

# Identification of a Two-Component System Important for Cell Division of the Rice Pathogen *Burkholderia glumae* in Response to Nutrient Conditions

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## Research Article

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## Abstract

Bacterial two-component regulatory systems control the expression of sets of genes to coordinate physiological functions in response to environmental cues. Here, we report that a genetically linked but functionally unpaired two-component system comprising the sensor kinase GluS (BGLU\_1G13350) and the response regulator GluR (BGLU\_1G13360) is critical for cell division in the rice pathogen *Burkholderia glumae* BGR1. The *gluR* null mutant, unlike the *gluS* mutant, formed filamentous cells in Luria–Bertani medium and was sensitive to exposure to 42°C. Expression of genes responsible for cell division and cell-wall (*dcw*) biosynthesis in the *gluR* mutant was elevated compared to the wild type, resulting in an imbalance between FtsZ and FtsA. GluR-His bound to the putative promoter regions of *ftsA* and *ftsZ*, indicating that repression of genes in the *dcw* cluster by GluR is critical for cell division in *B. glumae*. The *gluR* mutant did not form filamentous cells in M9 minimal medium, whereas exogenous addition of glutamine or glutamate to the medium induced filamentous cell formation. These results implicate glutamine and glutamate as external stimuli that modulate GluR-mediated cell division in *B. glumae*. Therefore, GluR controls cell division of *B. glumae* in a nutrition-dependent manner.

## Introduction

Two-component systems (TCS) consisting of a sensor kinase and a cognate response regulator are common in bacteria<sup>1</sup>. They are essential for the responses of bacteria to changes in environmental factors such as pH, osmotic pressure, antibiotics, and quorum-sensing (QS) signals<sup>1</sup>. The sensor kinases are autophosphorylated after sensing an environmental stimulus, which is followed by phosphotransfer from the phosphorylated sensor kinases to the response regulators<sup>1</sup>. The phosphorylated response regulators then undergo conformational changes to become active, thereby controlling the expression of target genes<sup>1</sup>. The genes encoding sensor kinases and response regulators are often genetically linked in bacterial genomes and functionally paired<sup>2,3</sup>. In addition to paired TCSs, sensor kinases and transcriptional regulators can crosstalk, thus modulating multiple biological processes in response to environmental signals irrespective of their genetic linkage<sup>2,4–6</sup>.

We study the social behavior and host interactions of the rice bacterial pathogen *Burkholderia glumae*, the cause of rice panicle blight<sup>7,8</sup>. A phytotoxin, toxoflavin, is the major virulence factor of *B. glumae* and exerts a toxic effect on photosynthetic organisms by generating radicals under light<sup>8,9</sup>. The virulence-factor biosynthesis and motility of *B. glumae* are dependent on QS<sup>8,10</sup>. As well as QS, we are interested in TCSs in *B. glumae* BGR1 because they coordinate and regulate the expression of genes critical for adaptation to stress, survival, fitness in the host, and virulence<sup>11–17</sup>. For instance, CpxAR of *Actinobacillus pleuropneumoniae*<sup>14</sup>, ArcAB of *Escherichia coli*<sup>15</sup>, and KdpDE<sup>16</sup> and PhoPQ<sup>17</sup> in a variety of bacterial taxa reportedly promote growth, fitness, and survival in the host. In addition, AgrAC, SsrAB, SaeRS, and ArlRS of *Staphylococcus aureus* and BvgAS of *Bordetella pertussis* are necessary for virulence<sup>11–13</sup>. Few studies have focused on TCSs in *B. glumae*, probably because of concern over

repeating works on other pathogens. However, Karki *et al.* reported that the PidS/PidR TCS is essential for the pigmentation and virulence of *B. glumae* 411gr-6<sup>18</sup>.

In this study, we identified a TCS composed of the sensor kinase GluS and the response regulator GluR, which was critical for normal cell division in *B. glumae* BGR1. *gluR* and *gluS* were cotranscribed, but GluR functioned independently of GluS in normal cell division. We report that GluR regulates the gene cluster involved in cell division and cell wall (*dcw*) biosynthesis to maintain balanced expression of FtsZ and FtsA for normal septum formation<sup>19</sup>. We conclude that external nutritional conditions modulate cell division in a TCS-dependent manner in *B. glumae*. These findings provide insight into how the recognition of external signals by TCS affects the sophisticated molecular mechanisms involved in controlling bacterial cell division.

## Results

**Identification of a TCS critical for normal cell division of *B. glumae* BGR1.** To identify a key TCS important for normal cell division of *B. glumae* BGR1, we first mutagenized it with mini-Tn5 and examined the morphology of the mutants. The mutant RT271 formed filamentous cells when grown in Luria-Bertani medium (LB) (Fig. 1a). To determine the insertion site of mini-Tn5 in the RT271 mutant, a mini-Tn5 insertion along with flanking sequences was rescued by digestion of its genomic DNA with *Eco*RI, self-ligation, and transformation into *E. coli* DH5a. Flanking sequences of mini-Tn5 from the rescued plasmid pRT271E revealed that an annotated gene BGLU\_1G13360 had an insertional mutation (Fig. 1b). This gene, *gluR*, encoded a 27.7 kDa protein that exhibited 99.6% similarity to known OmpR-type response regulators such as BURPS305\_7006 in *B. pseudomallei* 305, RisA (BMA10247\_1253) in *B. mallei* NCTC 10247, and BCENMCO3\_1962 of *B. cenocepacia* MCO-3 (Supplementary Fig. S1a). Downstream of *gluR* was a putative sensor kinase, *gluS* (BGLU\_1G13350), (Fig. 1b) that showed 96.7, 94.1, and 90.0% identities with known sensor kinases such as Envz1 (BGL\_1C23830) in *B. plantarii*, BGLA\_1G24110 in *B. gladioli* BSR3, and RisS (BMA1486) in *B. mallei* ATCC 23344, respectively (Supplementary Fig. S1b).

Due to the proximity of *gluS* and *gluR* in the BGR1 genome, we reasoned that these two genes might be co-transcribed into a polycistronic RNA. Therefore, we performed reverse transcription-polymerase chain reaction (RT-PCR) with specific primers (Fig. 1b). We found that *gluR* and *gluS* were indeed co-transcribed (Fig. 1c). We next mutagenized pBGH1, a cosmid carrying *gluS* and *gluR*, with Tn3-gusA to generate mutants of *gluR* and *gluS* followed by marker-exchange into *B. glumae* BGR1, resulting in BGLUR133 (BGR1 *gluR*:Tn3-gusA133) and BGLUS35 (BGR1 *gluS*:Tn3-gusA35) (Fig. 1b). Although *gluR* and *gluS* were co-transcribed, the insertion of Tn3gusA133 in *gluR* did not cause a polar effect (Fig. 1d).

**Aberrant cell division due to a mutation in *gluR*.** To determine whether the insertion of Tn3-gusA in *gluR* or *gluS* conferred a similar cell morphology to the RT217 mutant, we observed the morphology of the *gluR* and *gluS* mutants under a light microscope. The *gluR* mutant BGLUR133 showed extensive filamentous cells in LB medium (Fig. 2), consistent with the initial phenotype of the *gluR*:min-Tn5 mutant RT271 in LB

(Fig. 1a). However, the *gluS* mutant BGLUS35 formed normal cells in LB medium (Fig. 2). The *gluR* mutant BGLUR133 maintained a normal rod-shaped cell morphology similar to that of the *gluS* mutant BGLUS35 in M9 minimal medium (Fig. 2). Transmission electron microscopy (TEM) of ultrathin sections of the *gluR* mutant BGLUR133 revealed characteristic features of filamentous cells with multiple nuclei and indents along the cell membrane at points where the septum would have formed to separate dividing cells (Fig. 3a). The genetically complemented strain of the *gluR* mutant BGLUR133 with pBGH13, BGLUR133C, had morphologically uniform rod-shaped cells (Fig. 3a). The growth of the *gluR* mutant BGLUR133 and the wild-type BGR1 for 30 hours in LB medium was similar (Supplementary Fig. S2, Fig. 3b). Although filamentous cells of the *gluR* mutant BGLUR133 remained viable for 30 hours, their abundance decreased after 18 hours (Fig. 3b).

**Direct control of genes involved in cell division by GluR.** Because TEM suggested the involvement of GluR in cell division, we determined whether GluR influences the expression of genes in the *dcw* cluster involved in cell division. In *B. glumae*, there were 15 annotated genes; e.g., *ftsA*, *ftsI*, *ftsL*, *ftsQ*, *ftsW*, and *ftsZ* in the *dcw* cluster and *ftsB* and *ftsK* in other regions (Fig. 4a, b). The expression levels of *ftsA*, *ftsB*, *ftsI*, *ftsK*, *ftsL*, *ftsQ*, *ftsW*, and *ftsZ* in the *gluR* mutant BGLUR133 were significantly increased compared to those in the wild-type BGR1 (Fig. 4c). The expression levels of the eight genes in BGLUR133C were similar to those in the wild type (Fig. 4c). To determine whether GluR directly controls their expression, we performed electrophoretic mobility shift assays (EMSA) on the putative promoter regions of *ftsA* and *ftsZ* and purified His-tagged GluR (GluR-His). The binding of GluR-His to the putative promoter regions of *ftsA* and *ftsZ* confirmed that GluR-His directly represses the expression of cell division genes in *B. glumae* (Fig. 4d).

**Alleviation of aberrant cell morphology by constitutive expression of *ftsA* in the *gluR* mutant BGLUR133.** Because the FtsA to FtsZ ratio is critical for normal bacterial cell division, we evaluated the role of GluR in its maintenance. Taking the expression levels of *ftsA* and *ftsZ* in the wild type as 1.00, the expression levels of these two genes were 1.21 and 1.67, respectively, in the *gluR* mutant BGLUR133 (Fig. 4c). To confirm that imbalanced expression of *ftsA* and *ftsZ* causes abnormal cell division, we constitutively expressed *ftsA* under the control of the *trc* promoter in the wild type, the *gluR* mutant BGLUR133, and the complemented strain BGLUR133C; the resulting strains were designated BGR1(pFtsA), BGLUR133(pFtsA), and BGLUR133C(pFtsA), respectively. Cells of BGLUR133(pFtsA) showed normal cell division as well as a 5.2fold increase in *ftsA* expression (Fig. 5). Moreover, *ftsA* expression was increased more than 100fold in BGR1(pFtsA) and BGLUR133C(pFtsA), whose cells underwent abnormal division (Fig. 5).

**Influence of glutamate and glutamine on GluR-mediated control of cell division.** Because the *gluR* mutant BGLUR133 formed filamentous cells in LB medium but not in M9 minimal medium, we reasoned that the amino acids in LB medium might be the cause of filamentous cell formation. Therefore, we added 10% casamino acids to M9 minimal medium to evaluate their influence on the morphology of the *gluR* mutant BGLUR133. Adding casamino acids to M9 minimal medium transformed the morphologically normal cells of the *gluR* mutant BGLUR133 into filamentous cells (Fig. 6). To identify the amino acid(s) responsible for triggering filamentous cells in the *gluR* mutant BGLUR133, 20 amino acids were

individually added to M9 minimal medium. Of the 20 amino acids, only glutamine and glutamate individually or in combination triggered cells of the *gluR* mutant BGLUR133 to become filamentous in M9 minimal medium (Fig. 7a, b, c). When 13 amino acids excluding glutamine and its six amino-acid metabolites (glutamate, serine, alanine, proline, aspartate, and asparagine) were added to M9 minimal medium, the *gluR* mutant BGLUR133 maintained a normal morphology (Fig. 7d). These results confirmed the role of glutamine in the *gluR*-mediated control of cell division in *B. glumae*.

Because environmental glutamine and glutamate affected the cell morphology of the *gluR* mutant BGLUR133 in M9 minimal medium, we examined the expression levels of seven *fts* genes in M9 minimal medium with or without glutamine and glutamate. The expression levels of the seven *fts* genes were significantly lower in the *gluR* mutant BGLUR133 than in the wild type or the BGLUR133C complemented strain (Fig. 7e). However, addition of glutamine to M9 minimal medium increased the expression levels of the seven *fts* genes in the *gluR* mutant BGLUR133 (Fig. 7f).

**Heat sensitivity due to an imbalance of FtsA and FtsZ in the *gluR* mutant.** Because *fts* genes were identified in a temperature-sensitive filamenting mutant, we assessed whether the filamenting *gluR* mutant BGLUR133 is heat sensitive. The number of cells of the *gluR* mutant BGLUR133 decreased significantly after 6 hours at 42°C and they were entirely nonviable after 12 hours in LB medium (Fig. 8a). The wild-type BGR1, the *gluS* mutant BGLUS35, and the complemented strain BGLUR133C showed no growth but prolonged survival at 42°C (Fig. 8a). In M9 minimal medium at 42°C, the *gluR* mutant BGLUR133 mutant retained viability for 18 hours and subsequently lost viability (Fig. 8b). By contrast, the wild-type BGR1 and the complemented strain BGLUR133C increased in cell number during the static period of *gluR* mutant BGLUR133 in M9 medium (Fig. 8b).

## Discussion

In addition to QS systems, pathogens likely manipulate environmental factors. Here we investigated the response regulator, GluR, which is crucial for normal cell division in *B. glumae*. Although *gluR* and *gluS* were co-transcribed, GluS was not the counterpart of GluR because a mutation in *gluS* did not affect normal cell division of *B. glumae*. Such a genetically linked but functionally independent TCS system was reported for *risS* and *risA*, which encode a sensor kinase and a response regulator, respectively, in *B. pertussis*<sup>3</sup>. *risS* and *risA* were genetically linked but functionally independent<sup>3</sup>. Phosphorylation of RisA was mediated by crosstalk with a distant histidine kinase, RisK<sup>4</sup>. Therefore, an as-yet-unidentified sensor kinase may be responsible for phosphorylation of GluR in *B. glumae*.

Cell division involves ingrowth of the cell wall and membrane and septum formation in the chromosome of rod-shaped bacteria such as *B. glumae*<sup>20</sup>. To ensure equal partitioning of chromosomes into daughter cells, the expression of genes involved in cell division must be properly regulated<sup>19,20</sup>. In most bacteria, cell division and cell-wall synthesis are regulated by a series of genes in the *dcw* cluster<sup>19</sup>. Within bacterial groups of the same taxon and cell shape, the order and regulation of genes in the *dcw* cluster are highly conserved<sup>21</sup>. Therefore, it was not surprising that in *B. glumae*, the *dcw* cluster displayed

significant similarities to that of *E. coli*<sup>22</sup>. Pioneer studies of the *dcw* cluster genes in *E. coli* spotlighted *ftsZ* as the key element in cell division<sup>23,24</sup>. It was later noted that FtsZ is not sufficient to drive septation, leading to discovery of, for instance, *ftsA*, *ftsQ*, and *ftsP*<sup>2</sup>.

The mechanisms of regulation of the *dcw* cluster are unclear, despite the presence therein of several regulatory elements, *e.g.*, internal promoters, transcript stabilizers, and protein ratios<sup>22,25</sup>. Studies on the control of cell division have concentrated on FtsZ. Multiple promoter regions have been reported upstream of *ftsZ* in the *dcw* cluster, indicating regulation at the transcriptional level<sup>22,25,26</sup>. We found that GluR binds to the upstream promoter regions of *ftsZ* and *ftsA* located in the *ftsA* and *ftsQ* coding regions, respectively. Unlike positive regulators in *E. coli*, such as SdiA<sup>27</sup>, the phase-specific sigma factor<sup>28</sup>, and RcsB<sup>29</sup>, GluR negatively regulates cell division in *B. glumae*.

In the *dcw* cluster, biased expression of genes resulting from a mutation in *gluR* induced aberrant cell division. The GluR-controlled expression of *dcw* cluster genes was essential for normal cell division in *B. glumae*. FtsZ may be tethered to the membrane by two cytoplasmic membrane-associated proteins, ZipA and FtsA<sup>30</sup>, thus mediating cell division. Each *E. coli* cell is estimated to contain 3000–5000 molecules of FtsZ, 50–200 of FtsA, and 1500 of ZipA<sup>31</sup>. In addition, an imbalanced FtsZ and FtsA ratio is detrimental to *E. coli*<sup>19,32</sup>. In the filamentous cell-forming *gluR* mutant, expression of *ftsZ* was higher than that of *ftsA*, but the constitutive expression of *ftsA* restored a filamentous morphology. Although the optimal FtsZ and FtsA ratio is unknown, a 5.2fold increase in *ftsA* expression in the *gluR* mutant restored normal cell division to filamentous cells.

Because LB medium is rich in amino acids, and filamentation of the *gluR* mutant was facilitated by supplementation of extracellular glutamine or glutamate in M9, the glutamine- and glutamate-dependent filamentous cell formation at an early stage of growth in LB was explicable. However, the number of filamentous cells of the *gluR* mutant BGLUR133 decreased over time, possibly as a result of depletion of amino acids, including glutamine and glutamate, 12 hours after incubation (Supplementary Fig. S3). Because the *gluR* mutant formed filamentous cells in a glutamine or glutamate-dependent manner, we hypothesized that GluR phosphorylation is caused by extracellular glutamine or glutamate, which promotes proper cell division by repressing *dcw* cluster genes. Extracellular glutamine and glutamate reportedly alter the expression of genes involved in cell division and cell-wall synthesis of *B. subtilis*<sup>33</sup>. Beuria *et al.* reported that an increased FtsZ polymerization rate and extent in *E. coli* resulted from extracellular glutamine<sup>34</sup>. It was noted that FtsZ showed optimal polymerization as large, bundled filamentous structures in *E. coli* in the presence of 1 M glutamine<sup>34</sup>. Interestingly, FtsZ polymers formed in the absence of glutamine were ninefold less stable than those in its presence, emphasizing the roles of these amino acids in the stability of FtsZ polymers<sup>34</sup>.

Connections between TCS and glutamine metabolism have been reported in other bacterial taxa. For example, GlnK-GlnL of *Bacillus subtilis*<sup>35</sup>, GluR-GluK of *Streptomyces coelicolor*<sup>36</sup>, and AauR-AauS of *Pseudomonas putida*<sup>37</sup> reportedly sense and control glutamate uptake. In other bacteria, the TCSs

involved in glutamine sensing and uptake are located close to the glutamine ABC transporter<sup>35–37</sup>. GluR is not likely to be involved in glutamine uptake because we reported that GltI is responsible for glutamine uptake in *B. glumae*<sup>38</sup>. A *bona fide* sensor kinase responsible for glutamine sensing and GluR phosphorylation is yet to be identified in *B. glumae*.

The fact that the *gluR* mutant BGLUR133 was sensitive to heat treatment at 42°C was somewhat expected because the name *fts* was coined from filamentous temperature sensitive mutants in *E. coli*<sup>39</sup>. Mutations in septation genes conferred an elongated morphology on *E. coli*; similarly, filamentous *B. glumae* caused by a *gluR* mutation were heat sensitive. This supports the hypothesis that GluR is crucial for cell division and an optimum gene expression profile. Taken together, our findings indicate that GluR is key for maintaining the gene expression profile required for glutamine- or glutamate-dependent control of cell division in *B. glumae* BGR1.

## Materials And Methods

**Bacterial strains and growth conditions.** The bacterial strains and plasmids used are listed in Supplementary Table S1. Unless stated, the strains were grown in LB medium containing 0.1% (w/v) tryptone, 0.5% (w/v) yeast extract, 0.5% (w/v) sodium chloride, and 1.5% agar as required (Affymetrix®, Cleveland, OH) with the appropriate antibiotics at 37°C. Antibiotics were used at the following concentrations: rifampicin, 100 µg/mL; ampicillin, 50 µg/mL; tetracycline, 10 µg/mL; kanamycin, 25 and 50 µg/mL; and spectinomycin, 50 µg/mL. 5-Bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-gal) was added at 40 µg/mL as necessary.

**DNA manipulation and sequencing.** Basic DNA manipulation was conducted following standard protocols<sup>40</sup>. Plasmid DNA from *E. coli* was isolated using the Biomedic® Plasmid DNA Miniprep Kit (Ibiomedic, Korea) following the manufacturer's instructions. DNA sequencing was performed by Macrogen, Inc. (Seoul, Korea). The genetic information and gene IDs for DNA construction were obtained from the *B. glumae* BGR1 genome database (GenBank accession numbers: CP001503–CP001508; kropbase.snu.as.kr/cgi\_bg.cgi). A cosmid library of *B. glumae* BGR1 was constructed as described previously<sup>8</sup>.

**Rescue mini-Tn5, Tn3-gusA, and marker-exchange mutagenesis.** Using *E. coli* S17-1 (pRescue *mini-Tn5*), random mutations were created in *B. glumae* BGR1 as described previously<sup>41</sup>. Successful mutants were isolated by selection on LB agar containing kanamycin. The rescued *mini-Tn5* mutants were screened for phenotypic changes. Following a previous method<sup>42</sup>, the flanking regions were sequenced using the O-end primer (5'-GGTTTCACCGTCATCACCG-3'), and the TCS genes were disrupted using the identified rescue *mini-Tn5* insertions.

The pLAFR3 derivatives of pBGH1 carrying *gluR* (BGLU\_1G13360) and *gluS* (BGLU\_1G13350) were mutagenized using Tn3-gusA as described previously<sup>43</sup>. The Tn3-gusA insertion site and orientation in each mutant were mapped by restriction enzyme digestion analysis, and the plasmid sequenced using

the Tn3gus primer (5'-CCGGTCATCTGAGACCATTAAAAGA-3'). The plasmids carrying Tn3-gusA insertions were marker-exchanged into *B. glumae* BGR1 via tri-parental mating<sup>44</sup> to generate BGLUR133 and BGLUS35. All marker-exchange mutants were confirmed by southern hybridization analysis.

**Bacterial growth and viability assay.** Overnight liquid cultures of the *B. glumae* strains were adjusted to an OD<sub>600</sub> of 0.05 and subcultured into fresh LB medium. The cultures were incubated for 30 hours at 37°C with shaking at 250 rpm. At 6hour intervals, bacterial growth was assayed by spotting 10 µL of serial dilutions in triplicate on LB agar plates. Bacterial growth was expressed as log CFU/mL after 2 days of incubation at 37°C.

Cell viability was assayed using the LIVE/DEAD BacLight™ Bacterial Viability Kit, which contains SYTO® 9 green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide (Invitrogen™, Carlsbad, CA), following the manufacturer's instructions. Fluorescence images were captured using a confocal laser scanning microscope (Leica SP8X, Wetzlar, Germany) at excitation/emission wavelengths of 483/490–540 and 535/890–680 nm for green and red fluorescence, respectively.

**Transmission electron microscopy.** Bacterial cells were harvested from overnight cultures and prepared for observation by transmission electron microscopy (TEM) as reported previously<sup>38</sup>. Electron micrographs were acquired using a JEM 1010 microscope (JEOL, Tokyo, Japan) with acceleration voltages of 180 and 100 kV from a LIBRA 120 energy-filtration microscope (Carl Zeiss, Oberkochen, Germany).

**Quantitative reverse transcription-polymerase chain reaction.** Total RNA was isolated from *B. glumae* BGR1, BGLUR133, and BGLUR133C using the RNeasy® Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Genomic DNA was removed using DNase I (Thermo Fisher Scientific, Vilnius, Lithuania). From 1 µg of RNA, reverse transcription for cDNA synthesis was performed at 42°C for 1 hour with the Recombinant RNasin® Kit (Promega, Madison, WI). Using specific primer sets (Supplementary Table S2), *ftsA*, *ftsB*, *ftsI*, *ftsK*, *ftsL*, *ftsQ*, *ftsW*, and *ftsZ* cDNAs were synthesized. Transcription levels were determined using SsoFast EvaGreen Supermix (Bio-Rad, Hercules, CA) under the following conditions: 95°C for 30 seconds followed by 30 cycles of 95°C for 5 seconds and 55°C for 5 seconds. PCR was performed in triplicate and gene expression values were normalized to that of 16S rRNA using Bio-Rad CFX Manager software.

**Constitutive expression of *ftsA*.** To express *ftsA* under the control of the *trc* promoter in pKK38, we amplified the *ftsA*-coding region from the *B. glumae* strains BGR1, BGLUR133, and BGLUR133C using the primers FtsA\_Nco1 (5'-GGCCATGGAGCAAAGACTACAAAGATCT-3') and FtsA\_HindIII (5'-CCAAGCTTCAGAAATTGCTCAGGAACC-3') and a TaKaRa PCR Kit (TaKaRa Bio Inc., Kusatsu, Shiga, Japan) following the manufacturer's instructions. The PCR fragments were first cloned into the *Sma*1 sites of pBluescript II SK (+) and transferred to the *Nco*1-*Hind*III sites of pKK38 as described previously<sup>38</sup>. By means of tri-parental mating<sup>44</sup>, pKK38 derivatives were conjugated into the respective *B. glumae*

strains to generate strains with elevated *ftsA* expression—BGR1(pFtsA), BGLUR133(pFtsA), and BGLUR133C(pFtsA).

**Growth and viability of *B. glumae* strains at 42°C.** The *B. glumae* BGR1, TCS null mutants, and BGLUR133C strains were cultured overnight at 37°C, and the optical density at 600 nm ( $OD_{600}$ ) was adjusted to 0.05. The strains were incubated at 42°C with shaking at 250 rpm for 24 hours in LB and M9 minimal media, and the cell density was measured at 6hour intervals.

**Environmental stimuli driving GluR responses.** We cultured the wild type, *gluR* mutant BGLUR133, and BGLUR133C in M9 minimal medium (6 g of  $Na_2HPO_4$ , 3 g of  $KH_2PO_4$ , 0.5 g of NaCl, and 1 g of  $NH_4Cl$  in 1 L of deionized water containing 1 mM  $MgSO_4$  and 0.1 mM  $CaCl_2$ ) supplemented with 0.2% glucose. To evaluate whether amino acids are required for GluR activity, M9 minimal medium was supplemented with 10% Bacto™ Casamino Acids (Becton, Dickson & Co., Franklin Lakes, NJ) that comprises 20 essential amino acids. Individual amino acids (Sigma Aldrich™, St. Louis, MI) were analyzed at the concentrations in LB medium<sup>45</sup>.

**Glutamate utilization in *B. glumae*.** Overnight liquid cultures of the wild-type BGR1 were adjusted to an optical density of  $OD_{600}$  of 0.05 and subcultured in LB medium for 24 hours at 37°C with shaking at 250 rpm. At 3hour intervals, the cultures were centrifuged (14,000 rpm, 4°C, 10 minutes), and the supernatants were collected. Glutamate analysis was carried out by liquid chromatography-mass spectrometry (LCMS-2000, Shimadzu, Japan) at the National Instrumentation Center and Environment Management (Seoul National University, Seoul, Korea).

**Scanning electron microscopy.** *B. glumae* strains cultured overnight in LB or M9 minimal medium with/without amino acids were harvested, fixed with Karnovsky's fixative [2% glutaraldehyde, 2% paraformaldehyde in 0.05 M sodium cacodylate buffer (pH 7.4)], and post-fixed with 1% sodium tetroxide in 0.1 M sodium cacodylate buffer for 1 hour at 4°C as described previously<sup>46</sup>. Before imaging, the samples were coated with platinum at 10 mA for 270 seconds using a G20 Ion Sputter Coater (GSEM Co., Suwon, Korea) and electron micrographs were acquired using a Carl Zeiss microscope (Auriga, Zeiss Germany).

**Electrophoretic mobility shift assay.** GluR-His was purified using an established method<sup>10</sup>. Using the primer set gluR\_Nde1/gluR\_BamH1 (Supplementary Table S2), we amplified the promoter regions of the putative GluR targets, *ftsAp* and *ftsZp*. The resulting PCR products were labeled with biotin using Lightshift Chemiluminescent Electrophoretic Mobility Shift Assay Kits, as described by the manufacturer (Pierce, Appleton, Wisconsin). We used 329 bp upstream of *katE1* as a nonspecific competitor DNA amplified using KatE1-F and KatE1-R primers (Supplementary Table S2). Purified GluR-His (0.75  $\mu$ M) was incubated in binding buffer (10 mM Tris-HCl [pH 7.5], 100 mM NaCl, and 5% [v/v] glycerol) containing 1 nM biotin-labeled DNA as described previously<sup>10</sup>. For competition assays, unlabeled target DNA at 20fold molar excess was added to each reaction with the labeled DNA. Using 4% (w/v) polyacrylamide gels, the reactions were separated and transferred to nitrocellulose membranes. The bands were detected using

streptavidin/horseradish peroxidase-derived chemiluminescence kits, as described by the manufacturer (Pierce) and visualized using ChemiDoc XRS + and Image Lab Software (Bio-Rad).

**Statistical analysis.** All experiments were conducted in triplicate with the appropriate controls. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference *post hoc* analysis in SPSS software (ver. 25 x86-x64; IBM Corp., Armonk, NY) were conducted to detect significant differences. A value of  $p < 0.05$  was considered indicative of statistical significance.

## Declarations

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### Contributions

Designed the experiments: J.M. and I.H. Performed the experiments: J.M. and E.G. Analyzed the data: J.M., E.G., Y.K., and I.H. Contributed reagents/materials/analysis tools: J.M. and I.H. Wrote the paper: J.M. and I.H.

### Competing interests

The authors declare no competing financial interests.

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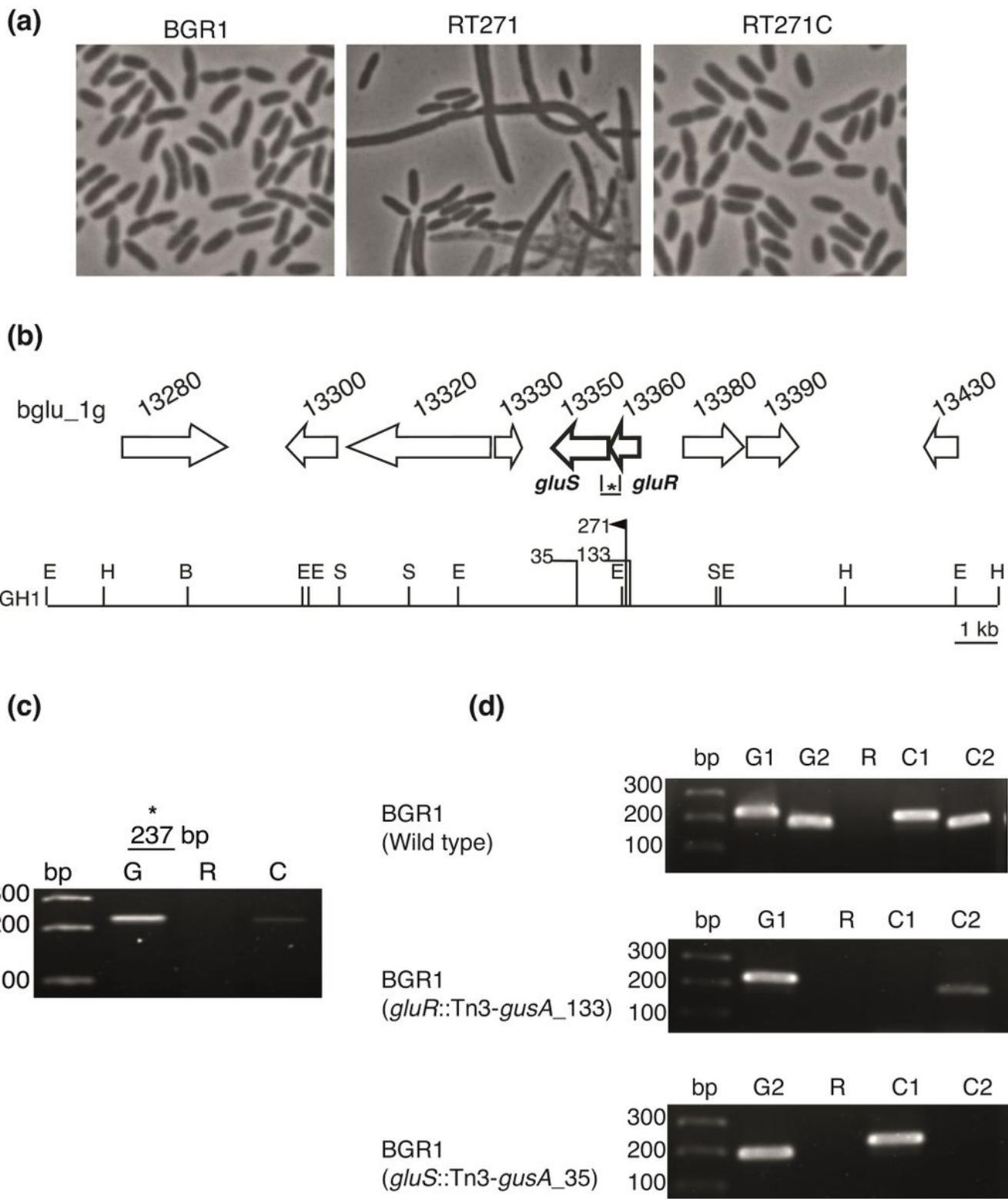
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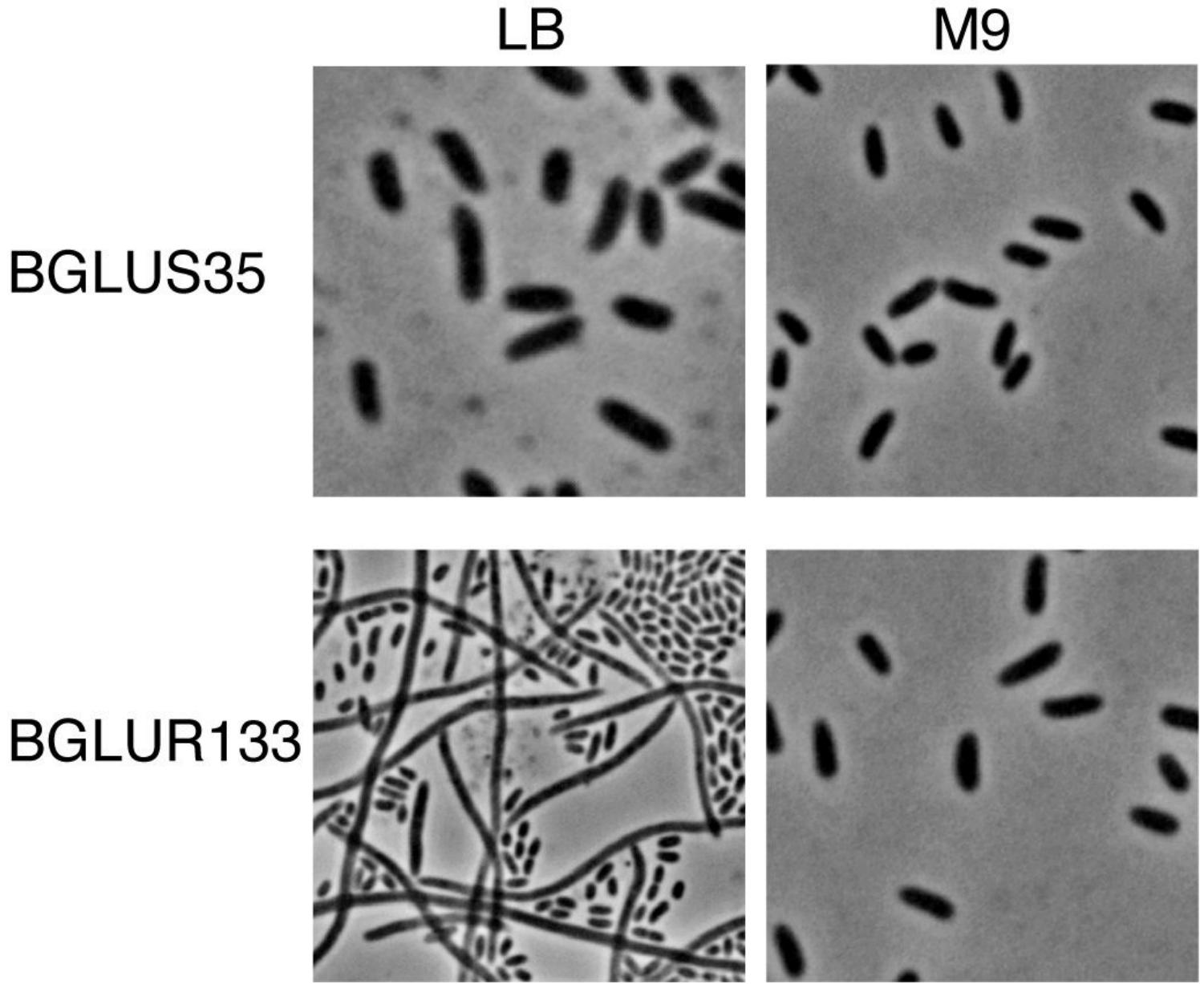
## Figures



**Figure 1**

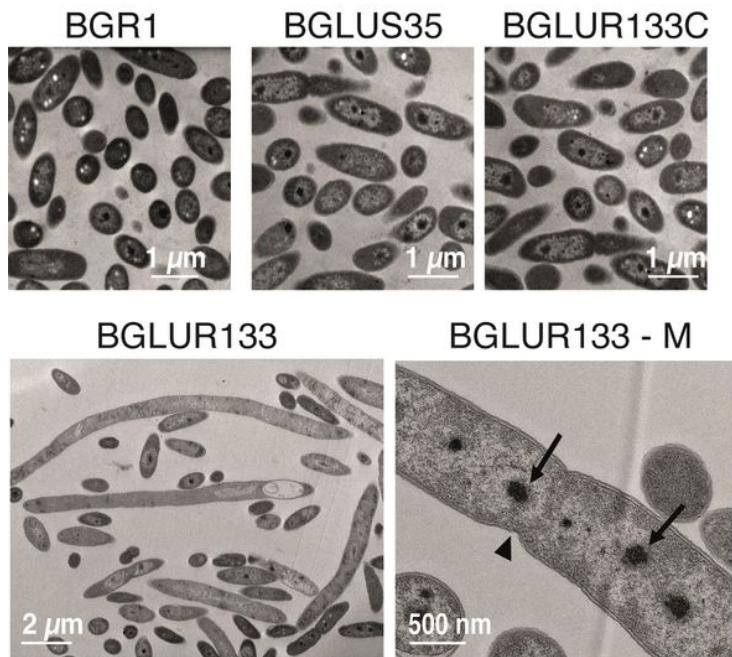
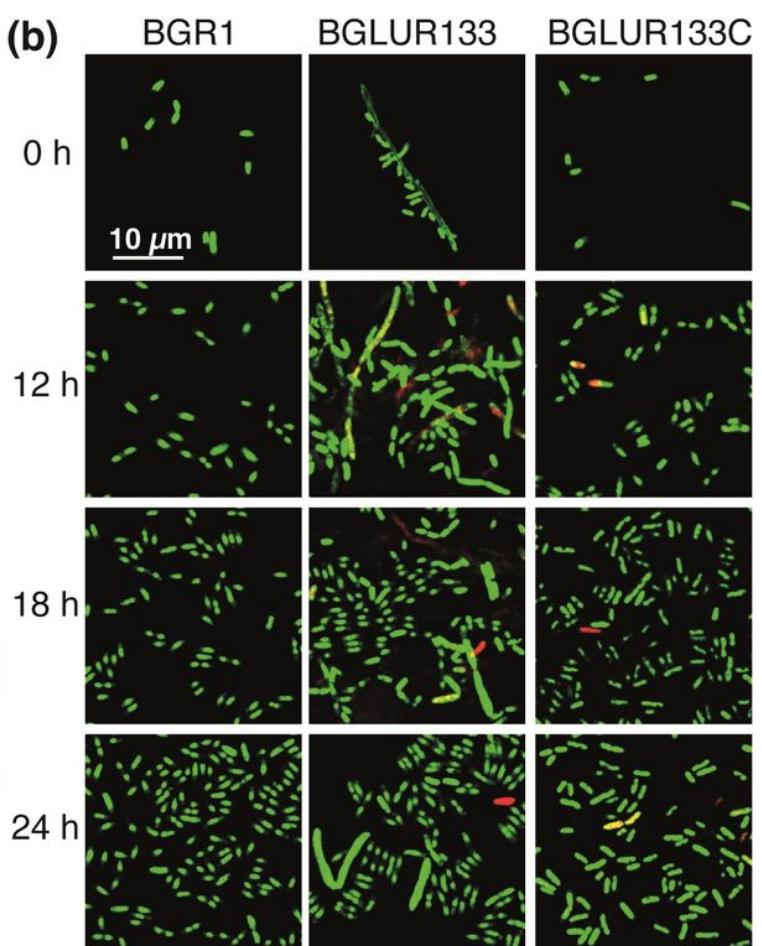
The GluS-GluR TCS of *B. glumae* BGR1. (a) Microscopic observation of the cell morphology of BGR1 (wild type), RT271 (BGR1, *gluR*::mini-Tn5rescue), and mutant complemented RT271C (BGR1, *gluR*::mini-Tn5rescue carrying pBGH1) strains in LB medium. (b) Organization scheme of the GluS sensor kinase and GluR response regulator in pBGH1. Vertical bars above the restriction map indicate the position of the Tn5 (271) and Tn3-gusA insertions (35 and 133). E, EcoRI; H, HindIII; B, BamHI; S, SacI. (c) RT-PCR

analysis showing that gluS and gluR genes are co-transcribed in BGR1. Primers were designed to amplify a 237 bp (\*) product encompassing the gluR and gluS genes in the wild type. Lane G, PCR product using genomic DNA as a template; Lane R, PCR product using RNA as a template; Lane C, PCR product using cDNA as a template. (d) No polar effect resulted from Tn3-gusA insertion. Lane bp, marker; Lane G1, PCR product from gluR chromosomal DNA as a template; Lane G2, PCR product from gluS chromosomal DNA as a template; Lane R, PCR product from total RNA as a template; Lane C1, PCR product from gluR cDNA as a template; Lane C2, PCR product from gluS cDNA as a template. Full gel images are presented in Supplementary Fig. S4 and S5.

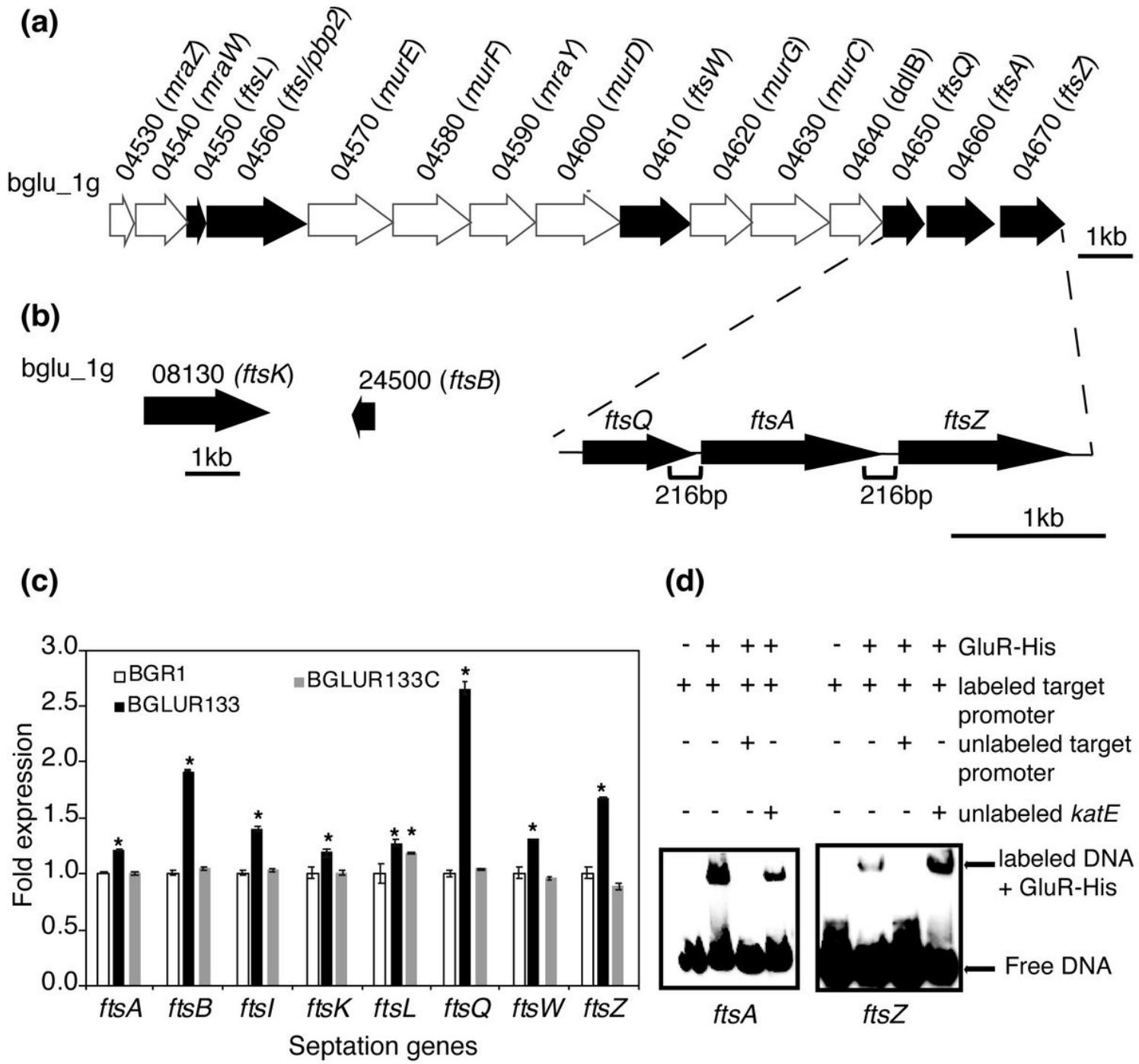


**Figure 2**

Tn3-gusA mutations in gluR resulted in nutrient-dependent cell filamentation. In LB medium, the gluR mutant BGLUR133 formed filamentous cells, but a normal rod-shaped cell morphology was observed in M9 minimal medium. No morphological defects were observed in the gluS mutant BGLUS35 in the different culture media.

**(a)****(b)****Figure 3**

The gluR mutant forms a heterogeneous population of viable filamentous and normal rod-shaped cells. (a) The indicated bacterial strains were grown to early stationary phase, and the morphological phenotypes of ultrathin sections were observed by TEM. BGLUR133-M shows that the filamentous cells formed by the gluR mutant contained multiple nuclei (arrows) with indents (arrowhead) along the cell wall, symbolizing failed septum formation. (b) Cell viability of the wild type, BGLUR133, and complemented BGLUR133C strains assessed by combination staining with propidium iodide (PI) and SYTO 9 green. Fluorescence images were obtained by confocal laser scanning microscopy. Dead cells stained with PI are red, and SYTO 9-stained viable cells are green.

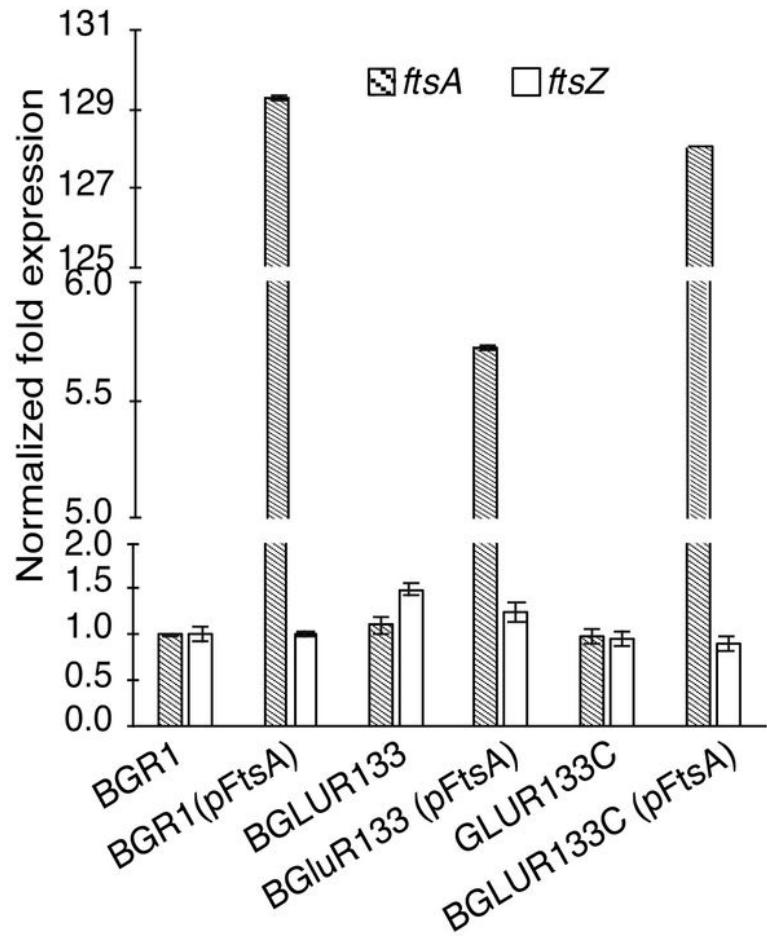


**Figure 4**

GluR represses cell division and septation genes in *B. glumae*. (a) Genetic organization of the dcw cluster in BGR1. Dark arrows represent genes involved in septation during cell division, and light arrows are genes involved in cell-wall synthesis or with no function. The section below the gene map indicates the positions and size of the respective putative promoters in this study. (b) Gene maps of additional cell-division genes outside the dcw cluster. (c) Expression levels of eight cell division genes in the wild type (BGR1), gluR mutant, and complemented BGLUR133C strains compared by qRT-PCR. mRNA levels were normalized to 16S rRNA, and the fold expressions are relative to those of the wild type. Data are means  $\pm$  standard error (SE) of triplicates; statistical analysis was performed by one-way ANOVA/Tukey's

correlation for multiple comparisons. \*, p < 0.05; ( $F(48,71) = 1536.273$ ;  $p = 0.00$ ). (d) Electrophoretic mobility shift assay (EMSA) showed direct control of *ftsA* and *ftsZ* by GluR-His binding to the respective putative promoter regions; 0.75  $\mu$ M GluR-His, 1 nM labeled target DNA, 1 nM unlabeled *katE* promoter DNA, and 20 nM unlabeled target promoter DNA were used for EMSA. Full blot images with multiple contrasts are shown in Supplementary Fig. S6.

(a)



(b)

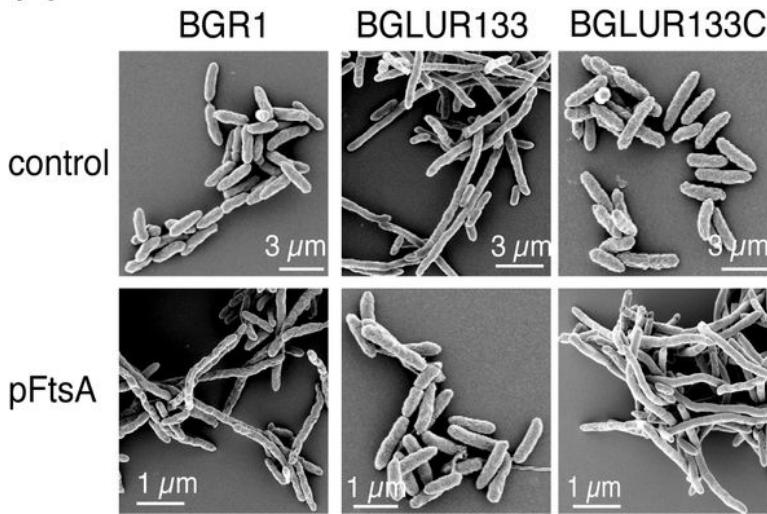
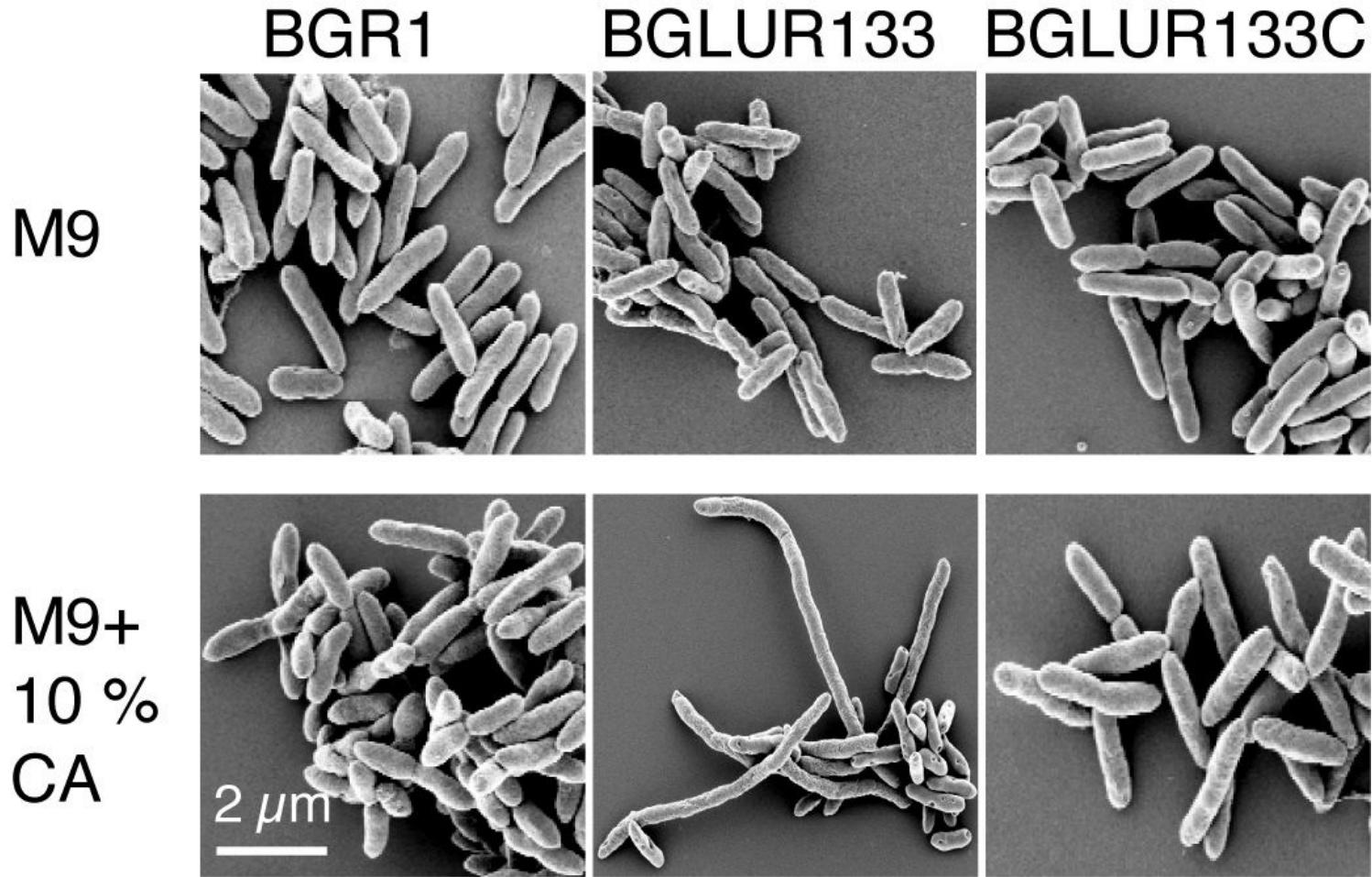


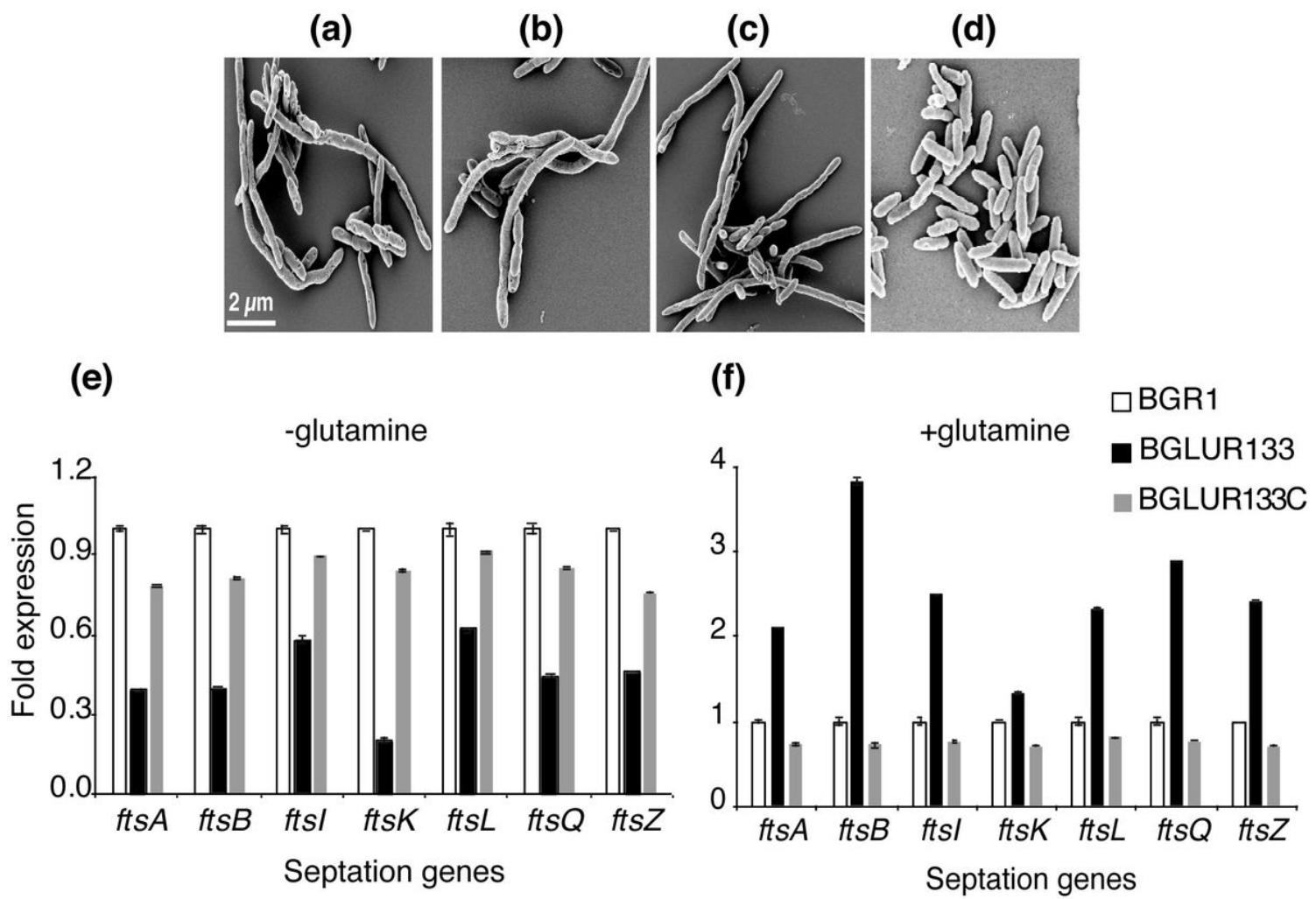
Figure 5

GluR maintains the molar ratio of ftsZ to ftsA to ensure normal cell division. FtsA was constitutively expressed to counteract ftsZ in the wild type (BGR1), BGLUR133, and BGLUR133C strains. (a) mRNA levels were quantified by qRT-PCR and are shown as normalized fold expression values. Data are means  $\pm$  standard error (SE) of triplicates. (b) Bacterial strains cultured to early stationary phase were visualized using a Carl Zeiss GmbH Auriga microscope. pFtsA represents constitutive expression of ftsA in the indicated bacterial strains.



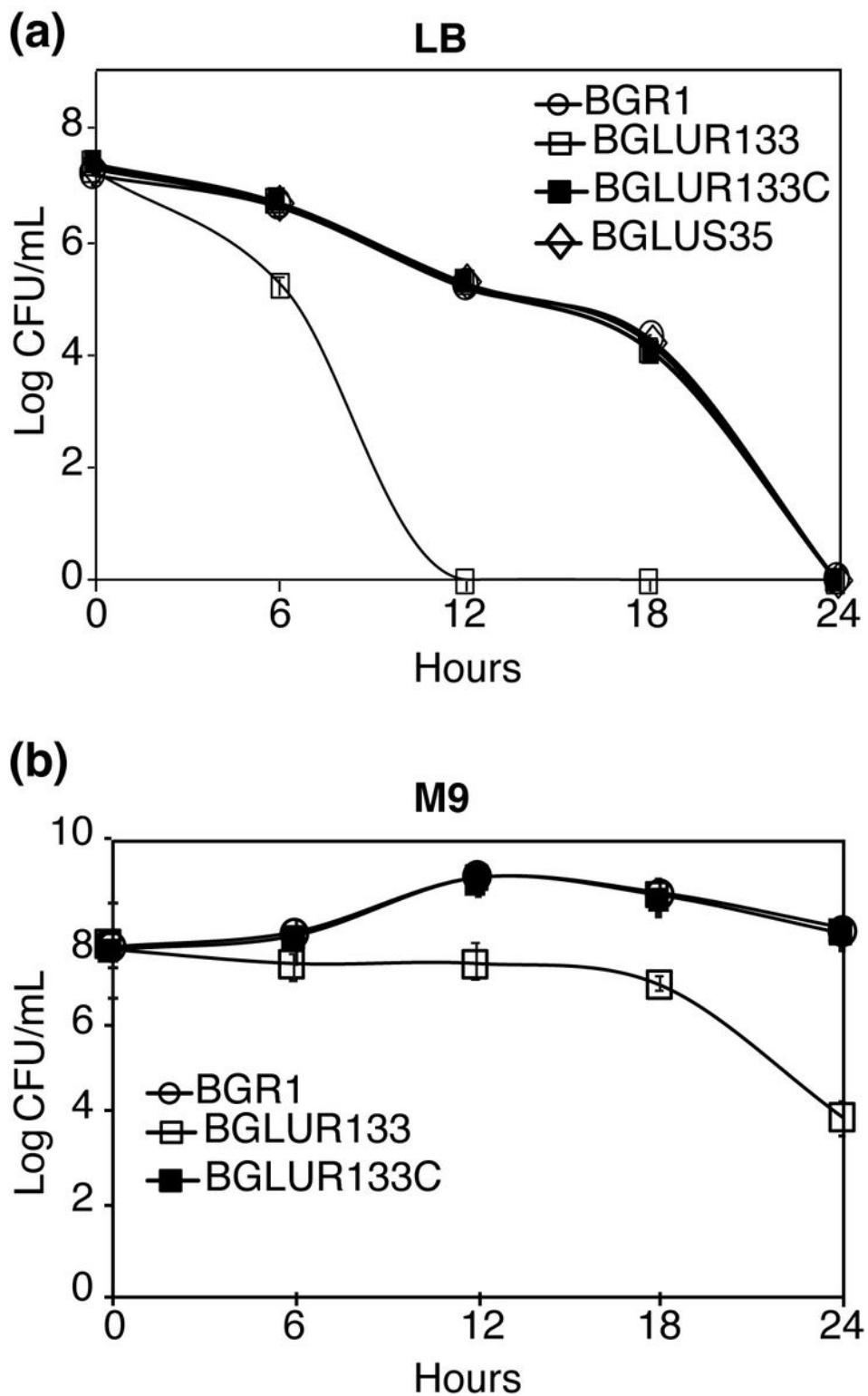
**Figure 6**

Extracellular amino acids promote filamentation in response to GluR mutation. The indicated bacterial strains were cultured overnight in M9 minimal medium with or without 10% casamino acids (CA), processed for SEM analysis, and their morphology observed using a Carl Zeiss GmbH Auriga microscope.



**Figure 7**

Extracellular glutamine and glutamate are required for GluR-mediated cell division. In an amino acid-rich M9 minimal medium, the gluR mutant BGLUR133 formed filamentous cells in (a) glutamine, (b) glutamate, and (c) several amino acids produced from glutamine (serine, alanine, proline, aspartate, and asparagine). (d) Removing glutamine and the respective metabolites restored normal cell division in the presence of the remaining 13 amino acids (arginine, cysteine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine). (e-f) Differences in the expression levels of septation genes in plain M9 (e) and glutamine-rich M9 minimal medium (f), analyzed by qRT-PCR using the wild-type BGR1 as the baseline. Data are means  $\pm$  standard error (SE) of triplicates.



**Figure 8**

Exponential population decline at 42°C as a result of mutations in GluR. At 6 hour intervals, the indicated strains' population densities in LB medium (a) and M9 medium (b), were quantified by CFU counting and the results expressed as log CFU/mL. Data are means  $\pm$  standard error (SE) of triplicates.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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