Involvement of Phosphoglucose Isomerase in Pathogenicity of *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

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Xanthomonas oryzae pv. *oryzae*, the causal agent of bacterial leaf blight of rice, was subjected to transposon mutagenesis to generate mutants defective in pathogenicity. A novel mutant 74M913 was attenuated in virulence but retained its ability to cause the hypersensitive response in leaf blight-resistant rice and tomato. Cloning and sequence analysis revealed that the transposon in 74M913 was inserted in a gene homologous

to the phosphoglucose isomerase (*pgi*) gene of *X. axonopodis* pv. *citri*. Growth of the mutant in a synthetic medium containing fructose or xylose as a sole carbohydrate source was much reduced, indicating the transposon disrupted *pgi* function. The interaction between expression of *pgi* and hypersensitive response and pathogenicity (*hrp*) genes was investigated because we had demonstrated previously that expression of *hrp* genes of *X. oryzae* pv. *oryzae* is induced in a synthetic medium containing xylose. However, *pgi* and the *hrp* gene (*hrpU*) were expressed independently. This study suggests that PGI is involved in pathogenicity of *X. oryzae* pv. *oryzae*.

Xanthomonas oryzae pv. *oryzae* causes bacterial leaf blight of rice (*Oryza sativa* L.) and has been reported in all rice-growing areas of the world (1,10,12,20,33). The bacterium enters its host through hydathodes or wounds around the leaf edges, and then multiplies and spreads in xylem vessels, causing disease (16,20,26).

As with other plant-pathogenic bacteria, hypersensitive response and pathogenicity (*hrp*) gene products are known as one of the most important pathogenicity factors in *X. oryzae* pv. *oryzae* (11,28,40). These products are involved in inducing disease symptoms in susceptible host plants and resistant responses, including a hypersensitive response (HR), in resistant hosts and nonhosts (2,38). Some virulence factors also have been found in *X. oryzae* pv. *oryzae*, such as extracellular polysaccharide (35) and a toxic compound (14). Also, a general secretory pathway, through which some extracellular hydrolytic enzymes (cellulase and xylanase, etc.) are secreted is shown to be required for the pathogenicity (15,22).

What *X. oryzae* pv. *oryzae* utilizes for nutrition in xylem vessels, especially as carbon sources, remains unknown. Hydrolytic enzymes produced by the bacterium might be involved in the acquisition of nutrition, colonization and, as a result, virulence against host plants. Our previous report (29) showed that glucose, which is an efficient carbohydrate source for growth of *X. oryzae* pv. *oryzae*, at least in culture, is not essential for bacterial growth in rice leaves because a mutant that could not utilize glucose grew in plants as well as the wild type.

Takeuchi et al. (27) reported that cell walls of rice leaf contain 60% xylan. Xylose is an efficient carbohydrate source for growth of *X. oryzae* pv. *oryzae* in culture (29). Moreover, *hrp* genes are

induced more effectively in xylose-containing media than media containing other carbohydrates (31). These results imply that xylose utilization is involved in the pathogenicity of *X. oryzae* pv. *oryzae*.

In this study, we isolated pathogenicity-deficient mutants using transposon mutagenesis, and demonstrated that one of the mutants, in which a phosphoglucose isomerase (*pgi*) gene was disrupted, could not grow in medium containing xylose as a sole carbohydrate source and had reduced virulence in rice.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and plasmids. Bacterial strains and plasmids used in this study are shown in Table 1. Strain T7174R is a spontaneous rifampicin-resistant mutant of *X. oryzae* pv. *oryzae* T7174 and has the same pathogenicity as the parental strain (7,35). T7174R and derivatives were usually grown at 28°C on nutrient-broth yeast extract (NBY) medium (34). For *hrp*-inducing conditions, XOM2 medium was used (31). *Escherichia coli* DH5αMCR was grown at 37°C in Luria-Bertani broth (23). Media were supplemented with antibiotics as follows: rifampicin at 20 µg/ml, ampicillin at 50 µg/ml, cycloheximide at 50 µg/ml, and kanamycin and spectinomycin at 25 µg/ml for *X. oryzae* pv. *oryzae* and 50 µg/ml for *E. coli*.

Plasmids constructed in this study are shown in Figure 1. An approximately 4.8-kb *SalI* fragment containing a *pgi* gene was cloned into pUC119 and pHM1, a broad-host-range plasmid, producing pUC913SI and pHM913SI, respectively. A 2.3-kb *EcoRI*-*NotI* fragment containing a *pgi* gene was inserted in pHM1, yielding pHM913EcNt. Transposon EZ::TNGUS (31), which contains a promoterless *gus* gene, was inserted in pUC913SI by *in vitro* transposition as described previously (31). Then a clone, in which the *gus* gene was inserted at +116 of a *pgi* gene (+1 represents A of the start codon) in the same transcriptional orientation, was selected by restriction enzyme analysis and sequence analysis, and named pUC913GUS. A 4.8-kb *SalI* fragment from

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pUC913GUS, which contains the 5' coding region of *pgi* and the preceding region followed by a *gus* gene, was cloned in pHM1, resulting in pHM913GUS. The introduction of plasmid DNA was performed by electroporation as described previously (28).

Transposon mutagenesis. For isolation of the *X. oryzae* pv. *oryzae* mutant with a transposon inserted into its genomic DNA, we used an EZ::TN transposome-mediated insertion system (Epicentre, Madison, WI). Transposon EZ::TN <KAN-2> (1 µl = 0.1 µg) was mixed with an equal volume of 100% glycerol and 2 µl (2 units) of EZ::TN transposase, and incubated at 37°C for 10 min. Aliquots (1 µl) were used for electroporation to *X. oryzae* pv. *oryzae*. Transposition clones were selected by plating on NBY-agar medium containing kanamycin.

Pathogenicity tests. Bacteria grown on NBY-agar media for 2 days at 28°C were suspended in distilled water (DW) at an absorbance at 600 nm (A_{600}) = 0.3. Inoculations of rice plants (cv. IR24) were performed by the clipping method (17). Lesion length was measured 2 weeks after inoculation. For examination of bacterial growth in rice leaves, 1-cm leaf sections containing the inoculation site were removed from plants every 24 h, homogenized in 1 ml of DW, and plated on NBY-agar medium containing the appropriate antibiotics. The HR-inducing ability in resistant rice (cv. IR-BB2) and tomato (cv. Momotaro) was tested by infiltration of bacterial suspension into the leaf parenchyma.

Recombinant DNA techniques. DNA manipulations were performed by standard procedures (23).

DNA sequencing. Genomic DNA of *X. oryzae* pv. *oryzae* strains was extracted and purified using Isoplat II (Wako, Osaka, Japan). Digestion with restriction enzymes and Southern blot analysis using transposon DNA as a probe were performed to obtain transposon-tagged DNA fragments of appropriate size for cloning. The fragments were cloned into pUC119 and used as sequence templates. The sequence reaction was done with a DYEnamic ET terminator cycle sequencing kit (Amersham Biosciences, Buckinghamshire, UK) using KAN-2 FP-1 forward primer or KAN-2 RP-1 reverse primer (Epicentre). These primers are hybridized with each end of the transposon. Electrophoresis and the subsequent sequence analysis were performed with an ABI373 sequencer (Applied Biosystems, Foster City, CA). Homology with known genes was identified using the BLAST program (4).

PGI enzyme assay. Bacteria grown on NBY-agar media for 2 days at 28°C were washed twice with 100 mM NaCl and resuspended to a concentration of 10^8 CFU/ml with buffer containing 10 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, and 1 mM dithiothreitol. Suspension (1 ml) was treated with an ultrasonicator, then centrifuged at $12,000 \times g$ for 10 min. The supernatant was

used for the spectrophotometric assay of PGI activity according to a method described by Gottlieb and Higgins (8).

Assay for bacterial growth in media. To investigate the growth of *X. oryzae* pv. *oryzae* strains under the restricted carbohydrate condition, we used a nutrient-poor *hrp*-inducing medium XOM2 with replacement of the carbohydrate source. Glucose, fructose, or xylose was added at a concentration of 1%. Bacterial strains grown on NBY-agar medium were washed twice and resuspended with DW at $A_{600} = 2.0$. Samples of 1 ml of bacterial suspension were added to 25 ml of the modified XOM2 and incubated (120 rpm, 28°C). The bacterial population of cultures (A_{600}) was measured every 24 h. As a control, the bacterial suspension was incubated in NBY and the population was measured every 2 h.

Assay of GUS activity. GUS activity of the bacteria was assayed as described previously (31). One unit of GUS activity is defined by the amount of *p*-nitrophenol released per hour.

RESULTS

Isolation of the pathogenicity mutant 74M913. Mutants of strain T7174R were obtained with transposon EZ::TN. More than 1×10^5 clones were obtained per 1 µg of transposon. One thousand kanamycin-resistant clones, that were similar to the parental strain in growth on NBY medium and production of extracellular polysaccharide and pigment, were selected and tested for their pathogenicity in rice. Lesion lengths were measured 2 weeks after inoculation, and nine pathogenicity-reduced mutants were selected. The ability of the nine mutants to induce HR also was tested using tomato leaves, and three of the mutants were HR⁻. Sequence analysis of the mutants revealed that the transposon was inserted in *hrpXo*, *hrcR*, or *hrpF* (data not shown). We selected one HR⁺ and pathogenicity-reduced mutant, named 74M913, for further investigation. The length of lesions formed on susceptible rice leaves (IR24) infected with 74M913 was reduced compared with that infected with control T7174R, but the pathogenicity was not completely lost (Table 2). The mutant retained the ability to induce HR in resistant rice leaves (IR-BB2) and in tomato leaves. On the other hand, the *hrpXo* mutant 74ΔHrpXo produced no disease symptoms in susceptible rice and did not induce HR in resistant rice or tomato. The growth of 74M913 in susceptible rice leaves was slower than that of T7174R (data not shown).

Identification of the gene mutated in 74M913. Southern blot analysis revealed that a single copy of the EZ::TN <KAN-2> transposon was inserted in the genome of 74M913 (data not shown). To identify the gene tagged by the transposon in the mutant, we digested the genomic DNA with *SalI*, whose recognition site is

TABLE 1. Bacterial strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	Reference or source
Strains		
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>		
T7174R	Spontaneous rifampicin-resistant mutant derived from T7174	7, 35
74ΔHrpXo	<i>hrpXo</i> -mutant	28
74M913	Transposon EZ::TN was inserted in <i>pgi</i>	This study
74M913GUS	Promoterless <i>gus</i> gene was inserted in <i>pgi</i>	This study
<i>Escherichia coli</i>		
DH5αMCR	F ⁻ <i>mcrA</i> Δ (<i>mrr-hsd RMS-mcrBC</i>) <i>recA</i> φ80 <i>dlacZ</i> ΔM15	Gibco-BRL
Plasmids		
pUC119	Plasmid vector, AmR	Takara
pHM1	Broad host range cosmid vector, SpR	R. Innes
pUC913SI	4.8-kb <i>SalI</i> fragment containing <i>pgi</i> in pUC119	This study
pHM913SI	4.8-kb <i>SalI</i> fragment containing <i>pgi</i> in pUC119	This study
pHM913EcNt	2.3-kb <i>EcoRI</i> - <i>NotI</i> fragment containing <i>pgi</i> in pHM1	This study
pUC913GUS	Transposon EZ::TNGUS was inserted in <i>pgi</i> A derivative from pUC913SI	This study
pHM913GUS	A <i>SalI</i> fragment containing 5' end of <i>pgi</i> and promoterless <i>gus</i> gene from pUC913GUS was cloned in pHM1	This study
pHMP1GUS2	A promoterless <i>gus</i> gene preceded by 5' end of <i>hrcU</i> and the promoter region was cloned in pHM1	31

located at the 3' end of the transposon, and inserted it into pUC119. The screening of the positive clones was conducted on kanamycin-containing medium. Sequence analysis using KAN-2 RP-1 reverse primer, which hybridizes with the 5' end of EZ::TN <KAN-2> and from which DNA is synthesized toward the genome DNA of the bacterium neighboring the transposon, revealed that the transposon was inserted into an open reading frame (ORF) homologous with a phosphoglucose isomerase (glucose-6-phosphate isomerase) gene (*pgi*) of *X. axonopodis* pv. *citri* (32) (Fig. 1A).

According to the nucleotide sequence analysis of the T7174 genome (H. Ochiai, Y. Inoue, M. Takeya, and H. Kaku, *unpublished data*), the ORF homologous with *pgi* was 1,515 bp (504 amino acids) in length and the putative translation product has 97.8 and 97.0% identity with *pgi* from *X. axonopodis* pv. *citri* and *X. campestris* pv. *campestris*, respectively (6). We also cloned a 3.5-kb *EcoRI*-*NotI* fragment containing a transposon from 74M913, and determined the sequence of the other side of the transposon-tagged region using KAN-2 FP-1 forward primer, which hybridizes with the 3' end of the transposon, confirming that the transposon was inserted at 1023 (1 is A of a putative initiation codon) of the *pgi* homolog with a 9-bp duplication in 74M913.

Sequence data also indicated that ORFs homologous with the pantoate-β-alanine ligase gene (*panC*) and with the aspartate 1-decarboxylase gene (*panD*) were located upstream of *pgi* and

ORFs homologous with XAC1789 and XAC1790 from *X. axonopodis* pv. *citri*, which are conserved hypothetical protein genes, were located downstream (Fig. 1A) (6). Thus, the region around *pgi* is well conserved between *X. oryzae* pv. *oryzae* and *X. axonopodis* pv. *citri*. The corresponding region from *X. campestris* pv. *campestris* also was conserved, except that the bacterium lacks an XAC1789 homolog (6).

To confirm that the ORF disrupted by a transposon encodes a PGI enzyme, we assayed bacterial strains for PGI activity. The spectrophotometric assay revealed that PGI activity was detected in control strain T7174R but not in 74M913 (data not shown).

TABLE 2. Virulence and hypersensitive response (HR) induction of *Xanthomonas oryzae* pv. *oryzae* mutant 74M913

Strains	LL on IR24 (mm) ^a	HR inductivity ^b	
		IR-BB1	Tomato
T7174R	57.0 ± 10.3	+	+
74ΔHrpXo (Hrp mutant)	Not detected	–	–
74M913	8.9 ± 5.8	+	+

^a Lesion length (LL) was measured 2 weeks after inoculation. Values are given as the mean and standard deviation (*n* = 5).

^b HR was observed 3 days after infiltration; + and – represent HR positive and negative, respectively.

Fig. 1. Physical map of **A**, a 4.8-kb *SalI* fragment containing a phosphoglucose isomerase (*pgi*) gene and **B**, plasmids constructed in this study. **A**, Arrows indicate open reading frames and corresponding genes from *Xanthomonas axonopodis* pv. *citri* are shown above. *panC*, *panD*, and *pgi* were considered to encode pantoate-β-alanine ligase, aspartate 1-decarboxylase, and glucose-6-phosphate isomerase, respectively, and XAC1789 and XAC1790 encode conserved hypothetical proteins. Amino acid identities between each of *X. oryzae* pv. *oryzae* and *X. axonopodis* pv. *citri* are shown in parentheses. Closed triangle represents a site for transposon insertion in 74M913. Ec, *EcoRI*; EV, *EcoRV*; Nt, *NotI*; Sc, *SacI*; SI, *SalI*. **B**, Lines corresponding to the map shown in **A** indicate regions inserted in the vector plasmid (pUC119 or pHM1). Plasmid pUC913GUS is inserted with transposon EZ::TNGUS (shown by a thick line with open arrows) in *pgi*. Plasmid pHM913GUS was constructed by digesting pUC913GUS with *SalI* (one of the sites is located at the right end of EZ::TNGUS) and inserting a 4.8-kb fragment in pHM1.

Complementation tests. To confirm that transposon insertion resulted in reduced pathogenicity of 74M913, complementation tests were conducted. We cloned a 4,756-bp *Sa*I fragment and a 2,251-bp *Eco*RI-*Not*I fragment containing *pgi* into a broad-host-range plasmid pHM1 to obtain pHM913SI and pHM913EcNt, respectively (Fig. 1B). Then strain 74M913 was transformed with each of the plasmids. PGI activity of the transformants was similar to that of the control strain, whereas 74M913 (pHM1) showed no activity (data not shown). Rice leaves were inoculated with the transformants and disease symptoms were examined 2 weeks after inoculation. Lesions formed by 74M913(pHM913SI) and 74M913(pHM913EcNt) reached lengths similar to those in the control, whereas lesions formed by 74M913(pHM1) were quite reduced in length (Fig. 2). Although the fragment cloned in pHM913SI contained some ORFs (homologs of *panC* [partial], *panD*, XAC1789, and XAC1790 from *X. axonopodis* pv. *citri*) other than *pgi*, plasmid pHM913EcNt contained only *pgi* (5' half of *panD* homolog and 3' end of XAC1789 were deleted). Therefore, PGI was considered to be the only product expressed from pHM913EcNt. Complementation by pHM913EcNt in the pathogenicity of 74M913 suggests that PGI is essential for the pathogenicity of *X. oryzae* pv. *oryzae*.

Growth of 74M913 in medium containing fructose and xylose. We examined the effect of the mutation in *pgi* on bacterial growth in culture by incubating 74M913 in the synthetic medium

containing glucose, fructose, and xylose as a sole carbohydrate source (Fig. 3). The mutant did not grow in the medium containing xylose or fructose, whereas T7174R did. The mutant grew in the medium containing glucose, but the growth was slower than the wild type. The growth of both strains was similar in the nutrient-rich complex medium NBY. The growth deficiency of 74M913 in the medium containing fructose, xylose, or glucose as a sole carbohydrate source was complemented by transformation with pHM913EcNt.

Expression of *pgi*. In a previous study (31), we showed that the expression of *X. oryzae* pv. *oryzae* *hrp* genes was induced in a xylose-containing medium. Now, *pgi* was revealed to be involved in utilization of xylose and pathogenicity of the bacterium. Therefore, we analyzed whether there are interactions between the expression of *pgi* and that of *hrp* genes.

We constructed a plasmid pHM913GUS, in which a promoterless *gus* gene was inserted into the *pgi* gene in the same transcriptional orientation (Fig. 1B). The plasmid was introduced into the control strain T7174R and the *hrp*-regulatory gene mutant 74ΔHrpXo (28), and both transformants were incubated in NBY (*hrp*-uninducing complex medium), XOM2 (*hrp*-inducing synthetic medium containing xylose), or modified XOM2 containing

Fig. 2. Virulence of 74M913 was complemented by transforming with pHM913SI or pHM913EcNt. Lesion length of five inoculated leaves was measured 2 weeks after inoculation and averages are shown.

Fig. 4. Expression of phosphoglucose isomerase (*pgi*) in nutrient-broth yeast extract (NBY) (a nutrient-rich complex medium), XOM2 (*hrp*-inducing synthetic medium containing xylose), and modified XOM2 containing glucose or fructose in place of xylose as a carbohydrate source. Bacteria T7174R and 74ΔHrpXo transformed with a plasmid containing a *pgi::gus* fusion gene (pHM913GUS) or an *hrpU::gus* fusion gene (pHM913GUS2) were incubated for 15 h and GUS activity was measured. Similar results were obtained from three independent experiments.

Fig. 3. Growth of 74M913 in medium containing glucose, fructose, or xylose as a sole carbohydrate source. Closed circles, open circles, and open triangles indicate T7174R, 74M913, and 74M913(pHM913EcNt), respectively. As a control, growth of T7174R and 74M913 in the nutrient-rich complex medium nutrient-broth yeast extract (NBY) was shown.

glucose or fructose in place of xylose. GUS activities from transformants incubated in each medium were measured 15 h after inoculation (Fig. 4). The activity was obtained from both transformants in all media although it was higher in NBY than in others. On the other hand, expression of an *hrp* gene (*hrcU*) was regulated by *HrpXo* and induced in XOM2 and the modified one but not in NBY as reported in previous work (31). We also tested GUS activity from a mutant 74M913GUS in various media, which has a *gus::pgi* fusion gene in the genomic DNA. The results were similar to those obtained using the transformant T7174R(pHM913GUS) (data not shown). These results suggested that *pgi* is expressed constitutively unlike *hrp* genes and that the expression is independent of the *Hrp* regulatory system.

Expression of an *hrp* gene in a *pgi* mutant was investigated (Fig. 5). Mutant 74M913 was transformed with pHMPIPGUS2 and incubated in XOM2 or modified XOM2, in which glucose was added in place of xylose. The GUS activities obtained from 74M913(pHMPIPGUS2) in xylose-containing XOM2 was low compared with that from T7174R(pHMPIPGUS2), due to the inability of the transformant to utilize xylose and, as a result, possibly due to reduced metabolism. On the other hand, 74M913(pHMPIPGUS2) and T7174R(pHMPIPGUS2) showed similar GUS activity in glucose-containing medium, indicating induction of *hrcU* expression in the *pgi* mutant.

DISCUSSION

It has been reported that *hrp* gene products, toxins, extracellular polysaccharide, extracellular hydrolytic enzyme, and their secretion pathway (type II secretion system) are involved in pathogenicity of *X. oryzae* pv. *oryzae* (11,14,15,22,28,35,40). In this study, we generated transposon-insertion mutants to isolate novel pathogenicity-related genes in the bacterium.

The pathogenicity-reduced mutant 74M913 had a transposon inserted in the region homologous with the *pgi* gene from *X. axonopodis* pv. *citri* (32). Complementation tests revealed that the *pgi* homologue is directly or indirectly involved in the virulence of *X. oryzae* pv. *oryzae*. PGI catalyzes the reversible

isomerization of glucose 6-phosphate and fructose 6-phosphate, and the enzyme is essential for fructose and glycerol catabolism in *Pseudomonas cepacia*, *P. aeruginosa*, and *X. axonopodis* pv. *citri* (3,21,32). Our results demonstrating that 74M913 could not grow in the synthetic media containing fructose as a sole carbohydrate source strongly suggest that the product of the ORF which is disrupted in the mutant functions as PGI. This was confirmed by the spectrophotometric assay for the PGI activity, showing loss of the activity in 74M913. Moreover, we also demonstrated the involvement of PGI in xylose utilization by *X. oryzae* pv. *oryzae*. On the other hand, the involvement of the gene product in glycerol catabolism was unclear because even a wild type did not grow in the synthetic medium containing glycerol.

Mutant 74M913 grew in the synthetic medium containing glucose as a sole carbohydrate source, although the growth was reduced compared with that of the control. The result is consistent with reports that glucose is catabolized mainly by the Entner-Doudoroff pathway in xanthomonads (39), and that PGI is not involved in this pathway (37).

Tung and Kuo (32) reported that loss of function of PGI in *X. axonopodis* pv. *citri* resulted in no bacterial growth in planta and no pathogenicity. In *X. oryzae* pv. *oryzae*, however, growth of the mutant and lesion formation in inoculated rice leaves was observed although they were less than those of the control. These results suggest that PGI of xanthomonads plays an important role in bacterial growth in host plants and the expression of disease symptoms; however, in the case of *X. oryzae* pv. *oryzae*, it is not necessarily essential for pathogenicity.

In our previous publications (29,30), we reported that *X. oryzae* pv. *oryzae* mutants, which are unable to utilize glucose and a glucose polymer, had a similar growth in plants and similar virulence to the wild type. Plant cell walls contain 60% xylan (polymer of xylose) in rice leaves (27), and mutants with a disrupted type II secretion pathway, which are unable to secrete xylanase, are also less virulent (22). Moreover, Miyazaki et al. (15) reported that xylanase activity increased in rice leaves infected with *X. oryzae* pv. *oryzae*. Thus, it is likely that the bacterium utilizes xylose as one of major carbohydrate sources for growth in rice leaves using PGI. However, the possibility that PGI is involved in the production of virulent factors cannot be excluded.

The expression of *hrp* genes, the most important genes for the pathogenicity of some gram-negative plant-pathogenic bacteria including *X. oryzae* pv. *oryzae*, is regulated by environmental conditions and reported to be induced only in plants or specific synthetic media (5,9,13,24,25,31,36). We had established an *hrp*-inducing medium, XOM2, for *X. oryzae* pv. *oryzae*, and showed that a more effective *hrp* induction is achieved when xylose is added to the medium as a carbohydrate source compared with glucose, fructose, or sucrose (31). In the present study, we showed that there was no specific induction of expression of *X. oryzae* pv. *oryzae* *pgi* between the *hrp*-inducing medium and the noninducing medium. Also, the expression of *pgi* was not repressed in the mutant deficient in *hrp* regulatory gene *hrpXo* (11, 19). The expression of *hrp* genes also is unlikely to be affected by *pgi*, because *hrcU* expression was observed in the *pgi* mutant when XOM2 containing glucose in place of xylose was used, and the mutant triggered HR in tomato (nonhost) and the resistant rice cultivar. Thus, the *pgi* and *hrp* genes might be expressed independently. On the other hand, the expression of *hrcU* was weaker in the *pgi* mutant than the wild type in the xylose-containing *hrp*-inducing medium XOM2. The most likely explanation for this is that the inability to utilize xylose repressed the most metabolism in the medium containing xylose as a sole carbohydrate source and, as a result, the expression of *hrp* genes also was repressed. Alternatively, the possibility exists of requirement of PGI for *hrp* expression in the presence of xylose.

X. oryzae pv. *oryzae* multiplies specifically in xylem vessels, considered to be an extremely nutrient-poor environment (16,20).

Fig. 5. Expression of *hrcU::gus* in *pgi* mutant 74M913. The mutant 74M913 and the control strain T7174R transformed with pHMPIPGUS2 were incubated in nutrient-broth yeast extract (NBY), XOM2, or the modified XOM2 containing glucose in place of xylose as a carbohydrate source and GUS activity was measured. Similar results were obtained from three independent experiments.

Noda and Kaku (18) reported that the growth rate of the bacteria was very low in guttation fluids collected from rice leaves, the components of which may be the same as those in the fluids in xylem vessels. They also indicated that addition of carbohydrate sources to the fluids was required for the bacterial growth. As described above, cell walls of rice contain much xylan (27) and this is likely to be the case in walls of xylem vessels. It is expected that, in infected tissues, *X. oryzae* pv. *oryzae* utilizes xylose obtained by xylanase for growth and/or as an inducer for the expression of *hrp* genes.

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