INTRODUCTION

To maintain regular protein synthesis, bacterial cells require a constant supply of amino acids. Most achieve this by taking up amino acids from the surrounding environment and/or synthesizing amino acids from simpler compounds (i.e. de novo synthesis). The synthesis of amino acids is considered a complicated and biologically expensive process and is therefore strictly regulated. It is known that among the efficient mechanisms of regulation is feedback control at the transcriptional or post-transcriptional level [1]. Of the 20 standard amino acids which are needed for bacterial cell viability, the sulfur-containing methionine [(formyl-)]methionine] is somewhat unique. This is because it is the first amino acid at the N-terminus of most proteins and plays an irreplaceable role in the initiation of protein biosynthesis. In addition, the methionine derivative S-adenosylmethionine (SAM) serves as a universal methyl group donor in a variety of methyltransferase reactions [2].

Most bacterial species, with the exception of some endosymbionts, are able to synthesize methionine using the trans-sulfuration pathway and/or the direct sulffhydrylation pathway from homoserine [3]. The trans-sulfuration pathway in *Escherichia coli* has been extensively characterized as having four steps: homoserine → O-succinyltransferase → cystathionine → homocysteine → methionine, which are catalysed by homoserine O-succinyltransferase (encoded by *metA*), cystathionine γ-synthase (encoded by *metB*), cystathionine β-lyase (encoded by *metC*) and methionine synthase (encoded by *metH or metE*), respectively [4] (Fig. 1). Furthermore, it is defined that the homoserine is derived from aspartate in three steps: aspartate → aspartyl phosphate → aspartate semialdehyde → homoserine, which are catalysed by bi-functional aspartate kinase/homoserine dehydrogenase (encoded by *metL*), aspartate-β-semialdehyde dehydrogenase (encoded by *asd*) and homoserine dehydrogenase (encoded by *hom*), respectively [5] (Fig. 1).

The way in which methionine biosynthesis is regulated has also been characterized in certain Gram-negative and -positive bacteria. The general principle for this is similar to the biosynthesis of most other amino acids, where methionine...
biosynthesis is inactive when methionine is available in the surroundings and active when methionine is limited. The availability of cellular methionine has an effect on the expression of the genes involved in methionine biosynthesis (most of these were called met genes). However, the detailed molecular basis of met gene regulation differs among bacteria, most likely due to the environment in which they are found. In general, Gram-positive bacteria utilize RNA regulators (riboregulators) that function at the post-transcriptional level, for example the S-box and T-box riboswitches. Conversely, Gram-negative bacteria employ protein regulators that function at the transcriptional level [6–9].

Although extensive studies have been carried out on E. coli and some other Gram-negative bacteria [4, 10], little is known about methionine biosynthesis and its regulation in Gram-negative phytopathogenic bacteria [11–13]. Xanthomonas campestris pv. campestris (Xcc) is a member of the large genus Xanthomonas, which comprises 27 species of Gram-negative bacteria, most of which are plant pathogens. As well as being an important plant pathogen, Xcc is considered a model pathogen for studying the molecular basis in microbe–plant interactions [14]. In the current study, we reconstructed the methionine biosynthesis pathway in an Xcc strain by integrating the genome information from Xcc and what is known regarding the E. coli methionine biosynthesis route. Using this information, we demonstrated that the synthesis of methionine is critical for Xcc to attain regular growth and full virulence in host plant tissues. Additionally, we identified the met operon consisting of three open reading frames (ORFs) that encode enzymes potentially involved in catalysing methionine biosynthesis. More importantly, we obtained direct evidence showing that the 5′UTR, but not the promoter of the met operon, is involved in feedback regulation of its expression in response to methionine availability. To the best of our knowledge, this provides the first example of a Gram-negative bacterium using 5′UTR to regulate methionine biosynthesis.

**Fig. 1.** Genetic organization and identification of Xcc met operon. (a) A schematic diagram showing the genetic organization of the met operon (XC1251-NC1252-NC1253). The sequences matched to the probes used in the Northern blotting analysis are indicated by thick, red lines above the genes. (b) Predicted methionine biosynthesis pathway in Xcc. (c) Detection of transcripts of the met operon by Northern blotting.

Zhang et al., Microbiology 2018;164:1146–1155
METHODS

Bacterial strains, plasmids and growth conditions

The bacterial strains and plasmids used in this work are listed in Table S1 (available in the online version of this article). Xcc and E. coli strains were grown under conditions described previously [15].

Construction of the mutant strain 1201PK2

The mutant 1201PK2 was constructed by the homologous suicide plasmid integration method as described by Windgassen and associates [16]. A 392 bp internal fragment of XCI251 was amplified using the DNA of the Xcc wild-type strain 8004 as template and the primer pair 1251-M-F/1251-M-R (Table S2). The DNA fragment was cloned into the suicide plasmid pK18mob, creating pK1251, which was introduced into strain 8004 by conjugation. The mutant transconjugants obtained with an insertion in XCI251 were confirmed by PCR using the primer pair P18con-F/1251con-R (Table S2). One of the confirmed mutants, named 1201PK2, was used for further study.

Determination of the transcription start site (TSS) of the met operon (XCI251-XC1253) by 5’-RACE

Total RNA was extracted from the wild-type strain 8004 and treated with DNase I. Five micrograms of the DNA-free RNA and 15 pmol of the gene-specific primer 1251GSP1 (CAACAGCGGTGTGACGAC) were incubated at 70 °C for 5 min and then 42 °C for 1 h in the presence of 1×M-MuLV-RT buffer, 1 mM dNTPs, 20 U RNase inhibitor and 200 U M-MuLV Reverse Transcriptase. After treatment with 2 U μl−1 RNase H for 30 min (to remove the remaining RNAs), the reaction product (cDNA) was purified using S.N.A.P. Column and finally resolved in 50 μl sterilized H2O. Then, 10 μl purified cDNA was incubated at 37 °C for 10 min in the presence of 0.2 mM dCTP and 20 U of TDT (terminal deoxynucleotidyl transferase) to add a poly (C) tail to the 3’-end of the cDNA. Finally, 5 μl poly(C) tail cDNA was used as template for PCR. To improve sensitivity, two rounds of semi-nested PCR were performed. The first of these was performed using 5 μl poly(C) tail cDNA as template, and AAP (GGCCACGCGGTGCAGACTAG TACGGGGIIGGGIGGGIIG) and an internal oligonucleotide, 1251GSP2 (CGCCGCGATCGCGGCTGCGCAATTGC) as primers; the second was performed using the product from the first PCR as template, and AUAP (GGCCACGCG TGACTAGTAC) and another internal oligonucleotide, 1251GSP3 (ATGCGCCGCCGCAACTGCAAC) as primers. After gel purification, the final PCR products were cloned into the pMD18-T vector and sequenced.

Construction of strain XC1251-3F expressing the recombinant protein XC1251-3FLAG

The strain XC1251-3F (Table S1) expressing a recombinant XC1251 protein (XC1251-3FLAG) with a 3×Flag tag at its C-terminus was constructed by in-frame insertion of the 3×Flag-coding sequence into the 3’-end (just before the stop codon TGA) of the XC1251 coding sequence in strain 8004, using the suicide vector-mediated unmarked allelic replacement method described by Patey et al. [17]. Briefly, a 781 bp DNA upstream flanking sequence of XCI251 and a 607 bp DNA fragment containing the 3×Flag-coding sequence, the stop codon TGA and downstream flanking sequence of XCI251 were generated by PCR amplification using the DNA of strain 8004 as template and the primer pairs 1251-3F-U-F/1251-3F-U-R and 1251-3F-D-F/1251-3F-D-R (Table S2), respectively. The DNA fragments thus obtained were linked by fusion PCR and cloned into the suicide plasmid pK18mobScb, generating the recombinant plasmid pK1251-3F, which was then transferred into strain 8004 by conjugation. Single-crossover integration transconjugants were selected and confirmed by sucrose-sensitive phenotype. A confirmed single-crossover transconjugant was checked for the in-frame insertion of 3×Flag-coding sequence before the stop codon of XC1251 by sequencing analysis of the PCR products generated by amplification of its genomic DNA with the primer pair 1251-3F-U-F/1251-3F-D-R (Table S2). The expression of XC1251-3FLAG protein was further confirmed by Western blotting.

Construction of promoter-gusA and 5’UTR-gusA fusion reporters

Two promoter-gusA transcriptional fusion reporter plasmids, named pP1251L and pP1251S, were constructed by fusing 109 and 52 bp DNA sequences upstream of the TSS with the 1826 bp DNA segment of the promoterless but SD-containing gusA gene of E. coli, respectively, and cloning the 1935 and 1878 bp fused DNA fragments thus obtained into the promoterless cloning sites of the vector pLAFR6, respectively. The 1935 and 1878 bp fused DNA fragments were obtained by PCR amplification using E. coli K12 genomic DNA as template and the primer pairs P1251L-SDgusA-F/P1251L-SDgusA-R and P1251S-SDgusA-F/P1251S-SDgusA-R (Table S2), respectively.

The 5’UTR of XC1251-XC1253 operon-gusA transcriptional fusion reporter p5UTR-SD+ and translational fusion reporter p5UTR-SD− were constructed by cloning the Plac5’UTR-SD+gusA and Plac5’UTR-SD−gusA fragment into the vector pLAFR6, respectively. Plac5’UTR-SD+gusA and Plac5’UTR-SD−gusA fragments were generated by ligation of the 235 bp DNA fragment Plac5’UTR with the 1847 bp DNA fragment SD+gusA and 1829 bp DNA fragment SD−gusA by fusion PCR, respectively. Plac5’UTR was obtained by PCR amplification using the DNA of strain 8004 as template and the primer pair Plac5’UTR-F/Plac5’UTR-R (Table S2), and SD+gusA and SD−gusA were obtained by PCR amplification using the DNA of E. coli K12 as template and the primer pairs SD+gusA-F/SD+gusA-R and SD−gusA-F/SD−gusA-R (Table S2), respectively.

Northern blotting and Western blotting

Xcc cells were cultured under specific growth conditions and collected at 24 h post-inoculation. Total RNA was isolated using the PureLink RNA Mini kit (Thermo Fisher Scientific, Shanghai, China), and 3–5 μg RNA was separated
on 6% denature (8 M urea) polyacrylamide gel and transferred to a positively charged nylon membrane (Roche Applied Science, Mannheim, Germany). After UV-cross-linking, the membrane was hybridized with a DIG-labelled RNA probe [prepared using a DIG RNA labelling kit (Roche Applied Science)] at 68°C for 8 h. Signal bands were detected using the DIG-Northern Starter Kit (Roche Applied Science) and visualized with an ImageQuant LAS 500 imager (GE Healthcare, Beijing, China). Western blotting was performed as described previously [15].

**GUS activity assay and plant test**

GUS activity was determined by measurement of the OD

**RESULTS**

**Xcc encodes a met (XC1251-XC1253) operon that is essential for growth in the absence of methionine**

Here, using the genome annotation of the Xcc strain 8004 [19] and the methionine biosynthetic pathway of *E. coli* [4], we constructed an *in silico* methionine biosynthetic pathway for *Xcc*. As shown in Fig. 1, all of the genes encoding the enzymes that accomplish the trans-sulfuration pathway from homoserine to methionine and the process from aspartate to homoserine are present in the genome of *Xcc* strain 8004. However, the gene (named *metY* in *E. coli*) encoding O-acetylhomoserine sulphhydrylase, which is indispensable for the direct sulphhydration pathway, is absent in the genome. This *in silico* model implies that *Xcc* synthesizes methionine via the trans-sulfuration pathway but not via a direct sulphhydration pathway. Notably, the genes encoding the enzymes converting aspartate semialdehyde to cystathionine, i.e. *XC1251* (*metA*), *XC1252* (*metB*) and *XC1253* (*hom*), are located together, while the genes encoding other enzymes involved in the biosynthesis of methionine from aspartate are scattered on the chromosome of *Xcc* strain 8004 (Fig. 1).

Bioinformatic analysis of the ORFs *XC1251-XC1253* suggests that these genes are transcribed in the same direction and potentially co-transcribed as a single operon (Fig. 1). This was verified by Northern blotting analysis (Fig. 1). We named this the *met* (*XC1251-XC1253*) operon for simplification. To validate the involvement of the *met* operon in methionine biosynthesis, an insertion mutant strain named 1201PK2 (Table S1) was constructed. This mutant has an insertion in the first ORF (i.e. *XC1251*) of the operon, thus blocking the transcription of the whole operon. As illustrated in Fig. 2, the mutant strain grew well in the nutrient-rich medium (NYG) but was unable to grow in the nutrient-limited medium (MMX), while the wild-type strain 8004 grew well in both media. However, supplementing MMX with methionine restored the mutant’s ability to grow as well as the wild-type (Fig. 2). Interestingly, none of the other 19 standard amino acids, which were used as supplements under the same test conditions, could rescue the growth of the mutant in MMX. These results demonstrated that the mutant is methionine auxotroph and the *met* operon is essential for the growth of *Xcc* in the absence of methionine.

**The met operon is required for full virulence and in planta growth of *Xcc***

To investigate whether the *met* operon (and by inference methionine biosynthesis) is important for the pathogenicity of *Xcc*, we tested the virulence of the mutant strain 1201PK2 on the host plant Chinese radish by leaf-clipping assay [15]. The results showed that the disease symptoms caused by the mutant were significantly weaker compared to the wild-type. As shown in Fig. 2(c), at 10 days post-inoculation the wild-type produced disease symptoms with a mean lesion length of 15.2 mm, while the mean lesion length caused by the mutant was only 6.4 mm. This finding indicates that the *met* operon is required for full virulence of *Xcc*.

The mutant grew poorly in environments lacking methionine (Fig. 2a, b). For this reason, we further assessed the growth of the mutant in * planta*. A group of five inoculated leaves were homogenized and plated on NYG medium supplemented with appropriate antibiotics. Growth was recorded after incubation at 28°C for 10 days. During the observation period, the number of mutant colonies recovered from the infected leaves was 10- to 100-fold lower than that of the wild-type (Fig. 2d). This finding demonstrates that the *met* operon (and by inference methionine biosynthesis) is important for *Xcc* to propagate regularly in host plants.

**met operon expression is controlled in a methionine-dependent manner**

To gain an insight into how the expression of the *met* operon is controlled, a wild-type strain expressing a recombinant *XC1251* protein (*XC1251-3FLAG*) was generated and named *XC1251-3F* (Table S1). This was achieved by adding 3×Flag tag to the C-terminus of *XC1251* by in-frame fusing a DNA segment encoding 3×Flag tag to the 3′-end (just before the stop codon TGA) of *XC1251*, and introducing it into the genome of strain 8004 using a suicide vector-mediated unmarked allelic replacement method [17]. The expression level of the recombinant protein *XC1251-3FLAG* was assessed using Western blotting when the *Xcc* strain was grown in MMX with or without methionine. The result showed that *XC1251-3FLAG* protein expression was elevated in cells grown in MMX without methionine (Fig. 3a). However, the level of *XC1251-3FLAG* was reduced dramatically in the presence of methionine. No detectable *XC1251-3FLAG* protein was observed when the concentration of methionine was increased to 500 µM (Fig. 3a). Moreover, the addition of other standard amino acids (such as 500 µM glycine) did not effect *XC1251-3FLAG* expression (Fig. 3b), indicating that the repression of *XC1251-3FLAG* expression by methionine was specific. These results suggest that the *met* operon is expressed
in a methionine-dependent manner, where activation and inactivation of the operon are dependent on the level of methionine in the surrounding environment.

**The 5’UTR, but not the promoter of the met operon, is involved in controlling expression in response to methionine availability**

The above findings suggest that Xcc may regulate the expression of the met operon by a feedback inhibition manner in response to methionine. To investigate whether feedback inhibition by methionine plays a role in controlling the transcriptional level of the met operon, we first determined the transcriptional start site (TSS) of the operon by 5’RACE (rapid amplification of cDNA ends) (Fig. S1). This analysis predicted putative -10 and -35 elements of the core promoter of the operon based on the identified TSS (Fig. 4a). Based on this, two promoter-gusA transcriptional fusion reporter plasmids, named pP1251L and pP1251S, were constructed by fusing 109 and 52 bp DNA sequences upstream of the TSS with the promoterless gusA gene (which still retained its Shine-Dalgano sequence) and cloning into the vector pLAFR6 (Fig. 4b). Subsequently, these reporter plasmids were introduced into strain 8004, generating reporter

![Figure 2](image-url)

Fig. 2. Inactivation of the met operon has an impact on Xcc virulence and growth in methionine-deficient medium and in planta. (a) and (b) Plate assay and quantitative liquid culture assay for detection of the growth of Xcc strains. NYG, nutrient-rich medium; MMX, minimal medium; MMX+Met or Gly, MMX supplemented with 1 mM of methionine or glycine. The photographs were taken at either 48 h (for NYG) or 72 h (for MMX and MMX+Met or Gly) post-incubation at 28 °C. The cell density was measured 72 h after incubation at 28 °C. (c) Black rot symptoms caused by Xcc strains on Chinese radish leaves. Images were taken at day 10 post-inoculation. Values under each leaf are the average lesion length (in mm) (mean±SD) from three repeats, each with 50 leaves. Different letters following the values indicate significant difference (t-test, P<0.01). (d) Growth rates of Xcc strains in inoculated leaves of Chinese radish. Bacterial cells were recovered from the inoculated leaves every day over a period of 10 days post-inoculation. Data are the mean±SD from a representative experiment, and similar results were obtained in two other independent experiments. WT, the wild-type strain 8004; 1201PK2, the met operon mutant strain.
feedback control of this operon may be at the post-transcriptional level. Notably, the operon has a very long (190 nt) 5’UTR (Fig. 4a), implying that the 5’UTR may be responsible for feedback control. To test this possibility, 5’UTR-gusA transcriptional and translational fusion reporter plasmids, named p5’UTR-SD+ and p5’UTR-SD−, respectively, were constructed (Fig. 4c, d), where both fusions were driven by the E. coli lac promoter whose expression is constitutive in Xcc. The reporter plasmids were introduced into strain 8004, generating the reporter strains named 8004/p5’UTR-SD+ and 8004/p5’UTR-SD− (Table S1). The GUS activities produced by the reporter strains grown in MMX with or without methionine were measured. The results showed that the GUS activity produced by the translational reporter was reduced by 98% when methionine was added (Fig. 4d). Addition of glycine to the medium had no effect on the GUS activity produced by either of the reporters (Fig. 4c, d), indicating that the reduction in GUS activity by methionine was specific. These data denote that the 5’UTR plays a key role in the feedback regulation of the met operon, whose regulation appears to be mainly at the translational level. Interestingly, only 75% GUS activity was produced by the translational reporter when methionine was added (Fig. 4c), suggesting that the 5’UTR may also employ a transcription attenuation mechanism to regulate the met operon.

The met operon is widely distributed among Xanthomonas species

A BLAST analysis revealed that the met operon containing metA, metB and hom genes is present in all completely sequenced Xanthomonas species, subspecies and pathovars (i.e. X. campestris pv. campestris, X. axonopodis pv. vesicatoria, X. axonopodis pv. citri, X. axonopodis pv. citrulmelo, X. oryzae pv. oryzae, X. oryzae pv. oryzicola, X. fuscans subsp. fuscans and X. albilineans) (Fig. 5). On the contrary, the location of the metA, metB and hom genes is scattered among the genomes of other bacteria. Interestingly, a further BLAST search showed that the 5’UTR of the met operon is highly conserved among all sequenced Xanthomonas genomes, including those with a draft sequence present in the NCBI sequence database (https://blast.ncbi.nlm.nih.gov/) (Fig. 5, Table S3). In addition to Xanthomonas species, the highly conserved 5’UTR sequence is also found in Pseudoxanthomonas suwonensis and Stenotrophomonas acidaminiphila (Table S3).

DISCUSSION

To establish a successful infection, a plant-parasitic bacterium must be capable of utilizing plant compounds as nutrients for growth in addition to overcoming plant defences. However, the infected plant tissue is generally a nutrient-deficient environment where nutritional compounds such as nucleotides and amino acids are insufficient for bacterial growth. Thus, having the ability to synthesize nucleotides and amino acids from simpler compounds is critical for plant-parasitic pathogens to proliferate efficiently and
Fig. 4. Characterization and functional analysis of the promoter region and the 5’UTR of the met operon. (a) Characterization of the promoter. The small green arrow represents the promoter (P1251) of the operon. TSS represents the identified transcriptional start site. The promoter-containing DNA sequences used for the construction of reporter plasmids are shown in green, while the 5’UTR sequence is in magenta. The –10/-35 region and the Shine-Dalgarno sequence (SD) are indicated. (b) Functional analysis of the promoter. The upper part shows the genetic organization of the reporter plasmids pP1251L and pP1251S. The lower part shows the GUS activities produced by the reporter strains 8004/pP1251L and 8004/pP1251S, as well as the negative control strain 8004/pNPgusA (pNPgusA) grown for 72 h in MMX alone (Met−) and MMX with 1 mM methionine (Met+). Data are from a representative experiment, and similar results were obtained in two other independent experiments. The letters above the columns represent no significant difference at P=0.05 by t-test. (c) and (d) Functional analysis of the 5’UTR. The upper part shows the genetic organization of the 5’UTR-gusA transcriptional fusion reporter plasmid (p5UTR-SD+). The lower part shows the GUS activities produced by the reporter strains 8004/p5UTR-SD+ (p5UTR-SD+) and 8004/p5UTR-SD− (p5UTR-SD−) grown in MMX alone (Met−) and MMX with the addition of 1 mM methionine (Met+) or 1 mM glycine (Gly+). The asterisks above the columns represent significant difference at P<0.05 by t-test. The data in blue and black above the columns are the absolute and relative values, respectively. One unit of GUS activity was defined as milligrams (mg) of p-nitrophenol released from p-nitrophenyl β-D-glucuronide per minute per ml of bacterial culture (cell density: OD600=1.0). Data presented are from a representative experiment, and similar results were obtained in two other independent experiments.
invade hosts successfully. *Xcc* is an intercellular parasite that generally invades, colonizes and multiplies in the vascular tissues of cruciferous plants. In this work, we showed that an *Xcc* mutant strain defective for the *met* operon (and by inference methionine biosynthesis) caused a severe reduction in virulence and growth rate in the host plant. Given that the mutant grew as well as the wild-type in nutrient-rich medium, this result suggests that there was insufficient methionine in the vascular tissues of the host plant tested for *Xcc* growth, and that *de novo* synthesis of methionine is crucial for *Xcc* to proliferate and establish an infection in the host.

As illustrated in Fig. 1, *Xcc* possesses genes encoding a complete set of enzymes required for methionine synthesis from aspartate via the trans-sulfuration pathway. Certain bacteria have developed an alternative pathway, called the direct sulfhydration pathway, to synthesize methionine, in which O-acetylhomoserine is directly converted to homocysteine by O-acetylhomoserine sulfhydrylase, encoded by *metY* [3]. However, it seems that members of the *Xanthomonas* genus do not employ the direct sulfhydration pathway to synthesize methionine, as the *metY* gene is missing in the genomes of all sequenced *Xanthomonas* strains as revealed by a BLAST search against the NCBI sequence database (https://blast.ncbi.nlm.nih.gov/).

Previous studies reported that in Gram-negative bacteria the expression of the genes involved in methionine synthesis is regulated by a mechanism of transcriptional-level
feedback inhibition [4]. In *E. coli* the *met* genes involved in methionine biosynthesis are regulated by the activator MetR and the repressor MetJ, which bind directly to the MetR- and MetJ-binding motifs present in the promoters of *met* genes [20]. The *Xcc* strain 8004 encodes a MetR homologue (XCO327) that shares 37% identity with the *E. coli* MetR protein, although *metJ* is missing in its genome [19]. However, bioinformatics analysis revealed that there is no typical MetR-binding motif present in any of the promoters of the genes involved in methionine biosynthesis, as illustrated in Fig. 1. Given that the promoter of the *Xcc met* operon does not respond to methionine availability (Fig. 4b), the MetR homologue in *Xcc* may not have a direct effect on the expression of *met* genes. It was reported that *Ralstonia solanacearum* MetR regulates the expression of *metE* but not *metH* [13], both of which are regulated by MetR in *E. coli* and *Salmonella typhimurium* [21]. Interestingly, *R. solanacearum* metE, metH and metR mutants are not auxotrophic for methionine [13]. Whether the MetR homologue in *Xcc* plays a role in methionine biosynthesis needs to be further investigated.

As mentioned above, Gram-positive bacteria generally utilize RNA regulators such as the S-box and T-box riboswitches to control the expression of genes involved in de novo methionine biosynthesis at the post-transcriptional level. Based on the results of promoter-*gusA* assay and 5’UTR-*gusA* fusion reporter analysis, we conclude that the *Xcc met* operon is constitutively transcribed regardless of whether methionine is sufficient or deficient in the environment, but that translation initiation of the operon mRNA is tightly controlled by its 5’UTR in response to methionine availability. Consistently, the predicted secondary structure of the 5’UTR shows that the SD sequence of the first ORF XCI251 is blocked (Fig. S2), suggesting that the 5’UTR can regulate the expression of XCI251 at the translational level. To the best of our knowledge, this is the first work showing that the 5’UTR of the *met* operon mRNA in a Gram-negative bacterium plays a key role in the regulation of de novo methionine biosynthesis. It is known that methionine’s downstream product SAM can be recycled. During methylation, SAM is converted to S-adenosylhomocysteine (SAH), which is then hydrolysed by SAH hydrolase to form the upstream metabolite of methionine, homocysteine [2]. It was demonstrated that a riboswitch at the 5’UTR of the gene encoding SAH hydrolase senses SAH and activates genes involved in co-enzyme recycling in Gram-negative bacteria, such as *Pseudomonas syringae* [22]. The riboswitch is highly conserved in the 5’UTRs of the genes predicted to encode SAH hydrolase in *Xcc* strains, suggesting that *Xcc* may also use the 5’UTR of SAH hydrolase-encoding gene to regulate SAM recycling [22].

Interestingly, an Rfam search (http://rfam.xfam.org/) showed that the 1–120 nt region in the 190 nt 5’UTR of the *Xcc met* operon displays 52% sequence identity with the aptamer domain of the *yifI* SAM-I riboswitch of *Bacillus subtilis*. However, there is no such SAM-I riboswitch-like sequence present in the 5’UTR regions of other genes involved in *Xcc* methionine biosynthesis. This suggests that the expression of the *met* operon and other *met* genes is differentially regulated in *Xcc*. Given that the *met* operon and its 5’UTR are highly conserved in *Xanthomonas* genus, to further investigate the detailed molecular mechanism by which the 5’UTR controls the expression of the *met* operon in *Xcc* will be very valuable in regard to our understanding of methionine biosynthesis regulation in *Xanthomonas*.

Funding information
This work was supported by the National Natural Science Foundation of China (31470237), the 973 Program of the Ministry of Science and Technology of China (2015CB150600) and the Ba Gui Scholar Program of Guangxi Zhuang Autonomous Region of China (2014A002).

Conflicts of interest
The authors declare that there are no conflicts of interest.

References
1. Serganov A, Patel DJ. Amino acid recognition and gene regulation by riboswitches. Biochim Biophys Acta 2009;1789:592–611.
2. Fontecave M, Atta M, Mulliez E. S-adenosylmethionine: nothing goes to waste. Trends Biochem Sci 2004;29:243–249.
3. McCutcheon JP, Moran NA. Extreme genome reduction in symbiotic bacteria. Nat Rev Microbiol 2012;10:13–26.
4. Ferla MP, Patrick WM. Bacterial methionine biosynthesis. Microbiology 2014;160:1571–1584.
5. Viola RE. The central enzymes of the aspartate family of amino acid biosynthesis. Acc Chem Res 2001;34:339–349.
6. Sherwood AV, Henkin TM. Riboswitch-mediated gene regulation: novel RNA architectures dictate gene expression responses. Annu Rev Microbiol 2016;70:361–374.
7. Breaker RR. Prospects for riboswitch discovery and analysis. Mol Cell 2011;43:867–879.
8. Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS. Comparative genomics of the methionine metabolism in Gram-positive bacteria: a variety of regulatory systems. Nucleic Acids Res 2004;32:3340–3353.
9. Gutiérrez-Preciado A, Henkin TM, Grundy FJ, Yanovsky C, Merino E. Biochemical features and functional implications of the RNA-based T-box regulatory mechanism. Microbiol Mol Biol Rev 2009;73:36–61.
10. Leyn SA, Suvorova IA, Kholina TD, Sherstneva SS, Novichkov PS et al. Comparative genomics of transcriptional regulation of methionine metabolism in Proteobacteria. PLoS One 2014;9:e113714.
11. Cubitt MF, Hedley PE, Williamson NR, Morris JA, Campbell E et al. A metabolic regulator modulates virulence and quorum sensing signal production in *Pectobacterium atroposumticum*. Mol Plant Microbe Interact 2013;26:356–366.
12. Andersen GL, Beattie GA, Lindow SE. Molecular characterization and sequence of a methionine biosynthetic locus from *Pseudomonas syringae*. J Bacteriol 1998;180:4497–4507.
13. Plener L, Boistard P, González A, Boucher C, Genin S. Metabolic adaptation of *Ralstonia solanacearum* during plant infection: a methionine biosynthesis case study. PLoS One 2012;7:e36777.
14. Mansfield J, Genin S, Magori S, Citovsky V, Srixaryanum M et al. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 2012;13:614–629.
15. Li L, Li RF, Ming ZH, Lu GT, Tang J. Identification of a novel type III secretion-associated outer membrane-bound protein from *Xanthomonas campestris pv. campestris*. Sci Rep 2017;7:42724.
16. Windgassen M, Urban A, Jaeger K. Rapid gene inactivation in *Pseudomonas aeruginosa*. FEMS Microbiol Lett 2000;193:201–205.
17. Patey G, Qi Z, Bourg G, Baron C, O’Callaghan D. Swapping of periplasmic domains between Brucella suis VirB8 and a pSB102 VirB8 homologue allows heterologous complementation. *Infect Immun* 2006;74:4945–4949.

18. Jefferson RA, Burgess SM, Hirsh D. beta-glucuronidase from *Escherichia coli* as a gene-fusion marker. *Proc Natl Acad Sci USA* 1986;83:8447–8451.

19. Qian W, Jia Y, Ren SX, He YQ, Feng JX et al. Comparative and functional genomic analyses of the pathogenicity of phytopathogen Xanthomonas campestris pv. campestris. *Genome Res* 2005;15:757–767.

20. Weissbach H, Brot N. Regulation of methionine synthesis in *Escherichia coli*. *Mol Microbiol* 1991;5:1593–1597.

21. Urbanowski ML, Stauffer LT, Plamann LS, Stauffer GV. A new methionine locus, *metR*, that encodes a trans-acting protein required for activation of *metE* and *metH* in *Escherichia coli* and *Salmonella typhimurium*. *J Bacteriol* 1987;169:1391–1397.

22. Wang JX, Lee ER, Morales DR, Lim J, Breaker RR. Riboswitches that sense S-adenosylhomocysteine and activate genes involved in coenzyme recycling. *Mol Cell* 2008;29:691–702.

**Five reasons to publish your next article with a Microbiology Society journal**

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.