

Transcriptional analysis of the *gum* gene cluster from *Xanthomonas oryzae* pathovar *oryzae*

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Abstract Genome sequence analysis of *Xanthomonas oryzae* pv. *oryzae* KACC10331 provides insight into the *X. oryzae* *gum* gene cluster that is composed of 14 open-reading frames (ORFs), designated *gumB*, *-C*, *-D*, *-E*, *-F*, *-G*, *-H*, *-I*, *-J*, *-K*, *-L*, *-M*, XOO3167, and *-N*. We analyzed the transcriptional linkage of the *X. oryzae* *gum* gene cluster by using RT-PCR. Analyses of the *gum* gene cluster by RT-PCR with the wild-type and mutant strains, which carried a deletion of the promoter-like region upstream of *gumB* or an insertion of the *rrnB* transcriptional terminator into the *gumF* gene, revealed that the ORFs of this gene cluster were transcribed as polycistronic mRNA, from *gumB* to *gumN*, and the secondary promoter was located upstream of *gumG*. Taken together, these results suggest that the genes of this cluster constitute an operon expressed from overlapping transcripts.

Keywords *gum* gene cluster · operon · RT-PCR · xanthan · *Xanthomonas oryzae*

Introduction

Xanthomonas oryzae pv. *oryzae* (*X. oryzae*) is a Gram-negative bacterium that causes blight on rice and produces copious amounts of exopolysaccharides, mainly xanthan gum, as a virulence factor. *X. oryzae* also can be used for industrial production of xanthan gum which has a variety of commercial applications as a speciality polymer (Becker et al. 1998). The *gum* gene cluster of *Xanthomonas campestris* pv. *campestris* (*X. campestris*) is mainly expressed as an operon from *gumB* to *gumM*, and is involved in xanthan gum biosynthesis (Katzen et al. 1996, 1998, 1999). Recent studies have also shown that a Tn5 insertion in the *gum* gene cluster of *X. oryzae* is associated with a loss of xanthan gum biosynthesis and that the *gum* gene cluster of *X. oryzae* is required for xanthan gum biosynthesis in a xanthan gum-deficient mutant (Dharmapuri and Sonti 1999). However, the *gum* gene cluster of *X. oryzae* has not been characterized at the molecular level. More recently, the complete genome sequence of *X. oryzae* KACC10331 was published (Lee et al. 2005), and a cluster of open-reading frames (ORFs) was identified in the *X. oryzae* genome that consisted of a tandem array of 14 ORFs, *gumB*, *-C*, *-D*, *-E*, *-F*, *-G*, *-H*, *-I*, *-J*, *-K*, *-L*, *-M*, XOO3167, and *-N*, which was similar to the *gum* operon of *X. campestris*. In both species, ORFs

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of the *gum* gene cluster were arranged in a similar genome structure.

As a first step toward understanding the molecular function of the *gum* gene cluster in *X. oryzae*, we analyzed its transcriptional organization by using RT-PCR and constructing mutants carrying a deletion of the promoter-like region upstream of the *gumB* or *gumF::T_{rrnB}* transcriptional block in association with plasmid integration mutagenesis.

Materials and methods

Bacterial strains and growth conditions

Escherichia coli DH5 α (Gibco BRL) (F^- *recA1 endA1 hsdR17*[r_{k}^- m_{k}^+] *supE44 thi-1 gyrA relA1*) was used for all recombinant DNA experiments that required a bacterial host. *E. coli* DH5 α cells were grown in Luria–Bertani (LB) broth or on LB-agar plates prepared as described previously (Sambrook et al. 1989). The wild-type *Xanthomonas oryzae* strain used in this study was KACC 10859. It was grown on SOC agar (Sambrook et al. 1989) and incubated at 28°C for 3–5 days. It was also cultured in NB medium (3 g beef extract and 5 g peptone/l) or XOL medium (Barreras et al. 2004) at 28°C and 200 rpm on a rotary shaker.

Site-specific gene disruption

Site-specific gene disruption was performed by using the nonreplicable integration vector, pK18mobsacB, which enables marker-free deletion of the target gene (Schäfer et al. 1994). A 2,348-bp DNA fragment containing the sequence of ORF *gumF* was amplified by PCR using *X. oryzae* KACC10859 genomic DNA as the template and primer pairs *gumF1* (5'-CTAGTCT-AGAACCGTTACTTCGATAGCGG-3'; *XbaI* restriction site is underlined) and *gumF2* (5'-CTAGTCTAGAGGAAGACCAACGTATGC-G-3'; *XbaI* restriction site is underlined). The resulting 2,348-bp PCR products were digested with *XbaI* and subcloned into the corresponding sites of pBluescript II KS+ (Stratagene). The resulting plasmid was digested with *HincII* and ligated with blunt-ended *EcoRI*-*ClaI* PCR

products of the *rrnB* terminator amplified by PCR using pTrc99A plasmid DNA (Amann et al. 1988) as the template and primer pairs *rrnB1* (5'-ACTCCATCGATGCTGTTTTGGCGGAT-GAGAG-3'; *ClaI* restriction site is underlined) and *rrnB2* (5'-CCGGAATTCAAAGGCCAT-CCGTCAGG-3'; *EcoRI* restriction site is underlined). The resulting plasmid was digested with *XbaI*, and the fragment containing the *rrnB* terminator encompassed by the sequence of ORF *gumF* was inserted into the corresponding sites of pK18mobsacB to generate the plasmid pSJ861. Plasmid pSJ861 was introduced into *X. oryzae* KACC 10859 by electroporation as described previously (Sun et al. 2003); integration of the introduced plasmid into the chromosome by a single crossover was monitored on SOC plates containing kanamycin. The single crossover was confirmed by the inability of the cells to grow on SOC plates containing 10 g sucrose/l and by diagnostic PCR (Kim and Cho 2005) using gene-specific primers outside the regions of the targeted gene. For deletion of the targeted gene and vector by a second round of a crossover, the kanamycin-resistant (Km^R) and sucrose-sensitive cells were grown overnight in the SOC medium and spread onto SOC plates containing 10 g sucrose/l. Cells growing on the plates were picked and verified for the deletion of the targeted gene and vector by diagnostic PCR using gene-specific primers outside the regions of the targeted gene.

DNA sequences located upstream (–1445 to –826) and downstream (+42 to +1177) from the ORF *gumB* in the genome of *X. oryzae* were amplified by PCR using *X. oryzae* genomic DNA as a template and primer pairs PromUF (5'-GGCCCAAGCTTAGGTGCAGTTGTTCCA-CC-3'; *HindIII* restriction site is underlined) and PromUR (5'-CCGGGGTACCGCTCCAGGCT-GGGGAATT-3'; *KpnI* restriction site is underlined), and PromDF (5'-CCGGGGTACCGCCTTGATCTGCTCGATG-3'; *KpnI* restriction site is underlined) and PromDR (5'-CTAGTCTAGAG-TCCAGCAACGTATCGGT-3'; *XbaI* restriction site is underlined), respectively. The PCR products were digested with *XbaI* and *KpnI*, and *KpnI* and *HindIII*, respectively, and were ligated into the *XbaI* and *HindIII* sites of pK18mobsacB. The resulting plasmid was introduced into *X. oryzae*

KACC 10859 by electroporation; deletion of the putative *gumB* promoter region in the genome of *X. oryzae* was monitored and verified as described above.

RT-PCR analysis

For RT-PCR experiments, total RNA was extracted from *X. oryzae* KACC10859 late-exponentially grown in XOL medium using the hot phenol method, and the extracted RNA was treated with DNase I. The DNase I-treated RNA samples were purified using an RNeasy column (Qiagen). Purified DNase I-treated RNA samples were subsequently incubated with a gene-specific primer for the *gumF*, ORF XOO3167, or *gumN* and 200 units of reverse transcriptase at 42°C for 30 min. Reverse-transcribed RNA samples were then added to the gene-specific primers (Table 1) and PCR mix. PCR was routinely carried out as described previously (Kim and Cho 2005).

Determination of xanthan

X. oryzae wild-type and mutant strains were inoculated in XOL medium and incubated for 72 h in a rotary shaker at 200 rpm and 28°C. The amount of xanthan in the supernatant was determined by the colorimetric method for estimation of pentoses and hexoses (DuBois et al. 1956), and was calculated with a standard curve constructed by using a known amount of xanthan (from Fluka Biochemika).

Results and discussion

The *gum* gene cluster of *X. oryzae* is transcribed as an operon

The *gum* gene cluster of *X. oryzae* spans nearly 16-kbp, from *gumB* through *gumN*, and the ORFs of this gene cluster are oriented in the same direction (Lee et al. 2005). RT-PCR was used to analyze the transcriptional linkage in the *gum* gene cluster; the primer pairs for each neighboring gene lying upstream of the primer used for reverse transcription were applied based on the general premise that sequences of an upstream

gene within a given transcript can be readily amplified with those of a downstream gene if these regions form a contiguous transcript. This method was used because Northern blot analysis to determine the size of specific mRNA from the gene cluster might be unsuccessful due to its likely large size. RT-PCR products generated by these primer pairs would, therefore, be contiguous and would be derived from a co-transcribed transcript.

As shown in Table 1, primer pairs for RT-PCR were then chosen to span each intergenic junction. The results from a series of control reactions confirmed the substrate quality and the specificity of the RT-PCR primers. For the negative control, no amplicons were consistently detected when total RNA that was treated with DNase I but was not reverse-transcribed served as the template, ensuring that residual genomic DNA had not contaminated the total RNA preparations. For the positive control, the presence of the expected amplicons when genomic DNA was included in the control samples demonstrated the reliability of the RT-PCR primers. As shown in Fig. 1, RT-PCR products of the predicted length were generated for all primer pairs spanning intergenic junctions from *gumB* to XOO3167. To determine whether ORF XOO3167 and *gumN* belong to the same transcriptional unit, RT-PCR experiments were performed as shown in Fig. 2. Pairs of primers specific to each individual gene (19F-14R for XOO3167; 20F-21R for *gumN*) or to the two adjacent genes (19F-20R for XOO3167-*gumN*) were chosen. RT-dependent products from each individual ORF were detected, and the expected 874-bp RT-dependent product encompassing both ORFs was obtained. These results suggest that these ORFs (*gumB* through *gumN*) are co-transcribed as a single polycistronic transcriptional unit.

However, no RT-dependent products of the predicted length were obtained when primers extending over 391-bp upstream from the predicted *gumB* start codon were used (Fig. 3). This indicates that the transcription start is located somewhere in a region between 436- and 391-bp upstream from the predicted *gumB* start codon, where putative promoter elements were identified. A comparison of the putative promoter

Table 1 Primers used for reverse transcription and subsequent PCR

Primer	ORF/specificity	Sequence (5' → 3')	Position ^a
1R	<i>gumB</i>	CTTCTCAATCTCGGCCAG	3416022–3416005
2F	<i>gumB</i>	GAGAAAATGGTGGCCGAC	3416280–3416263
2R*	<i>gumC</i>	GTCCAGCAACGTATCGGT	3415414–3415397
3F	<i>gumC</i>	TGTCGGAATCAAGGCCGA	3415070–3415053
3R	<i>gumD</i>	GATTCCGGTAAGCGACCAG	3414085–3414068
4F	<i>gumD</i>	ACCACTACATGCAGCGTC	3412965–3412948
4R	<i>gumE</i>	AACCCGAGACAAACCACC	3411506–3411489
5F	<i>gumE</i>	TTTGCTTGGTCTGGGCTC	3412498–3412481
5R	<i>gumF</i>	GGCATGGCAGAACCACC	3411253–3411236
6F	<i>gumF</i>	GTTGGCATTAGCGGTAGC	3410669–3410652
6R	<i>gumG</i>	ATCCCAACATCCATGGCG	3409223–3409206
7F	<i>gumG</i>	CGCATAACGTTGGTCTTCC	3409376–3409359
7R	<i>gumH</i>	AAAAACCCGCCATGCGTG	3408777–3408760
8F	<i>gumH</i>	ATCAAGTGCAGGCGCTTG	3408147–3408130
8R	<i>gumI</i>	TCGCATTGATGCGGATCC	3407571–3407554
9F	<i>gumI</i>	TGCTGGCCTATGTGGAAG	3407299–3407282
9R	<i>gumJ</i>	ATACTGCGTGGTCATGCC	3406507–3406490
10F	<i>gumJ</i>	TATTACGCATCCATCACGG	3405870–3405853
10R	<i>gumK</i>	GACGATCACATCCGACTC	3404994–3404977
11F	<i>gumK</i>	CATCACCGATCAACTGGC	3405259–3405242
11R	<i>gumL</i>	GCCCAACTGAAGACACTC	3403422–3403405
12F	<i>gumL</i>	CTGGCGTGAATATGGGAG	3404051–3404034
12R	<i>gumM</i>	GTATTGGCGAAGAACACCC	3403165–3403148
13F	<i>gumM</i>	CATGGCTTGATTCTCGCC	3403560–3403543
13R	XOO3167	AATGCCACGTCTCTTCGG	3402412–3402395
14R*	XOO3167	CTCGTCGAGCAGCGCAA	3402100–3402083
14F	Upstream of <i>gumB</i>	TGGTAGCGCACTAGTCTG	3417105–3417088
15F	Upstream of <i>gumB</i>	CGGTCTGTTCATGGGCTTT	3417028–3417011
16F	Upstream of <i>gumB</i>	CGGCAGATGTAGATCGGC	3416965–3416948
17F	Upstream of <i>gumB</i>	CCAGCTCGCTATGGACTT	3416899–3416882
18F	<i>T_{rrnB}</i>	GCTGTTTTGGCGGATGAGAG	
19R*	<i>gumF</i>	GCTACCGCTAATGCCAAC	3410669–3410652
19F	XOO 3167	TGATCCACGCGCTGGACC	3402471–3402454
20F	<i>gumN</i>	GTGGAAGGTCAGCAAGGG	3401800–3401783
20R	<i>gumN</i>	CAACAAGGTCAGCCTGCC	3401615–3401598
21R*	<i>gumN</i>	CTCAACCAATGGTCACGC	3401066–3401049

Reverse primers are marked by an asterisk

^a Numerical position on *X. oryzae* KACC10331 genome (GenBank accession no. AE013598)

region mapped by RT-PCR analysis with the consensus *Escherichia coli* σ^{70} promoter elements revealed similarities: 4 of 6 bp in –35 (CTGTCA vs. TTGACA) and 4 of 6 bp in –10 (AATATT vs. TATAAT). *E. coli* consensus hexamers were conserved in this region. There were also three putative stable RNA secondary structures found at the –416/–425, –438/–464, and –503/–523 regions, respectively. We do not know yet if these regions are involved in any regulatory processes; however, the long leader sequence and the presence of palindromic sequences in the putative promoter region suggest that some type of control may occur at the posttranscriptional level.

The *gum* gene cluster of *X. oryzae* constitutes an operon expressed from overlapping transcripts

Further experiments were performed to confirm that *gumB* through *gumN* belong to the same transcriptional unit, constituting an operon involved in xanthan biosynthesis. A mutant of *X. oryzae* KACC10859 was constructed by integrating the *rrnB* transcriptional terminator into the *gumF* gene to block transcriptional activity of this operon, as described in Materials and methods. RT-PCR was performed on total RNA isolated from the mutant strain. As expected, RT-PCR

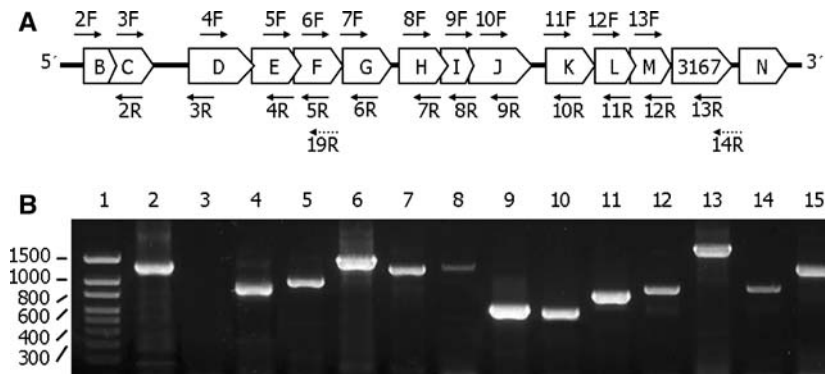


Fig. 1 Analysis of the *gum* gene cluster of *X. oryzae* by RT-PCR. **(A)** Schematic representation of the *X. oryzae* *gum* gene cluster, along with the approximate position and orientation of the gene-specific primers used for generating the cDNA (dotted arrows) and the subsequent PCR amplification step (black arrows). The thick black line represents the DNA sequence, and the overlying white arrows represent the ORFs. **(B)** RT-PCR products from primers designed to span the intergenic junctions from *gumB* through ORF XOO3167. The cDNA fractions obtained with the 19R RT primer were used for the

subsequent PCR reactions with primer pairs, 2F-2R, 3F-3R, 4F-4R, and 5F-5R (lanes 4, 5, 6, and 7, respectively). The cDNA fractions obtained with the 14R RT primer were used for the subsequent PCR reactions with primer pairs, 6F-6R, 7F-7R, 8F-8R, 9F-9R, 10F-10R, 11F-11R, 12F-12R, and 13F-13R (lanes 8, 9, 10, 11, 12, 13, 14, and 15, respectively). Genome-based PCR and a negative control without reverse transcriptase amplified with the 6F-6R primer pair were included (lane 2 and 3, respectively). DNA size markers in base pairs are indicated (lane 1)

products of the predicted length encompassing *gumB* and *gumC*, *gumC* and *gumD*, *gumD* and *gumE*, and *gumE* and *gumF* could be obtained. No transcriptional readthrough from the *rrnB*

terminator integration site appeared to occur, since RT-PCR experiments using primer pairs to *rrnB* terminator and *gumG*, or *gumF* and *gumG*, respectively, yielded no products (Fig. 4B, lane 8

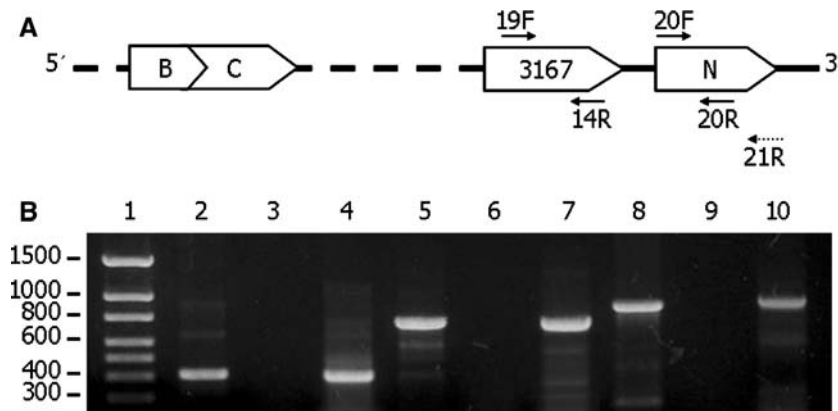


Fig. 2 RT-PCR analysis of the 3' end of the *X. oryzae* *gum* operon. **(A)** Schematic representation of the approximate position and orientation of the gene-specific primers for generating the cDNA (dotted arrow) and the subsequent PCR amplification step (black arrows) in respect to ORF XOO3167 and *gumN*. The thick black line represents the DNA sequence, and the overlying white arrows represent the ORFs. **(B)** RT-PCR products from primers designed to span the region of ORF XOO3167 and *gumN*. The cDNA fractions obtained with

the 21R RT primer were used for the subsequent PCR reactions with primer pairs, 19F-14R, 20F-21R, and 19F-20R (lanes 4, 7, and 10, respectively). Genome-based PCR products were obtained with primer pairs, 19F-14R, 20F-21R, and 19F-20R (lanes 2, 5, and 8, respectively). Controls without reverse transcriptase amplified with the primer pairs, 19F-14R, 20F-21R, and 19F-20R, were included (lanes 3, 6, and 9, respectively). DNA size markers in base pairs are indicated (lane 1)

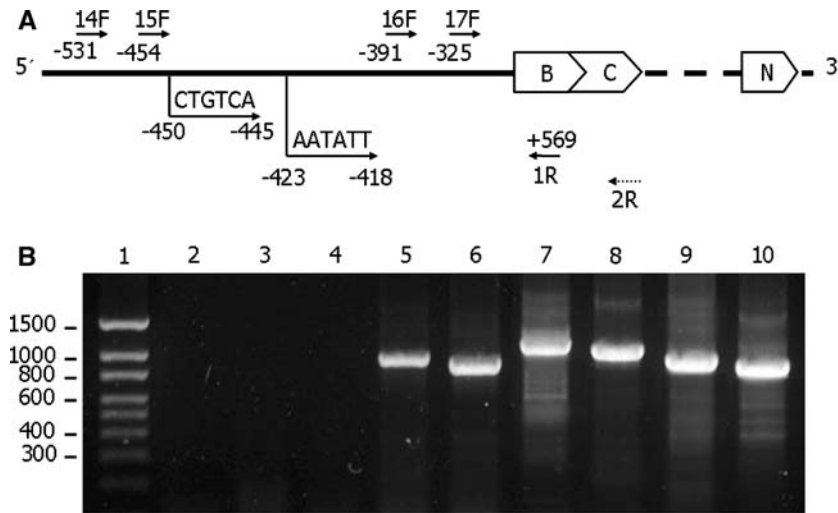


Fig. 3 RT-PCR analysis of the 5' end of the *X. oryzae* *gum* operon. **(A)** Schematic representation of the approximate position and orientation of the gene-specific primers for generating the cDNA (dotted arrow) and the subsequent PCR amplification step (black arrows) in respect to the upstream region of *gumB*. The thick black line represents the DNA sequence, and the overlying white arrows represent the ORFs. The predicted -10 and -35 consensus hexamers are indicated with arrows below the schematic. The number of the first nucleotide of the primer relative to the predicted *gumB* start codon is

indicated by arrows. **(B)** RT-PCR products from primers designed to span the upstream region of *gumB*. The cDNA fractions obtained with the 2R RT primer were used for the subsequent PCR reactions with primer pairs, 14F-1R, 15F-1R, 16F-1R, and 17F-1R (lanes 3, 4, 5, and 6, respectively). Genome-based PCR products were obtained with primer pairs, 14F-1R, 15F-1R, 16F-1R, and 17F-1R (lanes 7, 8, 9, and 10, respectively). A control without reverse transcriptase amplified with the primer pair, 14F-1R, was included (lane 2). DNA size markers in base pairs are indicated (lane 1)

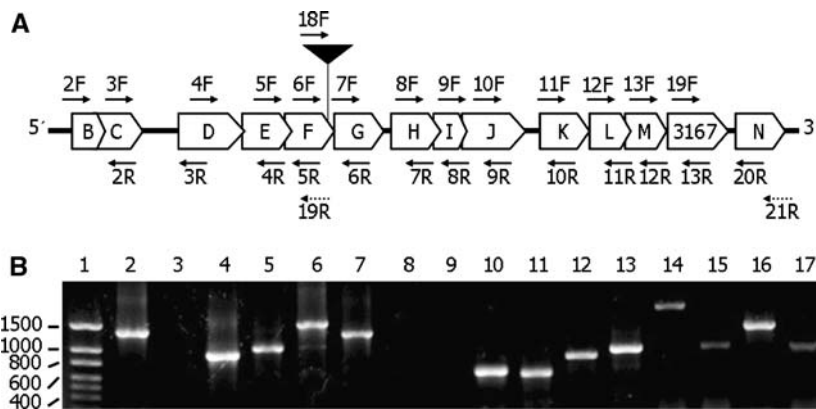


Fig. 4 Analysis of the *gum* gene cluster with an insertion of the *rrnB* terminator into the *gumF* gene by RT-PCR. **(A)** Schematic representation of the approximate position and orientation of the gene-specific primers for generating the cDNA (dotted arrows) and the subsequent PCR amplification step (black arrows). The thick black line represents the DNA sequence, and the overlying white arrows represent the ORFs. The *rrnB* terminator inserted into the *gumF* gene is indicated with a black triangle above the line. **(B)** RT-PCR products from primers designed to span the intergenic junctions from *gumB* through *gumN*. The cDNA fractions obtained with the 19R RT primer

were used for the subsequent PCR reactions with primer pairs, 2F-2R, 3F-3R, 4F-4R, and 5F-5R (lanes 4, 5, 6, and 7, respectively). The cDNA fractions obtained with the 21R RT primer were used for the subsequent PCR reactions with primer pairs, 6F-6R, 18F-6R, 7F-7R, 8F-8R, 9F-9R, 10F-10R, 11F-11R, 12F-12R, 13F-13R, and 19F-20R (lanes 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17, respectively). Genome-based PCR and a negative control without reverse transcriptase amplified with the primer pair, 6F-6R, were included (lane 2 and 3, respectively). DNA size markers in base pairs are indicated (lane 1)

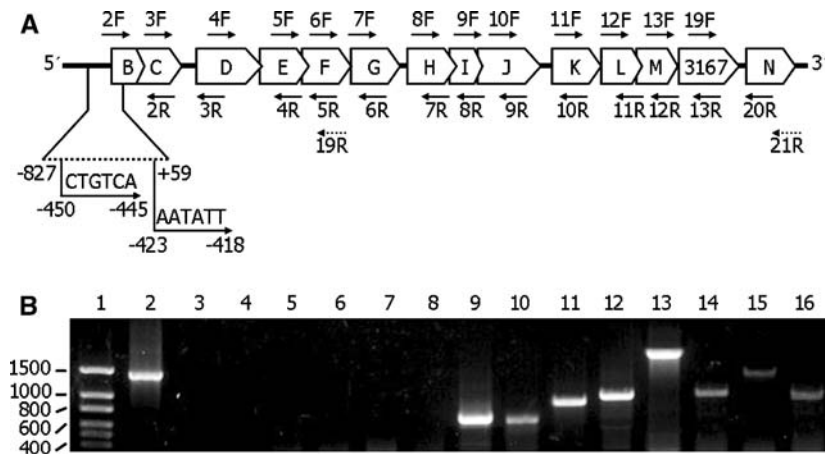


Fig. 5 Analysis of the *gum* gene cluster with deletion of the putative promoter region upstream of *gumB* by RT-PCR. **(A)** Schematic representation of the approximate position and orientation of the gene-specific primers for generating the cDNA (dotted arrows) and the subsequent PCR amplification step (black arrows). The thick black line represents the DNA sequence, and the overlying white arrows represent the ORFs. The putative promoter region upstream of *gumB* deleted in the *X. oryzae* wild-type strain was drawn out of the sequence and indicated with a dotted line below the schematic. The predicted -10 and -35 consensus hexamers are also indicated with arrows below the dotted line. The numbers of the nucleotides of the deleted region relative to the predicted *gumB* start codon are indicated by the dotted line. **(B)** RT-PCR

products from primers designed to span the intergenic junctions from *gumB* through *gumN*. The cDNA fractions obtained with the 19R RT primer were used for the subsequent PCR reactions with primer pairs, 2F-2R, 3F-3R, 4F-4R, and 5F-5R (lanes 4, 5, 6, and 7, respectively). The cDNA fractions obtained with the 21R RT primer were used for the subsequent PCR reactions with primer pairs, 6F-6R, 7F-7R, 8F-8R, 9F-9R, 10F-10R, 11F-11R, 12F-12R, 13F-13R, and 19F-20R (lanes 8, 9, 10, 11, 12, 13, 14, 15, and 16, respectively). Genome-based PCR and a negative control without reverse transcriptase amplified with the primer pair, 6F-6R, were included (lane 2 and 3, respectively). DNA size markers in base pairs are indicated (lane 1)

and 9). Surprisingly, RT-PCR products of the predicted length were generated for all primer pairs spanning intergenic junctions from *gumG* through *gumN* (Fig. 4B).

These results indicate that another type of promoter activity exists upstream of *gumG*. The effect of the integration of *rrnB* transcriptional terminator into the *gumF* gene on the transcriptional activity of *gum* operon was examined in a parallel study. The wild-type and the mutant strain that carried an insertion of *rrnB* terminator into the *gumF* gene were grown in XOL medium as described in Materials and methods. The amount of xanthan was quantified from the wild-type (positive control) and the *gumF* mutant strain. As expected, the transcriptional block of the *gumB* operon resulting from insertion of the *rrnB* terminator into the *gumF* gene had no discernible effect on xanthan accumulation. Analysis of xanthan accumulation from the mutant strain indicated that, in *X. oryzae*, the absence of

the *gumF* product, an acetyltransferase I homologue catalyzing the acetylation of the mannose residue, would not prevent assembly of xanthan, as seen in *X. campestris* (Katzen et al. 1998).

If we assume that the synthesis of xanthan in the *gumF* mutant strain requires the expression of genes downstream of *gumF*, then insertion of the *rrnB* terminator into the coding region of *gumF* would not be polar. We reasoned that this was likely to result from the presence of a secondary promoter internal to the *gum* gene cluster, particularly one that would lie upstream of *gumG*, and that it should be possible to test the requirement for secondary promoter in the transcription of genes downstream of *gumG* by constructing a *gumB* promoter deletion mutant and examining the consequence of this deletion on transcription of the *gum* gene cluster. Thus, a promoter deletion mutant carrying an 868-bp internal deletion upstream of *gumB* was constructed as described in Materials and methods. To confirm

whether all downstream genes of *gumG* could be transcribed from a secondary promoter, total RNA was extracted from the promoter deletion mutant and analyzed by RT-PCR. As shown in Fig. 5, twelve amplifications by RT-PCR covered all of the intergenic junctions of the *gum* operon, except the junctions between *gumB* and *gumG*, which confirms that the secondary promoter is located upstream of *gumG*.

The sequence comprising the intergenic junction of *gumF* and *gumG* contains a putative stable RNA secondary structure that differed from the Rho-independent terminator structures, which raised the intriguing speculation that this secondary structure may function as a decay terminator. Many RNA secondary structures serve as barriers to exonucleolytic degradation of the polycistronic mRNA by 3' to 5'-exonucleases, thereby increasing the stability of upstream mRNA. The functional significance of this transcriptional organization in the *gum* gene cluster as an overlapping transcriptional unit is not clear, but the overlapping transcriptional arrangement in the gene cluster might protect the downstream genes of *gumG* from being expressed poorly because of the processing of the primary transcript. Similar stem-loop structures that appear to impart stability to selected regions of polycistronic mRNAs have also been identified in several other bacterial operons (Newbury et al. 1987; Owolabi and Rosen 1990; Schramm et al. 1996; Khun et al. 2000; Maamar et al. 2006).

In conclusion, the results obtained suggest that the *X. oryzae gum* gene cluster exhibits an operon organization comprised of at least two overlapping transcripts. The predicted stem-loop structure of the intergenic junction of *gumF* and *gumG* may have a regulatory role and may partially account for the apparent overlapping transcriptional organization in this gene cluster. In the future, further investigations should be conducted to provide a better understanding of the proposed transcriptional organization of the *gum* gene cluster and its role in regulating the expression of the *gum* gene cluster.

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References

- Amann E, Ochs B, Abel KJ (1988) Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. Gene 69:301–315
- Barreras M, Abdian PL, Ielpi L (2004) Functional characterization of GumK, a membrane-associated β -glucuronosyltransferase from *Xanthomonas campestris* required for xanthan polysaccharide synthesis. Glycobiology 14:233–241
- Becker A, Katzen F, Pühler A, Ielpi L (1998) Xanthan gum biosynthesis and application: a biochemical/genetic perspective. Appl Microbiol Biotechnol 50:145–152
- Dharmapuri S, Sonti RV (1999) A transposon insertion in the *gumG* homologue of *Xanthomonas oryzae* pv. *oryzae* causes loss of extracellular polysaccharide production and virulence. FEMS Microbiol Lett 179:53–59
- DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
- Katzen F, Becker A, Ielmini MV, Oddo CG, Ielpi L (1999) New mobilizable vectors suitable for gene replacement in gram-negative bacteria and their use in mapping of the 3' end of the *Xanthomonas campestris* pv. *campestris gum* operon. Appl Environ Microbiol 65:278–282
- Katzen F, Becker A, Zorreguieta A, Pühler A, Ielpi L (1996) Promoter analysis of the *Xanthomonas campestris* pv. *campestris gum* operon directing biosynthesis of the xanthan polysaccharide. J Bacteriol 178:4313–4318
- Katzen F, Ferreiro DU, Oddo CG, Ielmini V, Becker A, Pühler A, Ielpi L (1998) *X. campestris* pv. *campestris gum* mutants: effects on xanthan biosynthesis and plant virulence. J Bacteriol 180:1607–1617
- Khun HH, Deved V, Wong H, Lee BC (2000) *fbpABC* gene cluster in *Neisseria meningitidis* is transcribed as an operon. Infect Immun 68:7166–7171
- Kim S-Y, Cho J-Y (2005) A modified PCR-directed gene replacements method using λ -Red recombination functions in *Escherichia coli*. J Microbiol Biotechnol 15:1346–1352
- Lee B-M, Park Y-J, Park D-S, Kang H-W, Kim J-G, Song E-S, Park I-C, Yoon U-H, Hahn J-H, Koo B-S, Lee G-B, Kim H, Park H-S, Yoon K-O, Kim J-H, Jung C-H, Koh N-H, Seo J-S, Go S-J (2005) The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice. Nucleic Acids Res 33:577–586
- Maamar H, Abdou L, Boileau C, Valette O, Tardif C (2006) Transcriptional analysis of the *cip-cel* gene cluster from *Clostridium cellulolyticum*. J Bacteriol 188:2614–2624
- Newbury SF, Smith NH, Higgins CF (1987) Differential mRNA stability controls relative gene expression within a polycistronic operon. Cell 51:1131–1143

- Owolabi JB, Rosen BP (1990) Differential mRNA stability controls relative gene expression within the plasmid-encoded arsenical resistance operon. *J Bacteriol* 172:2367–2371
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Schäfer A, Tauch A, Jäger W, Kalinowski J, Thierbach G, Pühler A (1994) Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene* 145:69–73
- Schramm H-C, Schneppe B, Birkenhäger R, McCarthy JEG (1996) The promoter-proximal, unstable IB region of the *atp* mRNA of *Escherichia coli*: an independently degraded region that can act as a destabilizing element. *Biochim Biophys Acta* 1307:162–170
- Sun Q, Wu W, Qian W, Hu J, Fang R, He C (2003) High-quality mutant libraries of *Xanthomonas oryzae* pv. *oryzae* and *X. campestris* pv. *campestris* generated by an efficient transposon mutagenesis system. *FEMS Microbiol Lett* 226:145–150