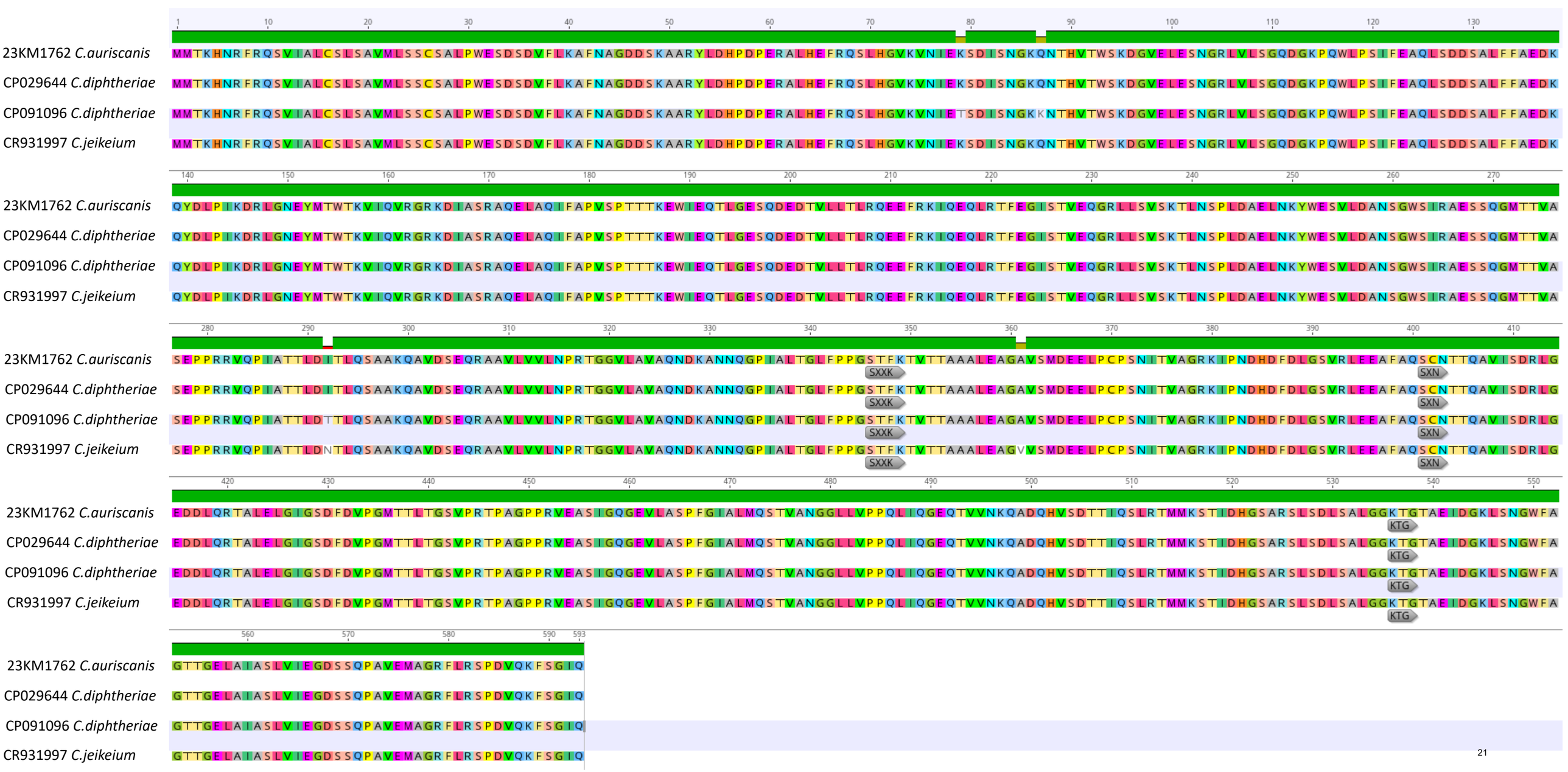
3,953
5,600
4,153
5,800
4,353
6,000
4,553
6,200
4,753
6,400
4,953
6,600
5,153
pbp2c CDS
source Corynebacterium auriscanis
ColE1 ori E. coli
aph CDS
source Shuttle-expression vector pPBEx2
pBL1 ori C. glutamicum
pmrdA_5
6,823 bp
Ligation
source Shuttle-expression vector pPBEx2
Ligation


pmrdA_7
8,226 bp
Ligation, Ligation
Ligation
-10
-35
Ligation
Ligation, Ligation
Ligation
aph CDS
source Shuttle-expression vector pPBEx2
ColE1 ori E. coli
source Shuttle-expression vector pPBEx2
tet(L) CDS
source Corynebacterium auriscanis
source Shuttle-expression vector pPBEx2
pBL1 ori C. glutamicum
hdfR CDS
source Corynebacterium auriscanis
blaB CDS
source Corynebacterium auriscanis


23KM1762 C.auriscanis
CP029644 C.diphtheriae
CP091096 C.diphtheriae
CR931997 C.jeikeium
SXXK
SXN
KTG

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Declaration of Originality

I hereby declare that I have written this work independently and that I have not used any sources other than those indicated. All passages, whose wording or meaning was taken from any sources, are all cited and distinctly noted as such.
I am aware that, should the foregoing not be true, the University Senate can revoke the doctor title, pursuant to Article 36(1)(r) of the University Act of September 5, 1996 (UniG) and Article 69 of the University of Bern Bylaws of June 7, 2011 (UniSt). For the purpose of grading and verifying compliance with this Declaration of Independent Work and with the University's Regulations on Plagiarism, I hereby grant the University of Bern the right to process the necessary personal data and to use the dissertation, in particular to make copies, to store it permanently in a database, and to use it to verify the independence of works by others or to make it available for such purpose.

Place, date	Signature of doctoral candidate
Bern, 20.06.2025	N. Gras

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