COMPARATIVE ANALYSIS OF VIRULENCE FACTORS IN THREE INDONESIAN *Xanthomonas oryzae* pv *oryzae* STRAINS AND A SIGNAL FACTOR BIOSENSOR STRAIN

Fatimah[1*], Iqbal Kusnandarsyah[1], Apon Zaenal Mustopa[2], and Nurlaili Ekawati[2]

[1]Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Indonesia
[2]Research Center for Biotechnology, Indonesian Institute of Sciences, Indonesia.

Abstract

Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the cause of bacterial leaf blight disease in rice (*Oryza sativa* L.). In understanding virulence determinants of three Indonesian *Xoo* strains, we identified extracellular polysaccharide (EPS) production, cellulose, and diffusible signal factor (DSF). The production of virulence factor was regulated by DSF signaling system in *Xoo*. In this study, we conducted a comparative analysis with the DSF biosensor isolate (*Xoo-dsf*). The comparative analysis of EPS production and cellulose activity in Xoo1130 and Xoo1122 showed similar results with *Xoo-dsf*. Xoo1130 produced three signals of DSF-family which are similar to *Xoo-dsf*. This indicates that the biosynthesis signals of DSF-family in Xoo1130 uses a similar autoregulation mechanism with *Xoo-dsf* which seems to be conserved in *Xoo* and in Indonesian *Xoo*.

Keywords: Cellulose activity, diffusible signal factor (DSF), Extracellular polysaccharide (EPS)

---

[*]Corresponding author & main contributor:
Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Jl. Tentara Pelajar No.3A Cimanggu, Bogor 16111.
Tel. +62-21-8754587, Fax. +62-21-87754588
E-mail. fatimahsuw@gmail.com

Introduction

Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the cause of bacterial leaf blight disease in rice (Shen & Ronald, 2002). *Xoo* infiltrated by means of hydathodes or wounds, proliferates in the epitheme, and passing through to the xylem vessels. The active propagation starts to develop the symptoms of blight disease on susceptible rice leaves. *Xoo* yielded factors of virulence that are simultaneously required for virulency which include iron chelating siderophores, extracellular enzyme, EPS, and the type III-secretion dependent effectors (Jha *et al.,* 2007).

The factors of virulence lead to an essential part in effective formation of *Xoo* in the leaves of the host plant including EPS, extracellular enzymes, and type III effectors (Liu *et al*. 2014). EPS present an important task in assisting the bacteria adhesion to the surface of host in the early stage of disease development during plant–pathogen interactions (Wang *et al.,* 2008). The plant pathogen cells secreted cell-wall degrading enzymes, as well as xylanases, pectinases, cellulases, and proteases to break the components of cell walls of the susceptible host. It leads an important task in bacterial nutrition and virulence (Temuujin *et al*. 2011). The cell wall is comprised of microfibrils of cellulose that implanted in the matrix of hemicellulose, pectic polysaccharides, and structural proteins in less amount (Zhao *et al.,* 2019). The *Xoo* type III secretion system as one of virulence factors enable *Xoo* to assault the rice leaves by secreting cell wall degrading enzymes such as cellulase, polygalacturonase, xylanase, lipase, and endoglucanase (Zou *et al.,* 2012).

It has been reported that the secreted protein such as adhesins was promoting the virulence of bacteria, as well as the diffusible factor signal (DFS) synthase, which belongs to the enoyl-co-A-hydratase family, taking a part

DOI: http://dx.doi.org/10.14203/ann.bogor.2021.v25.n1.8-14

in adhesion, conformation of biofilm, and virulence (Rai *et al.,* 2012).

There are more than nine DSF signal family members spread amongst bacteria (Zhou *et al*., 2017). Three of which were found in *Xanthomonas campestris* (*Xcc*) and *Xoo,* i.e., (1) cis-11-methyl-dodecenoic acid known as Diffusible Signal Factor (DSF), (2) cis-2-dodecenoic acid known as Burkholderia Diffusible Signal Factor (BDSF), and (3) cis, cis-11-methyldodeca-2,5-dienoic acid (CDSF) produced from *Burkholderia cenocepacia* complex (Wang *et al*., 2004; He *et al*., 2010; Deng *et al.,* 2011). While trans-2-decenoic acid known as Streptococcus Diffusible Signal Factor (SDSF) was produced by *Streptococcus* mutans (Vílchez *et al.,* 2010), 2-tetradecenoic acid and 2-cis-hexadecanoic acid were known as Xylella fastidiosa Diffusible Signal Factor (XfDSF1 and XfDSF2, respectively), produced by *Xylella fastidiosa* (Beaulieu *et al*., 2013; Ionescu *et al.,* 2016).

The assessement of DSF family signal is important for conception of its metabolic pathways, the networks of signaling, in addition to the role of regulatory enzyme. The expression of virulence gene is managed by DSF-dependent microorganisms as a response to environmental changes (Zhou *et al.,* 2017).

To understand the virulence determinants of three Indonesian *Xoo* strains, EPS production, cellulose, and DSF were identified. In this study, the comparative analysis was conducted with the DSF biosensor isolate (*Xoo-dsf*).

Materials and Methods

Bacterial growth conditions and morphology. Three field-strains of *Xoo* and control (Xoo-*dsf*) were selected for characterization (Table 1). *Xoo* strains were routinely grown at 28 °C in Wakimoto medium contains 20g/L Sucrose, 5 g/L Peptone, 0.5g/L $Ca(NO_3)_2$ $4.H_2O$, 1.82 g/L Na_2HPO_4 7 H_2O, 0.05 g/L $FeSO_4$ $7.H_2O$ (Ou, 1985) and 15 g of agar per liter. The appearance of the colony morphology was observed based on the shape, surface, consistency, and pigmentation.

Table 1. List of *Xoo* used in this comparative study.

Strain	Collection site	Cultivar source	Source
FE58 (Xoo-dsf)	-	-	IMCB*
Xoo93229	Harjobinangun, Yogyakarta	Cisadane	ICABIOGRAD
Xoo11-022	Maninjau, West Sumatera	Kuriak Putiah	ICABIOGRAD
Xoo11-030	Maninjau, West Sumatera	Kuriak Putiah	ICABIOGRAD

*Institute of Molecular and Cell Biology, Singapore

Production of EPS. The EPS production was carried out as explained by Jeong *et al.,* (2008). Each of the single colony of *Xoo* isolate was inoculated into 40 ml of Nutrient Broth (NB) medium and incubated with agitation at 28ºC for 72 hours. The cultures of bacterial were adjusted with NB medium at 600 nm for optical density (OD) value up to 1.0. The supernatants of culture were added with potassium chloride to a 1.0% (w/v) final concentration. The absolute ethanol was added two times volume to the supernatants and then store overnight at -20°C. The EPS was precipitated and spun down at 30.000 rpm for 30 minutes. The pellets were oven dried overnight at 55°C prior to dry weight measurement. The experiment was repeated twice with three replications.

Cellulolytic activity assay. The cellulolytic activity was assessed as explained by Jeong *et al.* (2008). The *Xoo* isolates were cultured in NB medium. After three days, the bacterial cultures were harvested at an OD_{600} of 1.0. The culture supernatant of thirty microliters was placed in a well in the agar medium, which comprised 50 mM sodium phosphate (pH 7.0), 0.02% sodium azide, 0.8% agarose, and 0.1% carboxymethyl cellulose. The plates were incubated at 28ºC for 20 hours. The incubated plates were stained for 10 minutes with 0.1% Congo Red and then washed with 1 M NaCl several times. The cellulolytic activity was measured by the clear zone diameter surrounding the well.

Growth curve. A 100 mL of potato sucrose broth (PSB) in a 250 mL Conical flask was inoculated with 1 ml of overnight *Xoo* culture. The flask containing culture was incubated in an orbital shaker at 28°C, 150 rpm. The aliquots of the culture were taken aseptically at regular intervals and turbidity measured in a spectrophotometer (Spectrophotometer UV

VIS SP-UV 200) at 600 nm using PSB medium as blank. The OD was evaluated every three hours until 48 hours of growth.

Purification of DSF, BDSF, and CDSF. The purification of DSF, BDSF, and CDSF was done using the method explained by He *et al.* (2010). Four *Xoo* strains were cultured for 48 hours in Yeast Extract Beef (YEB) medium. The centrifugation (Hermle Labortechnik) of five liters of bacterial supernatant were conducted at 3,800 rpm for 30 minutes at 4°C. The supernatants was adjusted by adding 1 M hydrochloric acid (HCl) to pH 4.0 before extraction. This mixture was added with two times volume of ethyl acetate. The layers of ethyl acetate were collected and dryness was conducted by removing the solvent using rotary evaporation at 40°C. A volume of twenty mililiter of methanol was used for dissolving the residue. The crude extract was analyzed for TLC. It was splited into four batches in TLC silica gel aluminum 60 F254. Three batches were eluted with compositions of hexane-ethyl acetate gradient (8:2, 9:1, and 7.5: 2.5 (v/v) 0.05% acetic acid) and one batch with composition of Chloroform: Methanol: H_2O (7:2:1, v/v). The collected active component was then applied to HPLC on a C18 reverse-phase column (4.6 × 250 mm, Phenomenex Luna), eluted with methanol: H_2O (80:20, v/v, 0.1% formic acid) at a flow rate of 1 ml/min in a Waters 2695 system with 996 PDA detector. The HPLC analysis was performed in BPPT (Agency for the Assessment and Application of Technology).

Results

Colony morphology of *Xoo* strains. Three Indonesian *Xoo* strains and *Xoo-dsf* strain were investigated based on their colony morphology. The colonies of *Xoo* were round ended, circular, rod-shaped, convex, faint yellow to dark yellow, shiny and vague surface (Figure 1). In comparison with other three *Xoo* strains (Xoo1130, Xoo1122, and *Xoo-dsf*) Xoo93229 was less shine and slimy.

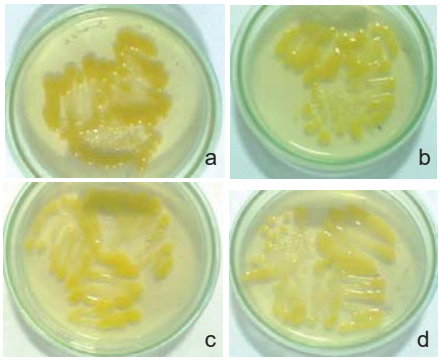

Figure.1. The appearance of colony morphologies of a) *Xoo-dsf*, b) Xoo1122, c) Xoo93229, and d) Xoo1130. The bacterial strains were cultured for four days on Wakimoto agar plates.

Measurement of EPS. In this study, three Indonesian *Xoo* strains produced different yields of EPS. The dry weight production of Xoo1130 and Xoo1122 strains were 80 mg similar with *Xoo-dsf* while Xoo93229 showed less production around 40 mg.

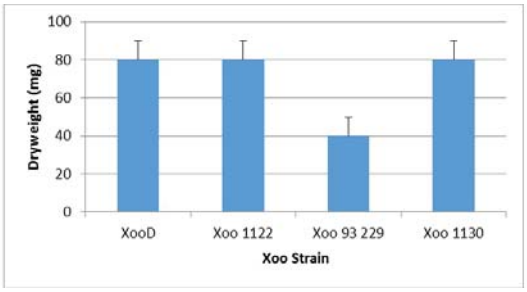

Figure 2. EPS production at OD_{600} = 1.0 of three Indonesian *Xoo* strains and *Xoo-dsf.*

Cellulase activity. The cellulose hydrolysis indicated by the appearance of clear zones/halo regions around the growing bacterial colonies. The average inhibition zone of four *Xoo* strains was 17-18 mm diameter of which Xoo1130 has similar inhibition zone with *Xoo-dsf* (Table 2).

Table 2. Diameter of inhibition zone on CMC agar plates of tested *Xoo* strains.

Xoo strains	Diameter (mm)
Xoo-dsf	18.0±0.4
Xoo1122	17.0±0.6
Xoo93229	17.5±0.2
Xoo1130	18.0±0.5

Annales Bogorienses Vol. 25, No. 1, 2021
DOI: http://dx.doi.org/10.14203/ann.bogor.2021.v25.n1.8-14

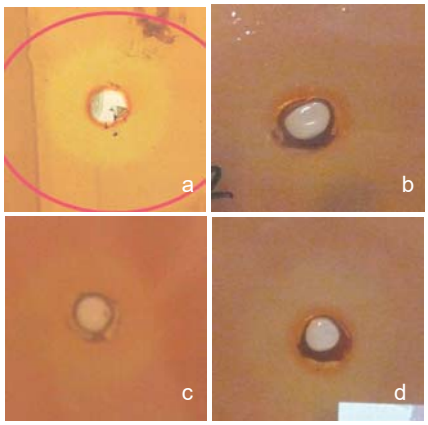

Figure 3. Halo region of four *Xoo* strains on CMC agar plates after incubation for 20 hours. a) *Xoo-dsf*, b) Xoo1122, c) Xoo93229, and d) Xoo1130.

Growth curve. The growth of *Xoo* population was analyzed by the growth curve of the *Xoo* culture. The growth of *Xoo* was plotted as the incubation time (time course) against the number of living cells logarithm (OD_{600}). The growth of the cells started at the early log exponential phase until 18 hours and reached its maximum at the early stationary phase (mid log) on 24 hours, then remained stable until 48 hours during stationary phase. The resulted curve has three different phases illustrating the increasing of cell density of four *Xoo* strains from 0.1 up to 2.7 (OD_{600}) in the course of 48 hours of growth at 28ºC (Figure 4). It revealed that the cell density of three Indonesian *Xoo* strains were higher as compared to *Xoo-dsf* during stationary phase in 24 hours steady until 48 hours.

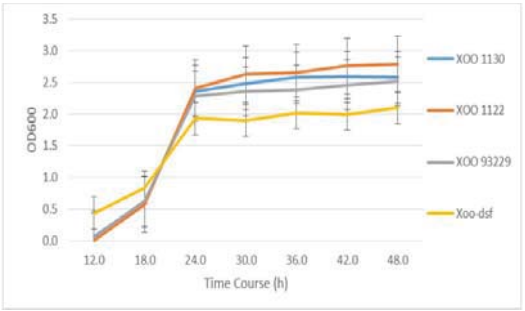

Figure 4. Growth curve of *Xoo-dsf* and three Indonesian *Xoo* strains.

Purification of DSF, BDSF, and CDSF. From our previous result, the Xoo1130 strain was more virulent than the other two Indonesian *Xoo* strains (Fatimah *et al.,* 2014). Therefore, the HPLC analysis was conducted in the comparation of Xoo1130 and *Xoo-dsf*. Based on the separation using HPLC, three active fractions were detected at retention time 16.0 16.9, and 22.0 minutes. The maximum UV absorption at 217 nm was exhibited. The results displayed that Xoo1130 yields three signals of DSF-family that shared similar pattern to *Xoo-dsf* of DSF and CDSF. However, the BDSF of Xoo1130 was sharper than *Xoo-dsf* (Figure 6).

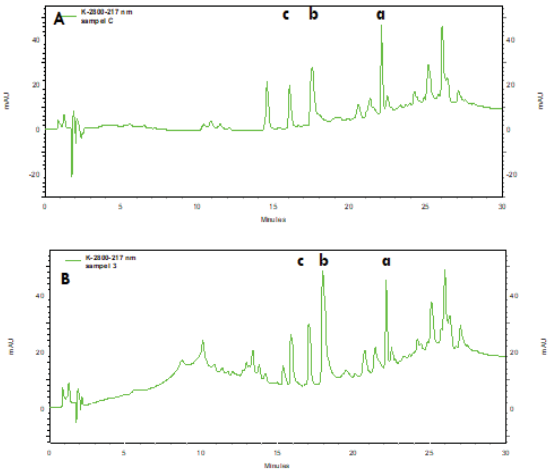

Figure 6. The chromatogram of of the active fractions from HPLC analysis (A) *Xoo-dsf* and (B) Xoo1130. *Xoo* yielded several signals of DSF-family, a= DSF, b=BDSF, and c=CDSF.

Discussion

The EPS of Xoo1130, Xoo1122 and *Xoo-dsf* strains gave higher yield as compared to Xoo93229. Based on their colony morphology, these three isolates were more shiny and mucoid as compared to Xoo93229. The characteristic was resulted from copious amounts of EPS production, known as xanthan gum and the yellow pigment xanthomonadin (Shen & Ronald, 2002).

The hydration on EPS provides defense mechanism towards the compounds of hydrophobic bacterio-static and desiccation in the intercellular space between mesophyll cells in leaf tissue. The attachment of pathogen in the surfaces of plant cell is assisted by the quality of adhesion during the initial phases of disease development in the plant–pathogen interactions (Vojnov *et al.,* 1998).

Xoo proliferates in xylem vessel of rice leaf tissue, in spite of that after 3–7 days, the *Xoo* isolates are penetrated into mesophyll tissue. The wounded leaves of susceptible rice

DOI: http://dx.doi.org/10.14203/ann.bogor.2021.v25.n1.8-14

varieties were resulted from these two events (Cao *et al.,* 2019). Cao *et al.* (2020) mentioned that *Xoo* and *Xoc* multiplied in the intercellular space between mesophyll cells in the leaf tissue, on the 5th day after inoculation. that caused the formation of bacterial exudates on wounded leaves and the lesions turning into yellow.

In addition, in this study, we obtained a consistent result for optical density through the used of NB medium because it does not contain sucrose. It has been reported that high background in assays of some extracellular enzyme activities is a result of high concentrations of sucrose in the medium (He *et al*., 2010; Jeong *et al.,* 2008).

In this study, Xoo1130 has similar inhibition zone with *Xoo-dsf*. The ability of the bacteria to hydrolyze cellulase was shown by the diameter of the clear zone. Degradation of CMC was determined indirectly by the bacterial isolate capacity to degrade cellulase, representing the activity of endoglucolytic (Soares *et al*. 2012).

The virulence of *Xoo* was regulated by the role of the genes encoding cellulase. It has been stated that the defense responses in rice was induced by purified cellulase. However, in *Xoo* with type III secretion system-dependent, this mechanism was suppressed. Consequently, the virulence characteristic associated with the expression of cellulase genes acts as a hint of pathogenicity-related gene directly or indirectly (Temuujin *et al.*, 2011; Temuujin & Kang, 2017).

In *Xcc,* the production of DSF at the late stationary phase was found to be maximal (Wang *et al*., 2004). In this study, the HPLC analysis displayed three profile of DSF-family signals production in Xoo1130, similar to *Xoo-dsf*. From these three DSF-family signals, two signals (DSF and CDSF) of Xoo1130 and *Xoo-dsf* gave similar profile. Based on the results of growth curve showed that four *Xoo* strains grew during the first 18 h and then multiplied exponentially at about 24 h. However, the bacterial growth of *Xoo-dsf* relatively slower (OD_{600}=2.0±0.2) as compared to three Indonesian *Xoo* strains (OD_{600}=2.5±0.4) during the stationary phase.

The results were different with He *et al.* (2010). It is reported that CDSF shared a similar production pattern as DSF while BDSF production occurred 6 hours earlier than DSF and CDSF however the production of BDSF was swoop down observed 6 h later (we named it BDSF-type 1). In contrast, in this study, the bacterial growth of Xoo1130 was faster and multiplied during the stationary phase as compared to *Xoo-dsf*. Moreover, the HPLC analysis profile of BDSF in Xoo1130 (we named it BSDF-type 2) was sharper than BDSF in *Xoo-dsf,* assuming that the production of BDSF-type 2 in Xoo1130 occurred maximum from the other two signals. It is suggested that these precursors induced tremendous bacterial growth and virulence factor production.

EPS production were induced by DSF, BDSF, and CDSF as functional signals. The structure of BDSF is different from DSF at the position of C-11 in the substitution of methyl. In comparison with other two signaling factors (DSF and BDSF), the EPS production in CDSF was slighter active. The utmost production of BDSF happenend afore as compared to DSF and CDSF signals, suggesting that BDSF was yielded distinctly throughtout the growth of bacterial (He *et al.,* 2010).

In addition, the medium we used in this study for bacterial growth of four *Xoo* strains were YEB medium. He *et al.* (2010) reported that YEB medium is a rich medium that supported the best bacterial growth as compared to LB, PSA, NYG, and XOLN medium. Furthermore, with regard to signaling factor, the composition of poor nutrinionally medium also influenced the proportion of the signals of three DSF-family.

As conclusions, the comparative analysis of three Indonesian *Xoo* and *Xoo-dsf* revealed high yield of EPS and cellulose activity of Xoo1130 and Xoo1122 strains, giving similar result to *Xoo-dsf*. The Xoo1130 strains yielded three signals of DSF-family, *i.e.* DSF, BDSF, and CDSF, similar to the *Xoo-dsf*. Taken together the results suggest that the mechanisms of the DSF signalling was autoregulate that likely conserved in *Xoo* and also in Indonesian *Xoo.*

Acknowledgements

The author would gratefully acknowledge Professor Ya-Wen He from the Institute of Molecular and Cell Biology, Singapore for providing DSF biosensor isolate. This research was funded by the 16th Research and Technology grant of Indonesian Toray Science Foundation. F are Main Contributor, I. K., A.

DOI: http://dx.doi.org/10.14203/ann.bogor.2021.v25.n1.8-14

Z. M. and N. E. are Member Contributor in this paper. F designed and performed experiments, analysed data, and wrote the paper. I. K performed experiments and manage culture, A. Z. M and N. E analysed data.

References

Beaulieu, E.D., Ionescu, M., Chatterjee, S., Yokota, K., Trauner, D., & Lindow, S. (2013). Characterization of a diffusible signaling factor from *Xylella fastidiosa*. *MBio, 4*(1), e00539-12. doi: 10.1128/mBio.00539-12.

Cao, J., Chu, C., Zhang, M., He, L., Qin, L., Li, X., & Yuan, M. (2020). Different cell wall-degradation ability leads to tissue-specificity between *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola. Pathogens*, *9*:187. doi:10.3390/pathogens9030187.

Cao, J., Zhang, M., Zhu, M., He, L., Xiao, J., Li, X., & Yuan, M. (2019). Autophagy-like cell death regulates hydrogen peroxide and calcium ion distribution in *Xa3/Xa26*-mediated resistance to *Xanthomonas oryzae* pv. *oryzae*. *International Journal Molecular Science, 21*: 194.

Deng, Y., Wu, J., Tao, F., & Zhang, L.H. (2011). Listening to a new language: DSF-based quorum sensing in Gram-negative bacteria. *Chemical Reviews, 111*:160-173. DOI: 10.1021/cr100354f.

Fatimah, Mustopa, A.Z., & Kusnandarsyah, I. (2014). Identification and characterization of virulence factor of several Indonesian *Xanthomonas oryzae* pv. *oryzae. Mikrobiology Indonesia, 8*(3):103-111. DOI: 10.5454/mi.8.3.3.

He, Y.W., Wu, J., Cha, J.S., & Zhang, L.H. (2010). Rice bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* produces multiple DSF-family signals in regulation of virulence factor production. *BMC Microbiology, 10*:187. doi: 10.1186/1471-2180-10-187.

Ionescu, M., Yokota, K., Antonova, E., Garcia, A., Beaulieu, E., Hayes, T., Lavarone, A.T., & Lindow, S.E. (2016) Promiscuous Diffusible Signal Factor Production and Responsiveness of the *Xylella fastidiosa* Rpf System. *MBio*,*7*(4), pii: e01054-16 DOI**:** https://doi.org/10.1128/mBio.01054-16**.**

Jeong, K.S., Lee, S.E., Han, J.W., Yang, S.U., Lee, B.M., Noh, T.H., & Cha, J.S. (2008). Virulence reduction and differing regulation of virulence genes in rpf mutants of *Xanthomonas oryzae* pv. *oryzae. Plant Pathology Journal, 24*(2):143-151.

Jha, G., Rajeshwari, R., & Sonti, R.V. (2007). Functional interplay between two *Xanthomonas oryzae* pv. *oryzae* secretion systems in modulating virulence on rice**.** *Molecular Plant-Microbe Interaction*, *20*:31-40.

Liu, W., Jinling, L., Lindsay, T., Jan, E.L., & Guo, L.W. (2014). Novel insights into rice innate immunity against bacterial and fungal pathogens. *Annual Review Phytopathology*. *52*:213-41 doi: 10.1146/annurev-phyto-102313-045926.

Ou, S. H. (1985). Rice Diseases. Kew, UK: Commonwealth Agricultural Bureaux.

Rai, R., Ranjan, M., Pradhan, B.B., & Chatterjee, S. (2012). A typical regulation of virulence-associated functions by a diffusible signal factor in *Xanthomonas oryzae* pv. *oryzae. Molecular Plant Microbe Interactaction, 25*: 789-801.

Soares, F.L., Itamar, S.M., Armando, C.F.D., & Fernando, D.A. (2012). Cellulolytic bacteria from soils in harsh environments. *World Journal Microbiology Biotechnolology, 28*:2195-2203. doi 10.1007/s11274-012-1025-2.

Shen, Y., & Ronald, P. (2002). Molecular determinants of disease and resistance in interactions of *Xanthomonas oryzae* pv. *oryzae* and rice. *Microbes and Infection, 4*: 1361-1367.

Temuujin, U., Kim, J.W., Kim, J.K., Lee, B.M., & Kang, H.W. (2011). Identification of novel pathogenicity-related cellulase genes in *Xanthomonas oryzae pv. oryzae*. *Physiology Molecular Plant Pathology, 76*: 152-157.

Temuujin, U., & Kang, H.W. (2017). Expressional regulation of the virulence gene eglXoA encoding endoglucanase, dependent on HrpXo and cyclic AMP Receptor-Like Protein (Clp) in *Xanthomonas oryzae* pv. *oryzae*. *Journal of Plant Pathology Microbiology, 8*:8 DOI: 10.4172/2157-7471.1000416.

Vílchez, R., Lemme, A., Ballhausen, B., Thiel, V., Schulz, S., Jansen, R., Sztajer, H., & Dobler, I.W. (2010). *Streptococcus* mutans inhibits *Candida albicans* hyphal formation by the fatty acid signaling molecule trans-2-decenoic acid (SDSF). *Chembiochem, 11*(11):1552-1562. DOI: 10.1002/cbic.201000086.

Vojnov, A.A., Zorreguieta, A., Dow, J.M., Daniels, M.J. & Dankert, M.A. (1998). Evidence for a role for the gumB and gumC gene products in the formation of xanthan from its pentasaccharide repeating unit by *Xanthomonas campestris*. *Microbiology,144*:1487-1493.

Wang, C.L., Xu, A., Gao, Y., Fan, Y.L., Liang, Y.T., Zheng, C.K., Sun, L.Q., Wang, W.Q., & Zhao, K.J. (2008). Generation and characterization of Tn5-tagged *Xanthomonas oryzae* pv. *oryzae* mutants that overcome *Xa23*-mediated resistance to bacterial blight of rice. *European Journal of Plant Pathology, 123*:343-351.

Wang, L.H., He, Y.W., Gao, Y.F., Wu, J.E., Dong, Y.H., He, C., Wang, S.X., Weng, L.X., Xu, J.L., Tay, L., Fang, R.X., & Zhang, L.H. (2004). A bacterial cell-cell communication signal with cross-kingdom structural analogues**.** *Molecular Microbiology, 51*:903-912.

Zhao, Y., Man, Y., Wen, J., Guo, Y., & Lin, J. (2019). Advances in imaging plant cell walls. *Trends in Plant Science, 24*:867–878.

Zhou, L., Zhang, L.H., Cámara, M., & He, Y.W. (2017). The DSF family of quorum sensing signals: diversity, biosynthesis, and turnover. *Trends Microbiology. 25*(4):293-303. DOI: 10.1016/j.tim.2016.11.013.

Zou, H.-S., Song, X., Zou, L.F., Yuan, L., Li, Y.R., Guo, W., Che, Y.Z., Zhao, W., Duan, Y.P., & Chen, G.Y. (2012). EcpA, An extracellular protease, is asSpecific virulence factor required by *Xanthomonas oryzae* pv. *oryzicola* but not by *X. oryzae* pv. *oryzae* in rice. *Microbiology,158*:2372–2383.