Journal of General Plant Pathology
https://doi.org/10.1007/s10327-026-01275-2

BACTERIAL AND PHYTOPLASMA DISEASES

Tabtoxin biosynthetic gene cluster in *Pseudomonas syringae* pv. *tabaci* 6605 genomic island 1 (GI-1$_{Pta6605}$) is required for severe disease symptoms

Kotomi Kunishi[1] · Norika Fujisawa[1] · Hidenori Matsui[1] · Nanami Sakata[1] · Yoshiteru Noutoshi[1] · Kazuhiro Toyoda[1] · Yuki Ichinose[1]

Received: 15 December 2025 / Accepted: 30 January 2026

Abstract

One of the genomic islands in *Pseudomonas syringae* pv. *tabaci* 6605 (GI-1$_{Pta6605}$) has been identified as a pathogenicity island required for virulence because the deletion almost completely eliminated disease symptoms in inoculation tests at 4×10^5 CFU/ml. GI-1$_{Pta6605}$ contains four cargo regions (CRs) named CR-1 to CR-4. The ΔCR-4 mutant did not produce tabtoxin like ΔGI-1 and disease symptoms did not develop in tobacco. However, it grew, although to a lesser extent than the wild-type strain. These results indicate that the tabtoxin biosynthetic gene cluster in GI-1 is required for virulence but not for establishment of compatibility.

Keywords GI-1$_{Pta6605}$ · Pathogenicity island · *Pseudomonas syringae* · Tabtoxin

GI-1$_{Pta6605}$, one of the genomic islands (GIs) in *Pseudomonas syringae* pv. *tabaci* 6605 (*Pta*6605), is a pathogenicity island required for virulence because its deletion almost completely eliminated disease symptoms in an inoculation assay (Watanabe et al. 2025). GI-1$_{Pta6605}$ is 110 kb in size and possesses a pseudogene and an intact tRNA^{Lys} gene at both borders. Genes in GI-1$_{Pta6605}$ can be classified into core genes (hereafter referred to as backbone regions) and accessory genes (hereafter referred to as cargo regions; CR). Backbone regions are conserved in a wide range of bacterial GIs that are essential for their transfer and maintenance, while the CR determine the function of GIs. GI-1$_{Pta6605}$ contains three type III effector genes, a tabtoxin biosynthetic gene cluster, and other unknown genes.

There are four cargo regions (CR) in GI-1$_{Pta6605}$. CR-1 (Pta6605_RS20475 to RS20530) encodes an integrase and several hypothetical proteins, and CR-2 (RS20590) shows homology to a gene for the metallophosphoesterase known as a type 9 secreted effector of *Riemerella anatipestifer* (Niu et al. 2019). *R. anatipestifer* is a bacterial causal agent of duck septicemic and exudative diseases. Recently, metallophosphoesterase, a T9S effector of *R. anatipestifer*, was identified as a crucial virulence factor. However, to our knowledge, metallophosphoesterase is not known as a virulence factor in plant pathogenic bacteria. CR-3 (RS28255 to RS20760) contains three type III effector (T3E) genes (*hopF1*: RS20695, *hopT1*: RS20735, and *hopO1*: RS20740). It is known that *Pta*6605 harbors at least 22 T3E genes (Kuroe et al. 2025). Kuroe et al. (2025) reported that Δ*hopF1* and Δ*hopO1*Δ*hopT1* caused severe disease symptoms in *Nicotiana benthamiana* comparable to those induced by the *Pta*6605 wild type (WT). Furthermore, it has been reported that mutant strains lacking more than 14 effector genes, including these three, exhibit reduced disease symptoms (Kuroe et al. 2025). However, the virulence of the strains lacking only three effector genes has not been investigated. Furthermore, Kuroe et al. investigated the effector genes of *Pta*6605 in their role in virulence in *N. benthamiana* but not in *N. tabacum*. Therefore, the importance of three genes in the interaction with *N. tabacum* cv. Xanthi remains unclear. The CR-4 (RS20815 to RS20880) is a tabtoxin biosynthetic gene cluster (Fig. 1). Tabtoxin is a non-specific phytotoxic dipeptide composed of tabtoxinine-β-lactam and either

✉ Yuki Ichinose
yuki@okayama-u.ac.jp

[1] The Graduate School of Environmental, Life, Natural Science and Technology, Okayama University, Tsushima-naka 1-1-1, Kita-ku, Okayama 700-8530, Japan

Published online: 19 February 2026

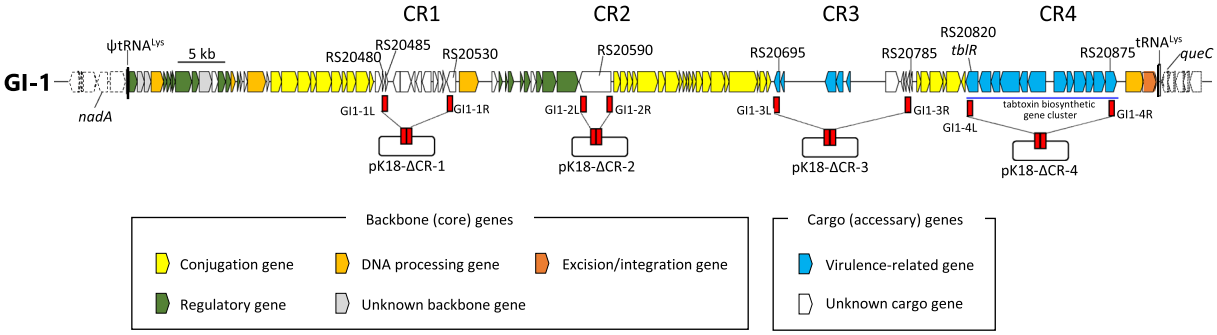

Fig. 1 Generation of each deletion mutant of cargo region in GI-1$_{Pta6605}$. A schematic model of GI-1$_{Pta6605}$ is shown. Backbone (core) regions and cargo (accessory) regions are categorized and shown with different colors as indicated. A pseudogene and an intact gene of tRNA^{Lys} are indicated by vertical lines at the boundaries of GI-1$_{Pta6605}$. To generate deletion mutants of each cargo region (CR), red DNA fragments were amplified by PCR, ligated, and inserted into a conjugative plasmid vector, pK18*mobsacB* (Schäfer et al. 1994) as shown below GI-1

serine or threonine (Arrebola et al. 2011). Tabtoxin is produced by some *P. syringae* pathovars including pvs. *tabaci*, *coronafaciens*, and *garcae* (Bender et al. 1999; Arrebola et al. 2011). *Pta*6605 WT produces tabtoxin, but its ΔGI-1 does not (Watanabe et al. 2025). Therefore, the tabtoxin biosynthetic genes are functional. Although deletion of GI-1 nearly abolished virulence, it remains unclear which CR is necessary for virulence. To identify the genes essential for GI-1 virulence, we generated a series of deletion mutants for each CR of GI-1.

To generate deletion mutant strains, we first amplified 0.5–0.6 kb DNA fragments at the left and right borders of each CR using set of PCR primers (Table S1), then cloned them into universal cloning vectors, pUC118 or pGEM-T Easy (Table S2). The left and right border DNA fragments of each CR was excised from the plasmids by *Eco*RI/*Bam*HI digestion and inserted them into pK18*mobsacB* (Schäfer et al. 1994; Fig. 1) at *Eco*RI or *Bam*HI sites. The resultant plasmids (Table S2) were introduced into *Escherichia coli* S17-1 for conjugation with the *Pta*6605 WT to obtain each CR-specific deletion mutant (Table S2). The target transconjugant was further selected by sucrose, and deletion mutants were confirmed by PCR and sequencing.

Production of tabtoxin was examined as described (Watanabe et al. 2025). Tabtoxinine-β-lactam, a compound converted from tabtoxin by peptidase, irreversibly inhibits glutamine synthetase, leading to abnormal ammonia accumulation and glutamine deficiency. In a bioassay of tabtoxin, the growth inhibitory effect of tabtoxin on *E. coli* is eliminated by the addition of exogenous glutamine. In our bioassay, clear growth inhibition zones of *E. coli* were appeared around *Pta*6605 WT, ΔCR-1, ΔCR-2, and ΔCR-3, indicating production of tabtoxin, but not around ΔGI-1 and ΔCR-4, as expected (Fig. 2).

The virulence of *Pta*6605 WT and mutant strains was assessed using *N. tabacum* cv. Xanthi by flood inoculation and infiltration methods as described (Fig. 3, Watanabe et al. 2025). For flood inoculation, tobacco seedlings grown on Murashige Skoog (MS) plates supplemented with 1% sucrose and vitamins for 2 weeks were transplanted into 0.1% sucrose MS plates and grown for a further 3 days. A bacterial suspension in 10 mM $MgSO_4$ at an OD_{600} of 0.004 (8×10^6 CFU/ml) supplemented with 0.025% (v/v) Silwet L-77 (OSI Specialties) was used for flood inoculation. Photos of seedlings were taken at 3, 5, and 7 days post-inoculation (dpi), as shown in Fig. 3A. *Pta*6605 WT caused severe symptoms, but the ΔGI-1 mutant remained healthy, as previously reported (Watanabe et al. 2025). On the other hand, the growth of seedlings inoculated by WT and all ΔCR strains was suppressed. Furthermore, tobacco seedlings inoculated with *Pta*6605 WT, ΔCR-1, ΔCR-2, and ΔCR-3 showed yellowing and necrosis at 5 and 7 dpi, whereas those inoculated with ΔCR-4 remained healthy without yellowing (Fig. 3A).

Bacterial growth of ΔGI-1 in tobacco leaves of cv. Xanti inoculated with flood assays was significantly lower than that of WT at 3 dpi, as previously reported (Fig. 3B). However, bacterial growth of ΔCR-1, ΔCR-2, and ΔCR-3 was also as high as that of WT strain at 3 dpi, and that of ΔCR-4 was significantly lower than that of WT strain. Because tobacco seedlings of cv. Xanthi inoculated with ΔGI-1 and ΔCR-4 appeared healthy, the bacterial growth was measured at 5 and 7 dpi. The population of ΔGI-1 and ΔCR-4 further increased at 5 dpi, but they subsided at 7 dpi (Fig. 3C).

Tobacco leaves of cv. Xanthi were also inoculated by infiltration of these bacteria as described previously (Watanabe et al. 2025). At a low inoculum concentration ($OD_{600} = 0.0002$, 4×10^5 CFU/ml), inoculated areas with WT and ΔCR-1, -2, and -3 turned yellow at 9 dpi and necrotic at 11 dpi; however, little change was observed in ΔGI-1- and ΔCR-4-inoculated areas up to 11 dpi (Fig. 3D). Similarly, at a high inoculum concentration ($OD_{600} = 0.02$, 4×10^7 CFU/ml), ΔCR-1, -2, -3, and -4 caused necrotic regions as well as WT at 4 dpi, and necrosis was also observed at 9 dpi in ΔGI-1 (Fig. 3D). These results indicate that although ΔGI-1

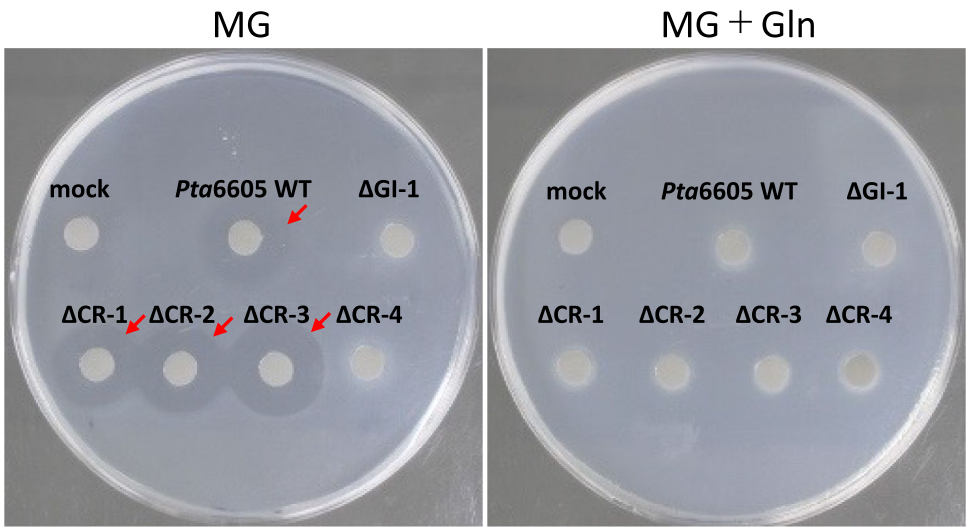

Fig. 2 Tabtoxin assay. Tabtoxin production of *Pta*6605 WT and mutant strains was assessed using *E. coli* DH5α as the indicator bacterium. *E. coli* suspension in MG (mineral salts–glucose) medium with 0.7% agar and with/without glutamine is overlaid onto the 1.5% agar MG plate. Five microliters of cultured *Pta*6605 WT and each mutant was put on the sterilized filter paper. Although tabtoxin irreversibly inhibits glutamine synthetase, exogenous addition of glutamine (Gln) can eliminate the growth inhibitory effect of tabtoxin on *E. coli*. MG + Gln and MG indicate the presence (34 μM at final concentration) or absence of glutamine in MG media. Photographs were taken after 48 h of incubation at 37°C, and representative results obtained from two independent experiments are shown. Red arrows indicate the growth inhibition zone

loses most of its virulence due to the GI-1 deficiency, it still exhibits symptoms upon inoculation at high concentrations, so it retains its pathogenicity. Furthermore, inoculation with ΔCR-4 caused symptoms earlier than inoculation with ΔGI-1, and flood inoculation caused a slight growth defect in tobacco seedlings (Fig. 3A), suggesting that in addition to CR-4, CR-1 to -3 may also contribute to virulence. In this study, we demonstrated the importance of CR4 in virulence. However, we have not confirmed that virulence is restored by complementation, because the region is over 16 kb in size. It will be an important future challenge to perform HGT of GI-1, with or without CR-4, into a strain that does not have GI-1.

These results indicate that tabtoxin production-defective *Pta*6605 is still compatible with tobacco plants, and that tabtoxin is required for the virulence of this pathogen. *P. syringae* BR2, a causal agent of bean wildfire, is also known to produce tabtoxin (Kinscherf et al. 1991). In this report, a tabtoxin-deficient BR2 derivatives did not cause disease, suggesting that tabtoxin is the major virulence factor of *P. syringae* BR2 in common beans. Furthermore, tabtoxin production was not required for growth of *P. syringae* BR2 in planta, and tabtoxin-producing *P. syringae* epiphytic bacterium Cit7 was non-pathogenic to both tobacco and bean, indicating that tabtoxin production alone is not sufficient to cause disease (Kinscherf et al. 1991).

Tabtoxin-deficient *P. syringae* pv. *tabaci* was reported as the *P. syringae* pv. *tabaci* variant '*angulata*', a causal agent of angular leaf spot of tobacco (Tanaka 1973; Willis et al. 1989). Turner and Taha (1984) reported that a non-toxigenic mutant (Tox^{-}) of *P. syringae* pv. *tabaci* was isolated after repeated in vitro culture. The Tox^{-} mutant might be generated by spontaneous deletion of GI-1. Recently, *Pta*8018, which lacks GI-1, was found to be a hypovirulent bacterium for tobacco plants (Hiraki et al. unpublished). *Pta*8018 did not cause any symptoms at low inoculation concentration like *Pta*6605 ΔGI-1 and ΔCR-4 mutant strains. However, different from these mutant strains, *Pta*8018 WT grew better than *Pta*6605 WT after inoculation (Hiraki et al. unpublished). These results suggest that, in compatible tobacco-*Pta* interactions, tabtoxin significantly contributes to disease development but plays a lesser role in bacterial proliferation. We have ever attempted to characterize pathogenicity and virulence factors of *P. syringae* and concluded that three bacterial abilities are necessary to cause disease (Ichinose et al. 2013). The three bacterial abilities are: (1) the ability to invade the host, (2) the ability to suppress the host's resistance, and (3) the ability to harm and cause disease in the host (Ichinose et al. 2013). In the case of *P. syringae*, type III effectors (T3Es) are thought to be the primary factors of the ability to suppress host resistance, and compatibility is thought to be primarily determined by T3Es, whereas toxins such as tabtoxin are a major factor in host harm and disease induction.

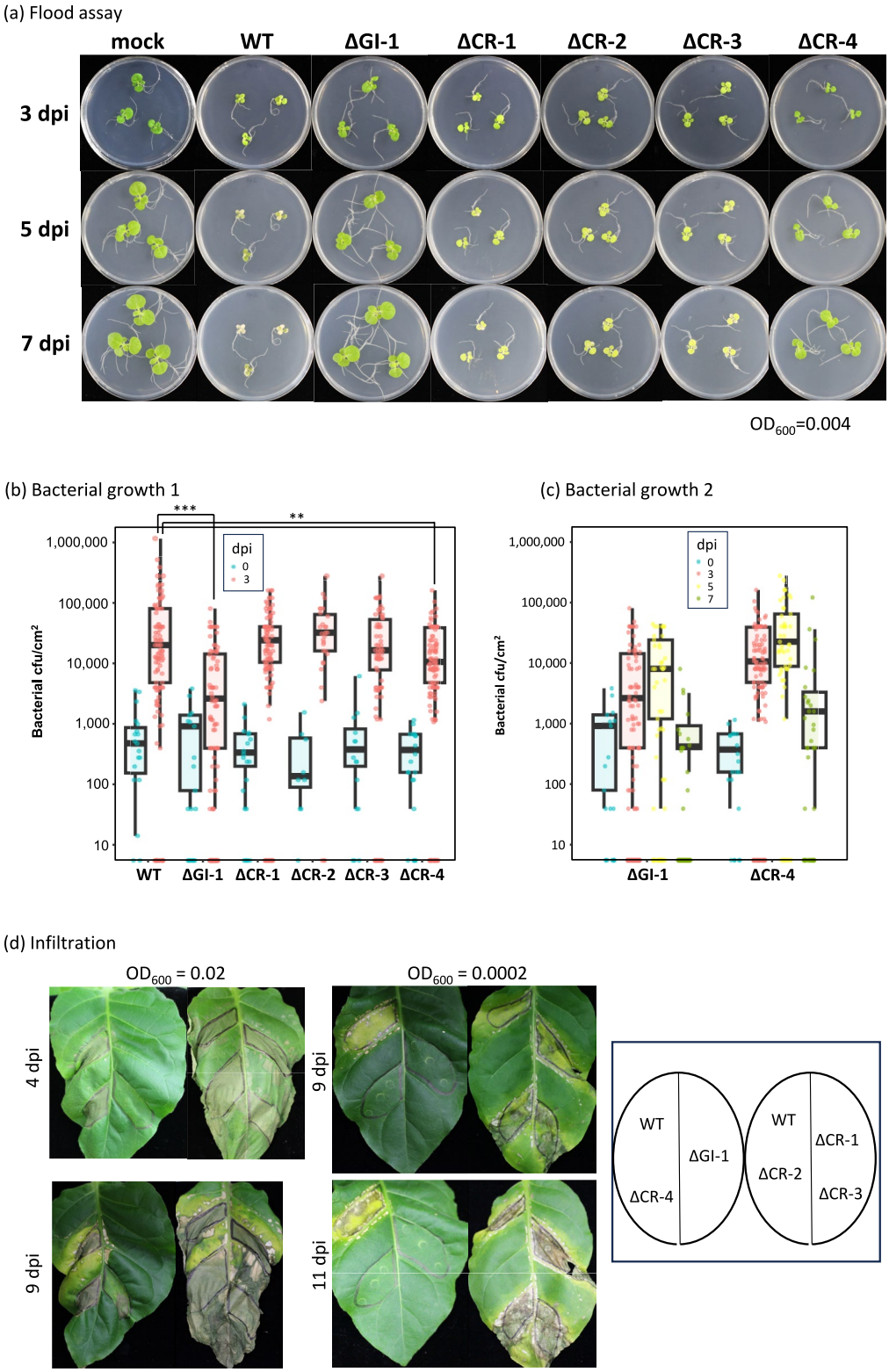

Fig. 3 Virulence assay. *Nicotiana tabacum* cv. Xanthi was used in all experiments. **a** Tobacco seedlings were inoculated by flooding 8×10^6 CFU/ml of the bacterial suspension of each strain, followed by incubation at 22 °C. Photographs taken at 3, 5, and 7 dpi show representative results from three independent experiments. **b** The bacterial population after flood inoculations (8×10^6 CFU/ml) was counted at 0 dpi (3 hpi) and 3 dpi. Bars represent standard errors from 4 independent experiments. Bacterial CFUs for each strain in one experiment from 4 individuals were pooled (**$p<0.01$; ***$p<0.001$ by a one-way ANOVA followed by Dunnett's multiple comparisons test). **c** The bacterial population of ΔGI-1 and ΔCR-4 was counted at 0 to 7 dpi in flood inoculations (8×10^6 CFU/ml). Whiskers represent the maximum and minimum values from three independent experiments. **d** Infiltration inoculation at $OD_{600}=0.02$ (left) and $OD_{600}=0.0002$ (right). Photos were taken at 4 dpi and 9 dpi after the inoculation of $OD_{600}=0.02$, and at 9 dpi and 11 dpi after the inoculation of $OD_{600}=0.0002$

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10327-026-01275-2.

Acknowledgements This work was supported in part by Grants-in-Aid for Scientific Research (B) (no. 22H0234814) from the Ministry of Education, Culture, Sports, Science and Technology of Japan to YI. This work was supported by JSPS Program for Forming Japan's Peak Research Universities (J-PEAKS) Grant Number JPJS00420230010.

Author contributions HM, NS, YN, KT, and YI conceived the study and designed the experiments. KK and NF performed experiments and analyzed data. YI drafted the manuscript, and HM, NS, YN, and KT contributed to data interpretation and the critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

Funding Open Access funding provided by Okayama University

Declarations

Conflict of interest All authors declare that there is no conflict of interest.

Ethical approval This article does not include any experiments with animals or humans conducted by any of the authors.

References

Arrebola E, Cazorla FM, Pérez-García A, de Vicente A (2011) Genes involved in the production of antimetabolite toxins by *Pseudomonas syringae* pathovars. Genes 2:640–660. https://doi.org/10.3390/genes2030640

Bender C, Alarcón-Chaidez F, Gross D (1999) *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiol Mol Biol Rev 63:266–292. https://doi.org/10.1128/mmbr.63.2.266-292.1999

Ichinose Y, Taguchi F, Mukaihara T (2013) Pathogenicity and virulence factors of *Pseudomonas syringae*. J Gen Plant Pathol 79:285–296. https://doi.org/10.1007/s10327-013-0452-8

Kinscherf TG, Coleman RH, Barta TM, Willis DK (1991) Cloning and expression of the tabtoxin biosynthetic region from *Pseudomonas syringae*. J Bacteriol 173:4124–4132. https://doi.org/10.1128/jb.173.13.4124-4132.1991

Kuroe K, Nishimura T, Kashihara S, Noutoshi Y, Sakata N, Yamamoto M, Toyoda K, Ichinose Y, Matsui H (2025) *Pseudomonas syringae* pv. *tabaci* 6605 requires seven type III effectors to infect *Nicotiana benthamiana*. Mol Plant Pathol 26:e70091. https://doi.org/10.1111/mpp.70091

Niu P, Chen Z, Ren X, Han W, Dong H, Shen R, Ding C, Zhu S, Yu S (2019) A *Riemerella anatipestifer* metallophosphoesterase that displays phosphatase activity and is associated with virulence. Appl Environ Microbiol 87:e00086-21. https://doi.org/10.1128/AEM.00086-21

Schäfer A, Tauch A, Jäger W, Kalinowski J, Thierbach G, Pühler A (1994) Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. Gene 145:69–73. https://doi.org/10.1016/0378-1119(94)90324-7

Tanaka Y (1973) Occurrence of angular leaf spot disease of tobacco in Japan. Ann Phytopathol Soc Jpn 39:312–317

Turner JG, Taha RR (1984) Contribution of tabtoxin to the pathogenicity of *Pseudomonas syringae* pv. *tabaci*. Physiol Plant Pathol 25:55–69. https://doi.org/10.1016/0048-4059(84)90017-1

Watanabe Y, Kunishi K, Matsui H, Sakata N, Noutoshi Y, Toyoda K, Ichinose Y (2025) Genomic islands of *Pseudomonas syringae* pv. *tabaci* 6605: identification of *Pta*GI-1 as a pathogenicity island with effector genes and a tabtoxin cluster. Mol Plant Pathol 26:e70087. https://doi.org/10.1111/mpp.70087

Willis DK, Kinscherf TG, Coleman RH, Barta TM (1989) Identification and isolation of a region required for tabtoxin production: apparent deletion in *Pseudomonas syringae* pv. *tabaci* variant '*angulata*' and tox$^-$ mutants. Graniti et al (eds) NATO ASI Series vol H27, phytotoxins and plant pathogenesis. Springer, pp 61–70