Title:

The TetR-type transcriptional repressor RolR from *Corynebacterium glutamicum* regulates resorcinol catabolism by binding to a unique operator *rolO*

Running title:

RolR binds to *rolO* and regulates resorcinol catabolism

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Appendixes:

Table A1. Primers and dsDNA used in this study. Restriction enzyme cutting sites are underlined. Ribosome binding site is given in boldface.

Primers (5'-3')		
Q11F	GCACCCGATACTGGTTCTA	For real-time qRT-PCR analysis of *rolH*
Q11R	AGTGACAATGCCGTGAATG	
Q12F	TATGGCTGCTGAGGGAATT	For real-time qRT-PCR analysis of *rolM*
Q12R	CAGGTAGGCACCGTAGAGC	
Q13F	AAAACCCTCGCCTCAAACT	For real-time qRT-PCR analysis of *rolD*
Q13R	GTCGGTGATATGCCCAACC	
QrpoBF	CGTACTGCTGAAGGCTCTT	For real-time qRT-PCR analysis of *rpoB* as an internal control
QrpoBR	TTTGCTACACCATCGGACT	
11a	GAAGCAGGGCATAGTGGA	To amplify *rolHM* transcript with 12b
12b	CTAATTTCAGCGGCGAGA	
12c	CAGCGACTATCCGATTACG	To amplify *rolMD* transcript with 13d
13d	TCTTTGACCGTTCCCTCC	
1110F	ACACATATGCCCACGCCTTCG (*Nde*I)	To generate pET28a-*rolR*
1110R	CTTGAGCTCCCTGAGTCTGGTGCTT (*Sac*I)	
PE10	TGAAGCGTCCTTGTGCTG	For primer extension of *rolR*
PE11	ACCTCCACCGACGATGCAG	For primer extension of *rolHMD*
FP11F	CGAAGGCGTGGGCATAGGG	For DNase I footprinting of the *rolHMD* sense strand
FP11R	TTCGTAGAATGTTGGCCAC	
P10F	GGCGCCCAGACATCGGTATCGAAG (*Nar*I)	To generate pXMJ19-P_{rolR}-*lacZ*
P10R	CTGCAGCATAGGGAAAACCTTAGC (*Pst*I)	
TRC10F	TAAGAATTC**AAAGGAGGA**ATGCCCAC GCCTTCGCAGC (*Eco*RI)	To generate pTRCmob-*rolR*
TRC10R	GGCGGATCCCTACTGCGGTTTGACTGG (*BamH*I)	
For EMSA		
EMSAF1	TAGCTGATCTGCGGTG	To generate the M/F1 fragment

		with EMSAR1
EMSAR1	CGAAGTTATTGGGTGA	
EMSAF2	TGTAAAATCTGAACCCTTGTTCAT	To generate the F2 fragment with EMSAR2
EMSAR2	CTGAACAACATTATCTAGATCACA	
EMSAF3	ATTTATGAATCATGATTCAGAATG	To generate the F3 fragment with EMSAR2
EMSAR3	CATTCTGAATCATGATTCATAAAT	To generate the F4 fragment with EMSAF1
EMSAF4	AATGGGGGTATTGTAAA	To generate the F5 fragment with EMSAR3
EMSAR4	TGATTCATAAATGAACA	To generate the F6 fragment with EMSAF4
EMSAF5	AATGGGGGTATTGTAAAATCTGAACCC TTGTTCATTTATG	To generate the F7 fragment with EMSAR5
EMSAR5	CATAAATGAACAAGGGTTCAGATTTTA CAATACCCCCATT	
EMSAF6	ACCCTTGTTCATTTATGAA	To generate the F8 fragment with EMSAR6
EMSAR6	TTCATAAATGAACAAGGGT	
EMSAFU	AGGTTCCCGTTCCGATGTTT	To generate the U fragment with EMSARU
EMSARU	CTAAGGTTTTCCCTATGCCC	
EMSAFD	GATCCCATGTCACCCAATA	To generate the D fragment with EMSARD
EMSARD	CCAATCGACTTCCAAAAGC	
dsDNA in SPR		
REP1	TGAACCCTTGTTCA ACTTGGGAACAAGT	The 1st inverted repeat
REP2	TTCATTTATGAA AAGTAAATACTT	The 2nd inverted repeat
REP3	TGAATCATGATTCA ACTTAGTACTAAGT	The 3rd inverted repeat
rolO	AAAATCTGAACCCTTGTTCATTTATGAAT TTTTAGACTTGGGAACAAGTAAATACTTA	The operator *rolO*

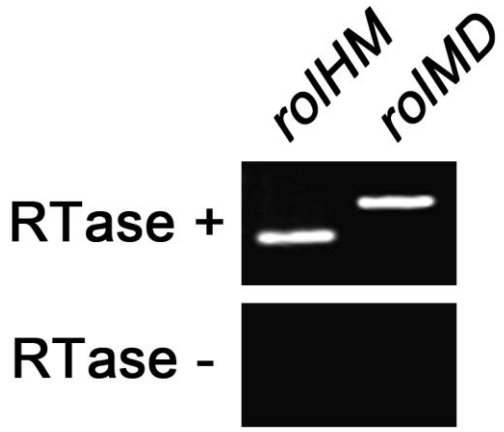
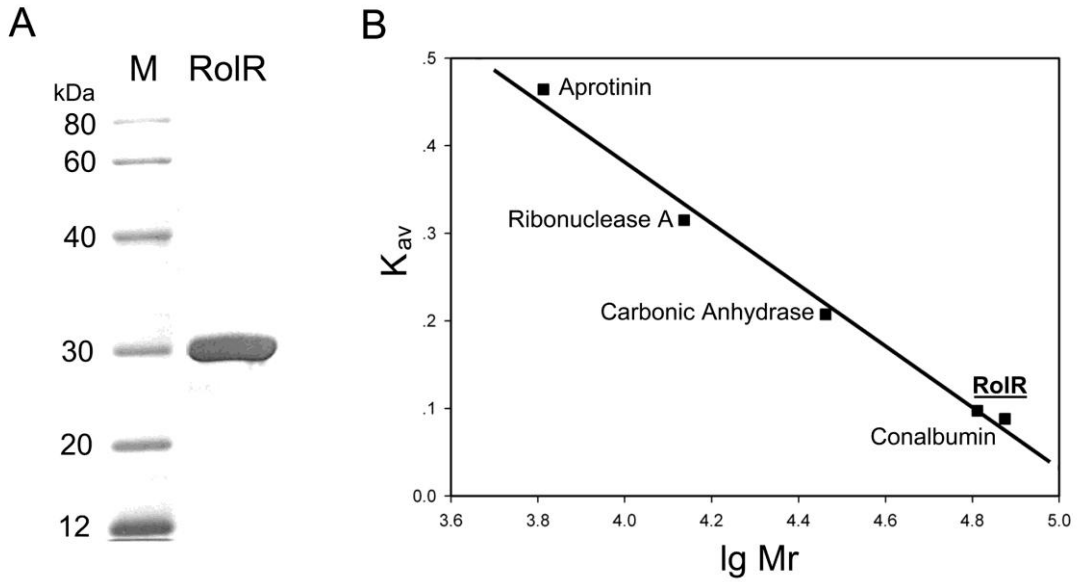


Figure A1. RT-PCR analysis of the co-transcription of genes *rolH*, *rolM*, and *rolD*. RTase+ indicates addition of reverse transcriptase and RTase- indicates no addition of reverse transcriptase. The primers inside the coding region of genes were used to amplify the fragments spanning the intergenic regions of *rolH* and *rolM* (lane, *rolHM*), *rolM* and *rolD* (lane, *rolMD*).



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36 **Figure A2.** Purification of His_6-RolR and determination of its native molecular mass.
37 **A:** Coomassie-stained SDS-PAGE of His_6-RolR purified by Ni^{2+}-NTA
38 chromatography and FPLC. **B:** Determination of the native molecular mass by gel
39 filtration. For calibration, a premixed protein molecular mass marker containing the
40 following proteins was used: Aprotinin (6,500 Da), Ribonuclease A (13,700 Da),
41 Carbonic anhydrase (29,000 Da), Conalbumin (75,000 Da). V_o was determined with
42 blue dextran (2,000 kDa).

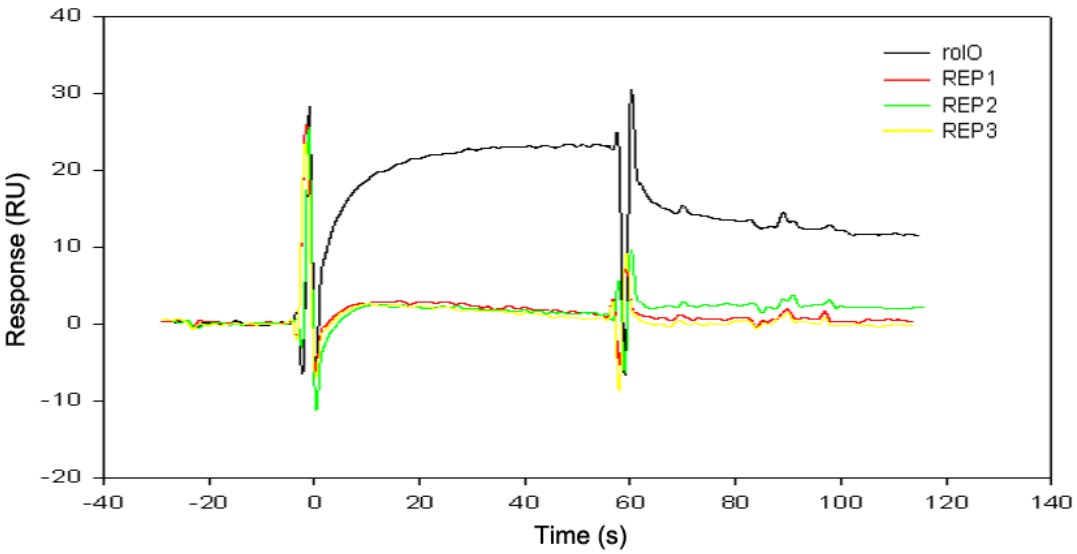

Figure A3. Surface Plasmon Resonance assay of RolR binding to different dsDNA fragments. His_6-RolR was immobilized on a CM5 sensor chip and the individual dsDNA fragment (50 µM, 20 µl) was injected at a flow rate of 20 µl/min.

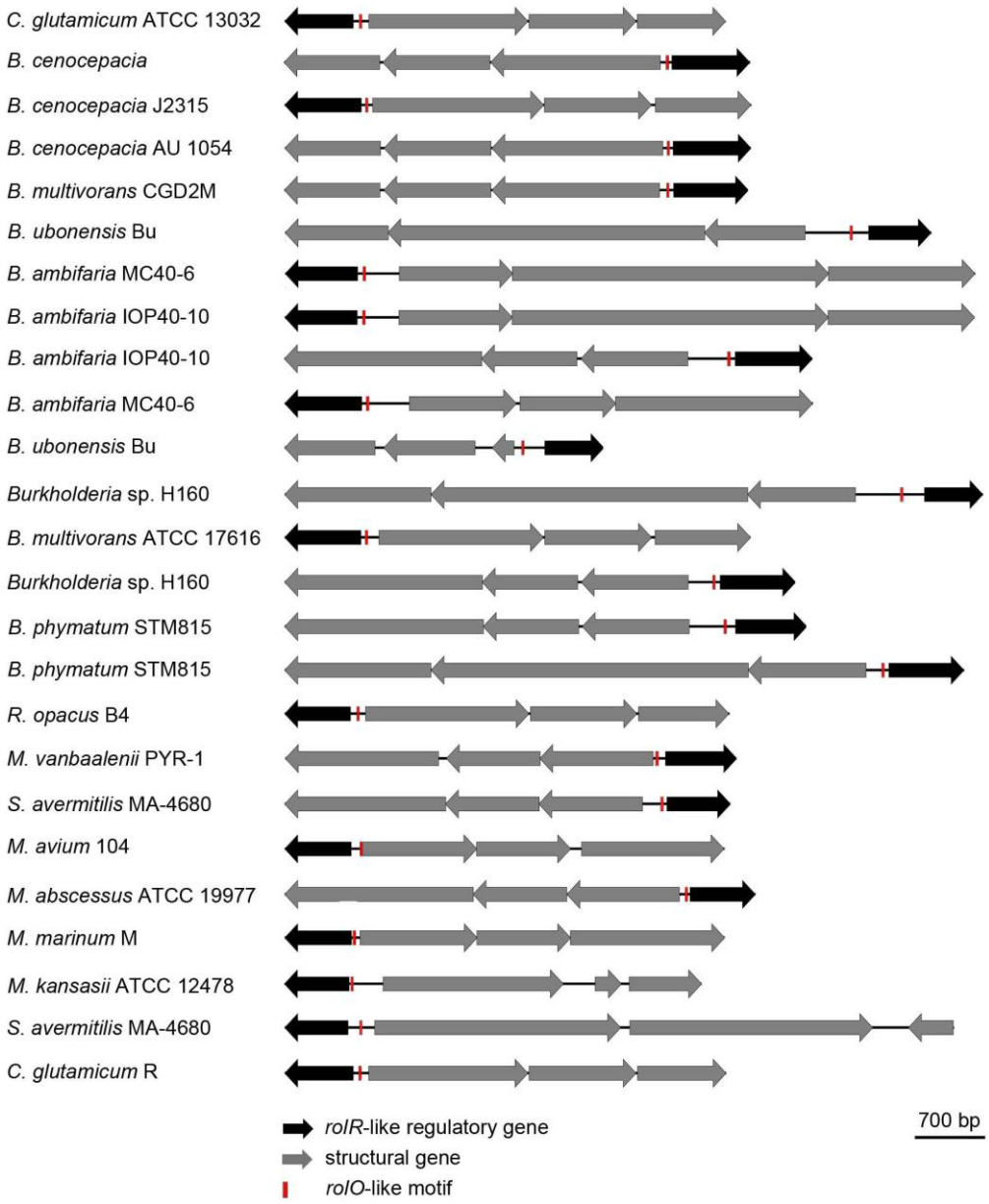

Figure A4. Genome data-mining of *rolR*-like genes and *rolO*-like motifs (red tangles) from species of *Corynebacterium*, *Burkholderia*, *Mycobacterium*, *Rhodococcus*, and *Streptomyces*. The lengths and distances of gene coding regions are drawn to scale based on genome annotation.