Hyperinduction of pectate lyase in *Dickeya chrysanthemi* EC16 by plant-derived sugars

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ABSTRACT

Pectate lyase (Pel) synthesis in *Dickeya chrysanthemi* has been reported to be hyperinduced in planta and also in the medium containing plant extract in addition to polygalacturonate. In this study, the major components of Pel-hyperinducing fractions were found to be glucose, fructose, and sucrose by TLC and NMR. From the analysis of the sugars and their derivatives, it was found that acyclic D-hexoses with the *trans* relationship between C-3 and C-5 hydroxyl groups were found to be basic structure required for hyperinducing the expression of a major isozyme in infected plants (i.e. *pelE*). From the fact that some non-metabolizable sugars such as 2-deoxy-D-glucose and D-fucose could lead to hyperinduction and that the hyperinduction was observed only in the medium containing low concentration (<0.25%) but not higher of the sugars was added, these sugars may be considered to participate in hyperinduction as the signal rather than through their metabolism.

Introduction

*Dickeya* species (formerly *Erwinia chrysanthemi*) are members of the soft-rot enterobacteriaceae family that provokes severe damage on numerous economically important crops and ornamental plants worldwide (Ma et al. 2007; Joko et al. 2014). Until recently, *E. chrysanthemi* was divided into multiple *Dickeya* spp. including *D. chrysanthemi*, *D. dadantii* subsp. *dadantii*, *D. dadantii* subsp. *dieffenbachiae*, *D. dianthicola*, *D. paradisica*, *D. zeae* (Samson et al. 2005), and an emerging potato pathogen *D. solani* (Toth et al. 2011; Golanowska & Łojkowska 2016). Under the complexity of its phylogenetics, the well-characterized *E. chrysanthemi* strain EC16 (originally isolated from *chrysanthemum*) was transferred to *D. chrysanthemi* EC16, while strain 3937 (isolated from African violet) was changed to *D. dadantii* 3937 (Sandy & Butler 2011).

Pathogenicity of *Dickeya* spp. is mainly determined by the production of a large amount of plant cell wall-degrading enzymes and, in particular, pectate lyases (Barras et al. 1994; Toth et al. 2003) that cleave randomly α-1,4 galacturonosyl linkages of polygalacturonate by a β-elimination mechanism (Collmer and Keen 1986). Among the several pectate lyases (Pel), PelE appears to be the most important isozyme in plant maceration (Payne et al. 1987), followed by PelB, PelC, and PelA (Hugouvieux-Cotte-Pattat et al. 1996). Apart from these important virulence factors, *Dickeya* spp. also respond to small molecules in their environment such as those released by plants, after plant cell wall degradation and pectate metabolism (Charkowski et al. 2012).

Plants employ abundant small molecules in signaling and regulatory processes (Tholl & Aharoni 2014) and plant signals have been thought to play the key role in pathogen–plant interaction. For example, *Agrobacterium tumefaciens* responds to plant-derived molecules such as sugars and phenolic compounds as a signal to regulate its virulence genes (Stachel et al. 1985; Bolton et al. 1986; Ankenbauer & Nester 1990; Subramoni et al. 2014). Likewise, *D. chrysanthemi* recognizes the plant signals for the synthesis of Pel at maximum level (>200-fold) higher than the basal level by adding plant extract together with their substrate (polygalacturonate) compared to only a 9-fold induction occurred with polygalacturonate alone, a process called hyperinduction (Bourson et al. 1993). A novel regulatory protein, called Pir (plant inducible regulator), was found to be responsible for Pel-hyperinduction in responding to the plant-derived signals (Nomura et al. 1998; 1999). However, it has not been elucidated what is (are) the signal molecule(s) responsible for Pel-hyperinduction. Here we report the characterization of plant components required for *pelE*-hyperinduction in *D. chrysanthemi* EC16 as the acyclic D-hexoses with the *trans* relationship between C-3 and C-5 hydroxyl groups.

Materials and methods

**Bacterial strains, growth media, and chemicals**

Bacterial strains used in this study are described in Table 1. The bacteria were grown in YP medium (1% tryptone, 0.5% yeast extract, pH 6.8) at 27°C. M63 minimal medium was supplemented with 0.2% glycerol. When required, polygalacturonic acid (PGA, 0.2%), kanamycin (Km, 30 μg/ml), nalidixic acid (Nal, 50 μg/ml), streptomycin (Stm, 25 μg/ml) were added (Joko et al. 2007a). Plate media were solidified by the addition of 1.5% agar. Optical density of the bacterial culture was read by BactoMonitor BACT-500 at OD₆₆₀ nm (Intertech Inc., Tokyo) to estimate the number of bacterial cells (Joko et al. 2012).
Table 1. Bacterial strains used in this study.

| Strains | Relevant characteristics | Source or reference |
|---------|-------------------------|---------------------|
| Dickeya chrysanthemi | | |
| EC16 | Wild type | Laboratory collection |
| EC16n | Nal\(^{r}\), selected by spontaneous | P. Jitareerat |
| EPELE16 | EC16 integrants of Tn7-luc:pelE promoter construct, Sm\(^{r}\) | K. Nomura |
| EPELA16 | EC16 integrants of Tn7-luc:pelA promoter construct, Sm\(^{r}\) | K. Nomura |
| EPELB16 | EC16 integrants of Tn7-luc:pelB promoter construct, Sm\(^{r}\) | K. Nomura |
| EPELC16 | EC16 integrants of Tn7-luc:pelC promoter construct, Sm\(^{r}\) | K. Nomura |

\(^{a}\) Nal: nalicidic acid; Sm: streptomycin; \(^{r}\): resistance.

Enzyme assays

Plate assays for Pel were done as previously described (Joko et al. 2007b). The plate assay medium contained 1% PGA, 1% yeast extract, 0.38 \(\mu\)M CaCl\(_2\) in 100 mM Tris–HCl (pH 8.5). The medium was supplemented with 100 \(\mu\)g/ml ampicillin and solidified with 0.8% (w/v) agarose. Each petri plate (\(\phi\)100 mm) contained 25 ml of the Pel assay medium. Wells were made in the agarose medium with a cork borer (\(\phi\)5 mm), and the bottoms were sealed with 10 \(\mu\)l of 0.8% (w/v) agarose containing 100 \(\mu\)g/ml ampicillin.

To determine the total activity of Pel, bacterial cells were grown at 27°C until early stationary phase by measuring optical density at OD\(_{600}\) with BACT-500 (Intertech. Inc., Japan). One ml of the culture was sonicated two times for 20 s at 70 pulse/min (Ultrasonic Disrupter UD-200, Tomy Inc., Tokyo) on the ice and then centrifuged at 12,000 rpm for 5 min to remove cell debris. Ten \(\mu\)l of this supernatant was applied to each well in the plates and incubated at 27°C for 16–18 h. The clear zone around the wells was observed due to the degradation after flooding with 5N of H\(_2\)SO\(_4\).

Spectrophotometric assay of Pel activity was performed as done by Collmer et al. (1982) with minor modification. After a thorough mixing, the optical density at 230 nm was measured every min (Ultrospect 3000, Pharmacia Biotech, Cambridge, England). One unit of Pel activity was defined as the enzyme required for an increase of 1 \(\times\) 10\(^{-3}\) optical density at 230 nm in 1 min.

Pel-hyperinduction by various plant extracts

Extracts of several vegetables (potato tuber, radish root, turnip root, carrot root, Chinese cabbage, cauliflower, celery, green pak choy, asparagus, and broccoli) and fruits (tomato, apple, banana, cantaloupe melon, avocado, strawberry, pineapple, kiwifruit, oranges, and mango) were tested for hyperinducing ability of Pel. Extracts were obtained by cutting 1 g of vegetables and fruits into small pieces, placed into a test tube containing 10 ml of DDW, then autoclaved for 1 min at 121°C. One-tenth volume of this extract was added into the growth medium. After growth in M63 + 0.2% glycerol and 0.2% PGA with or without plant extract, total activity of Pel was assayed spectrophotometrically at OD\(_{230}\) nm as described above. When the ratio of Pel-specific activity in PGA + plant extract to that in PGA was over fivefold, we considered it as hyperinduced level.

Fractionation of pel-hyperinducing ability in potato extract

The hot-water extract was obtained after the filtration of the autoclaved chopped potato tuber in DDW through filter paper (\(\phi\)90 mm, Toyo 2500) and the filtrate was fractionated by molecular weight using sequential centrifugal filter devices with the cutoffs of 100, 50, 30, 10, and 3 kDa (Centricon, Millipore, Bedford, USA). To test the possibility of the involvement of macromolecules, the active fraction was treated with either one of lipase, protease K, and DNase I for 60 min at 37°C followed by boiling for 15 min to inactivate those enzymes. The filtrate was concentrated by freeze-drying (FDU-830, Eyela, Tokyo) and the debris was dissolved in 100% methanol. After centrifugation of the suspension at 10,000 \(\times\) g for 10 min, the supernatant and pellet were used as a methanol-soluble fraction and insoluble fraction, respectively. The centrifugation was repeated once more. Both fractions were then evaporated using a rotary evaporator at 30°C; the pellet was dissolved in sterile DDW for testing Pel-hyperinducing ability. The hyperinducing activity in each fraction was assayed by adding each fraction to an equal volume of 2\(\times\) concentrated M63 minimal medium containing 0.4% glycerol and 0.4% PGA.

Elution from Toyopearl HW-40C column

The methanol-soluble fraction, in which the hyperinducing activity was found, was loaded into the vertical column (\(\phi\)2 cm \(\times\) 30 cm) of Toyopearl HW-40C (Tosoh) silica gel and eluted with 100% methanol. The fractions after elution were evaporated using rotary evaporator at 30°C. The pellet was dissolved in 5 ml of sterile DDW and 0.5 ml of this solution was added into M63 + 0.2% glycerol + 0.2% PGA.

Thin-layer chromatography

The fractions showing Pel-hyperinducing ability after the elution from HW-40C silica gel column were spotted onto TLC plate. Then, it was developed in the mixtures of solvents (methanol/DDW, acetic-acid/ethyl-acetate/methanol/DDW, or ethyl-acetate/hexane). TLC plate was allowed to thoroughly air dry and the spots were observed under UV light and after spraying with 5% sulfuric acid in ethanol or with 0.2% naphthoresorcinol in ethanol (Mahfut et al. 2016).

Analysis with nuclear magnetic resonance

The samples for NMR were prepared by dissolving an analyze in a deuterium solvent [methyl-d8-alcohol-d (CD\(_2\)OD) and deuterium oxide (D\(_2\)O)]. Sample dissolved in 100% methanol was evaporated using rotary evaporator for 30 min. A deuterium solvent was added into the flask, mixed carefully, and evaporated for 30 min. Several drops of deuterium solvent were added to the flask, mixed thoroughly, and poured into NMR microtube (\(\phi\)5 mm) for the analysis with FT-NMR (JNM-EX270, Jeol).

Effectiveness of various sugars on pelE-hyperinduction

D. chrysanthemi EC16 constructs in which luxA-E of Vibrio fisheri (Shen et al. 1992) was fused into the genes of Pel
isozymes (pelA::lux, pelB::lux, pelC::lux, and pelE::lux) (Matsumoto et al. 2003) were cultured in M63 minimal medium containing 0.2% glycerol and 0.2% PGA supplemented with various concentration of each of the following sugars: D-arabinose, L-arabinose, D-fucose, L-fucose, D-galactose, D-glucose, L-glucose, D-mannose, D-mannitol, L-rhamnose, D-ribose, L-ribose, D-sorbitol, L-sorbitol, D-xylose, L-xylose, 2-deoxy-D-glucose, D-cellobiose, \( \beta \)-lactose, maltose, maltotriose, trehalose, melibiose, sucrose, raffinose, the enzymatically formed rare sugars (D-fructose, L-fructose, D-psicose, L-psicose, D-sorbitose, L-sorbitose, D-tagatose, L-tagatose) (Izumori 2006), and methylated sugars (methyl-\( \alpha \)-D-glucopyranoside, methyl-\( \beta \)-L-arabinopyranoside, 3-0-methyl-D-glucopyranose, \( \alpha \)-methyl-D-(+)-glucoside, methyl-\( \alpha \)-D-mannopyranoside, methyl \( \alpha \)-L-rhamnopyranoside, 3-0-methyl-D-glucopyranoside, methyl-\( \alpha \)-D-galactopyranoside, methyl \( \beta \)-D-xylopyranoside, 3-0-methyl-D-glucopyranoside). Bacterial suspension was incubated at 27°C on an orbital shaker (Dwimartina et al. 2017) (FMC-100, Eyela, Tokyo) at 160 rpm. The cell density was checked every 2 h by reading its optical density (OD660 nm) with Bact-Monitor 500 (Intertech.Inc., Japan), and the expression of pelE was assayed by reading the light production from the fused lux genes using chemiluminescence reader (Hamamatsu Photonics, Hamamatsu, Japan). The mean of 10 times of 1 s readings in 500 \( \mu \)l samples at every 2 h was expressed as the specific activity in terms of cell density (cpm/OD660).

Results

Effects of plant extract on pel-hyperinduction

When D. chrysanthemi EC16 was grown in M63 minimal medium containing 0.2% glycerol and 0.2% PGA together with 1% (w/v) potato extract, the hyperinduction of Pel was confirmed (Figure 1). Besides potato extract, the extract of various vegetables such as radish, turnip, carrot, Chinese cabbage, cauliflower, celery, green pak choy, asparagus, and broccoli and those from many fruits except avocado and mango were confirmed to hyperinduce the synthesis of Pel too (Figure 2(A,B)).

Identification of pel-hyperinducing compound(s) in potato

When a hot-water extract of potato was treated with lipase, proteinase K, and Dnase I, the hyperinducing ability of Pel was not affected. Thus, the component(s) responsible for Pel-hyperinduction seem not to be these macromolecular substrates or their breakdown products. Also, as the extract could be autoclaved at 121°C for 10 min without losing the Pel-hyperinducing ability, the compound(s) may be heat stable (Figure 3(A)). In the treatment with centrifugation-dependent filtration (Centricon) with several sizes of molecular sieves, the Pel-hyperinducing ability was found in the fraction smaller than 3 kDa (Figure 3(B)). When potato extract was dried by rotary evaporator and redissolved in 100% methanol, the Pel-hyperinducing ability was found in methanol soluble fraction (Figure 3(C)).

Silica gel column and TLC

When the above methanol-soluble fraction was loaded onto Toyopearl HW-40C silica gel (Tosoh Corp., Tokyo) vertical column (\( \varphi 2 \) cm \( \times 30 \) cm) and eluted with 100% methanol, Pel-hyperinducing activity was mainly found in 5 ml fractions with 4, 5, 6, and 7; these fractions were analyzed by ascending TLC on silica gel and were developed with chloroform/methanol (1:2) then were sprayed with \( \text{H}_2\text{SO}_4/\text{EtOH} \) (5:95). The small tailing spots appeared on the silica TLC plate suggested the presence of sugars in these fractions. Fractions 5 and 6, which showed similar Rf values at ca. 0.35 on the TLC plate, were collected and processed again with a silica gel column. The re-collected fractions were then analyzed by
spraying with naphthoresorcinol reagent onto TLC after the development with the solvents: ethyl acetate/2-propanol/ DDW (6:6:1). Few clear spots with small tailing were observed. When the samples were treated with acetate anhydride and pyridine (1:2), they showed the similar spots at known Rf values as those of authentic D-glucose, D-fructose, and sucrose on TLC plate (Figure 4).

**NMR analysis**

$^{13}$C NMR (nuclear magnetic resonance) analysis of the above-mentioned Pel-hyperinducing fractions also indicated the fraction contained mainly a mixture of sugars (Figure 5 (A)). These NMR peaks were compared with those of several authentic sugars (Figure 5(B)). The peak pattern and comparative analysis of spectra of NMR data indicated that this active fraction contained D-glucose, D-fructose, and sucrose as the major components.

**Hyperinducibility of Pel by various sugars**

Based on the results of TLC and NMR analyses, we tested the effects of several commercially available sugars on Pel production of *D. chrysanthemi* EC16. At their concentration, less than 0.25% (w/v), D-glucose, D-fructose, and sucrose in addition to 0.2% PGA and 0.2% glycerol could hyperinduce the synthesis of Pel. However, at higher concentration of

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**Figure 3.** Synthesis of pectate lyase (Pel) in *D. chrysanthemi* EC16. Bacterial cells were grown until early stationary phase at 27°C in M63-0.2% glycerol minimal medium with the indicated supplements (arrows). Heat tolerance after autoclaving (A), molecular weight range after treatment with Centricon (B), and solubility in methanol (C) of potato extract were tested for hyperinducibility of Pel synthesis.

**Figure 4.** Thin-layer chromatography (TLC) spots of active fractions for Pel-hyperinduction. The samples were developed in the mixture of acetate anhydride and pyridine (1:2). The TLC spots of the active fractions (8 + 9 and 11 + 12) together with those of authentic samples of D-glucose (G), D-fructose (F), and sucrose (S) were shown.

**Figure 5.** $^{13}$C Nuclear magnetic resonance (NMR) assays. The samples for NMR were prepared by dissolving the analyte in a deuterium solvent and it was analyzed using FT-NMR. The peaks of the sample fraction no. 8 and 9 (A) and that of a mixture of D-glucose and D-fructose (B) were shown.
these sugars (>0.25%), they seemed to have repressed it (Figure 6A–C).

When the expression of pelE isozyme was examined using gene fusion construct with promoter-less luxA-E cassette of Vibrio fischerii, low concentration (0.1%) (w/v) of the mono- saccharides such as L-arabinose, D-fructose, D-glucose, D-galactose, D-mannose, 2-deoxy-D-glucose and D-fucose, Dribose, D-xylene, the disaccharides such as melibiose, sucrose, and the trisaccharides such as raffinose, and sugar alcohol such as D-mannitol were found to hyperinduce the expression of pelE (Table 2).

Discussion

We demonstrated here that several sugars were major com- pounds in the fraction of potato extract showing Pel-hyperin- ducing after elution of the methanol-soluble fraction from the silica gel column. Bourson et al. (1993) suggested that Pel- hyperinducing compounds from carrot root could be an oligosaccharide though they failed to determine the exact chemical nature of that molecule. Furthermore, Hugouvieux-Cotte-Pattat (2004) showed that rhamnose, a neutral sugar available in two types of ramified regions of pectin was shown to hyperinduce rhiT and rhiN genes in D. dadantii 3937 after simultaneous addition with PGA. Similar mechan- ism may be responsible for hyperinduction of the expression of pel and that of the rhi genes.

D-glucose, D-fructose, and sucrose are known as major carbon sources on the surfaces of leaves or stem and they are thought to be excreted from the plant mostly from injury or from glandular trichomes (Tukey 1970). Also, plant cell walls contain a variety of glycosidases which are presumably involved in plant defence mechanisms, expansion of the plant, and disruption of the cell wall structures. Free sugars and oligosaccharides may be generated both by mechanical means and by the enzymatic cleavage of these glycosidases (McNeil et al. 1984). TLC and NMR analyses of Pel-hyperin- ducing fractions of potato hot-water extract indicated the presence of D-glucose, D-fructose, and sucrose as the major components. This result is somewhat surprising because it is already known that Pel production of D. chrysanthemi is subjected to catabolite repression (Reverchon et al. 1997). Catabolite repression occurs by lowering cyclic-AMP due to a rapid catabolism of efficient energy sources such as glucose (Magasanik 1961). In fact, a high concentration of most of these sugars (>0.25%) together with PGA caused repression on Pel synthesis probably through catabolite repression (Tsuyumu 1979). However, when their concentration was lowered to less than 0.25%, the Pel-hyperinduction began to be noticed. Considering the low concentration of sugars on the surface and in the apoplastic region of plants, the low concentra- tion of sugars mimics these environments.

| Table 2. Expression of pelE::lux transcriptional fusions in D. chrysanthemi EC16. |
|---------------------------------|------------------|
| Growth condition               | Lux specific activity<sup>a</sup> |
| M63-glycerol                   | 2.9 ± 0.7         |
| M63-glycerol + PGA             | 31.7 ± 6.1        |
| M63-glycerol + Potato extract  | 2203.4 ± 418.4    |
| M63-glycerol + D-arabinose     | 0.5 ± 0.02        |
| M63-glycerol + D-fructose      | 4458.5 ± 688.4    |
| M63-glycerol + L-arabinose     | 5171.9 ± 358.9    |
| M63-glycerol + D-fructose      | 4.9 ± 0.3         |
| M63-glycerol + PGA + L-fructose| 646.9 ± 73.7      |
| M63-glycerol + PGA + L-fructose| 2.2 ± 0.08        |
| M63-glycerol + D-galactose     | 18419 ± 723.4     |
| M63-glycerol + D-glucose       | 5316.5 ± 535.5    |
| M63-glycerol + L-glucose       | 1.4 ± 0.07        |
| M63-glycerol + PGA + 2-deoxy-D-glucose| 744.9 ± 9.7 |
| M63-glycerol + PGA + D-mannose | 2745 ± 751.5      |
| M63-glycerol + PGA + D-mannitol| 4470.6 ± 475.1    |
| M63-glycerol + PGA + L-fructose| 0.8 ± 0.2         |
| M63-glycerol + PGA + L-fructose| 1.6 ± 0.3         |
| M63-glycerol + D-mannose       | 5.7 ± 0.8         |
| M63-glycerol + D-rhamnose      | 47616 ± 759.9     |
| M63-glycerol + D-rhamnose      | 0.3 ± 0.08        |
| M63-glycerol + D-sorbitose     | 1.1 ± 0.06        |
| M63-glycerol + D-sorbitose     | 0.9 ± 0.08        |
| M63-glycerol + D-tagatose      | 38.6 ± 8.8        |
| M63-glycerol + D-tagatose      | 0.3 ± 0.03        |
| M63-glycerol + L-tagatose      | 3.0 ± 0.3         |
| M63-glycerol + D-xylate        | 10031 ± 96.5      |
| M63-glycerol + L-rhamnose      | 2.9 ± 0.07        |
| M63-glycerol + L-rhamnose      | 18.4 ± 0.5        |
| M63-glycerol + L-arabinose     | 45.3 ± 0.8        |
| M63-glycerol + L-arabinose     | 12.9 ± 2.4        |
| M63-glycerol + L-arabinose     | 4.9 ± 0.4         |
| M63-glycerol + L-arabinose     | 17908 ± 217.6     |
| M63-glycerol + L-arabinose     | 14481 ± 139.6     |
| M63-glycerol + L-arabinose     | 10.2 ± 0.7        |
| M63-glycerol + L-arabinose     | 9588.8 ± 81.2     |
| M63-glycerol + L-arabinose     | 0.09 ± 0.006      |

<sup>a</sup>Lux-specific activity was expressed as the (cpm/OD<sub>660</sub>) of the bacterial culture.
In this study, low concentration of many of the tested monosaccharides, disaccharides, and trisaccharides was shown to hyperinduce the expression of pelE in the simultaneous presence of PGA at a similar level as that of Pel in the medium containing PGA and plant extract. However, the other pel isozymes did not show hyperinduction in the same medium (Table 3). We found that pelA::lux expression was increased in acidic conditions (data not shown), and thus the different isozymes of Pel may recognize different signals for its hyperinduction.

The inducible monosaccharides commonly exist both as cyclic and acyclic form (D-form) and share common trans relationship between C-3 and C-5 hydroxyl group. There were some exceptions, for example, D-ribose, D-xylose, and L-arabinose which possess only five carbons (pentose) and did not share the configuration at C-3 and C-5 (Figure 7) were able to hyperinduce pelE expression. Methylated monosaccharide glycosides such as methyl β-L-arabinopyranoside, methyl α-D-galactopyranoside, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl α-L-rhamnopyranoside, methyl β-D-xylpyranoside which cannot perform acyclic (Figure 8) form failed to hyperinduce the expression of pelE. When these sugars are esterified with a methyl group, which cannot form the acyclic anymore, lost also the ability to Pel-hyperinduction. So, the minor acyclic form of the above hexose may be effective for its hyperinduction. 3-O-Methyl-D-glucose which OH was methylated at C-3 still exists as acyclic form but non-inducible, indicating that C-3 hydroxyl group would be essential for the hyperinduction. Other monosaccharides such as D-arabinose, L-glucose, L-ribose, L-xylose, L-rhamnose, and rare sugars (D-psicose, D-sorbose, L-fructose, L-psicose, L-sorbose, L-tagatose) except D-tagatose and D-sorbitol which is able to form acyclic but having different a configuration of C-3 and C-5 hydroxyl groups from the above failed to hyperinduce the expression of pelE (Figure 9).

The oligosaccharides such as sucrose and raffinose, which exist only as cyclic (Figure 10), were effective for the hyperinduction. This may be due to their cleavage which leads to the above acyclic hexose with the same configuration at C-3 and C-5 hydroxyl groups. In the case of melibiose, it also possesses same C-3 and C-5 hydroxyl configuration and can perform an acyclic form of its monosaccharide chain, but it could hyperinduce pelE. This may be due to the effect of the linkage to other monosaccharide at C-6. However, non-effective oligosaccharides such as D-cellobiose, lactose, maltose, and maltotriose which share common configuration at C-3 and C-5 having linkage to other monosaccharide at position C-4 (Figure 11). This may be the reason why they were found

### Table 3. Expression of pel::lux isozymes in D. chrysanthemi EC16.

| lux fusion | Growth condition | Lux specific activity a (x10^5) |
|------------|------------------|--------------------------------|
| pelB::lux  | M63-glycerol + PGA + Potato extract  | 0.1 ± 0.04                      |
|            | M63-glycerol + PGA + L-arabinose    | 0.3 ± 0.06                      |
|            | M63-glycerol + PGA + D-fructose     | 0.2 ± 0.06                      |
|            | M63-glycerol + PGA + Melibiose      | 1.8 ± 0.5                       |
| pelB::lux  | M63-glycerol + PGA + Potato extract  | 0.1 ± 0.04                      |
|            | M63-glycerol + PGA + L-arabinose    | 0.2 ± 0.02                      |
|            | M63-glycerol + PGA + D-fructose     | 0.2 ± 0.05                      |
|            | M63-glycerol + PGA + Melibiose      | 0.6 ± 0.08                      |
| pelB::lux  | M63-glycerol + PGA + Potato extract  | 0.09 ± 0.008                    |
|            | M63-glycerol + PGA + L-arabinose    | 0.2 ± 0.03                      |
|            | M63-glycerol + PGA + D-fructose     | 0.4 ± 0.03                      |
|            | M63-glycerol + PGA + Melibiose      | 0.1 ± 0.006                     |

aLux-specific activity was expressed as the (cpm/OD660) of the bacterial culture. The data were expressed as the mean of peak Lux-specific activities from at least three independent experiments.

![Figure 7](image_url). Chemical structure of inducible monosaccharides. These monosaccharides commonly exist both as cyclic and acyclic form (D-form) and share common trans relationship between C-3 and C-5 hydroxyl group. D-ribose, D-xylose, and L-arabinose which possess only five carbons (pentose) and did not share the configuration at C-3 and C-5 are exception.
to be not inducible. Trehalose which cannot form acyclic form was also found to be non-effective. These findings suggested the importance of acyclic form existence and OH configuration of sugars for hyperinduction of pelE in *D. chrysanthemi* EC16.

The non-metabolizable sugars for *D. chrysanthemi* EC16 such as 2-deoxy-D-glucose and D-fucose (6-deoxy-D-galactose) which share common configuration at C-3 and C-5 hydroxyl groups were also shown to be equally effective for hyperinduction of the synthesis of Pel. Thus, these sugars may act as signals probably in a similar manner as the interaction with periplasmic binding protein and a transmembrane signal protein as shown by ChvE of *A. tumefaciens* or galactose/glucose-binding protein (GBP) of *E. coli* (Shimoda et al. 1993). Likewise, Shimoda and associates (1990) investigated the effect of 2-deoxy-D-glucose on the vir genes expression. Although *A. tumefaciens* did not use this sugar analog for growth, it was shown that this sugar was required for a marked increase in the expression of vir genes. Non-effective sugars for the induction of vir genes in *A. tumefaciens* have a different C-3 stereochemical structure from that of the effective sugars.

In *A. tumefaciens*, the monosaccharides that induce the expression of vir genes are clustered in aldoses group. Periplasmic loop of VirA protein, a member of two-component regulatory system virA-virG (Stachel & Zambrysky 1986; [Figure 9](#)),

![Chemical structure of non-inducible monosaccharides. D-arabinose, L-glucose, L-ribose, L-xylose, L-rhamnose, and rare sugars (D-psicose, D-sorbose, L-fructose, L-psicose, L-sorbose, L-tagatose) except D-tagatose and D-sorbitol which is able to form acyclic but having different configuration of C-3 and C-5 hydroxyl groups was non-effective.](#)

![Chemical structure of methylated monosaccharide glycosides. Methyl β-D-arabinopyranoside, methyl α-D-galactopyranoside, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl α-L-rhamnopyranoside, methyl β-D-xylopyranoside which cannot perform acyclic form failed to hyperinduce the expression of pelE.](#)
Leroux et al. 1987), and the periplasmic sugar-binding protein ChvE (Huang et al. 1990) are required to specifically recognize the stereorestrictions of aldoses (Cangelosi et al. 1990).

The effective sugars for Pel-hyperinduction in D. chrysanthemi seemed to share similarity with those for vir induction in A. tumefaciens, though there were some exceptions. L-arabinose, D-fucose, D-galactose, D-glucose, D-xylose, and 2-deoxy-D-glucose were shown to be effective for both plant pathogenic bacteria; however, D-fructose, D-ribose, D-mannitol, and sucrose were shown to be effective only for D. chrysanthemi EC16 but not for A. tumefaciens as described by Cangelosi et al. (1990). This variability might be due either to the difference in their degradation or to the difference in the structure of some sensor protein(s) in D. chrysanthemi and that in A. tumefaciens. The elucidation of this specific recognition of sugars by such sensor (or receptor) proteins will lead to a better understanding of the mechanism of in planta hyperinduction of Pel.

**Figure 10.** Chemical structure of effective oligosaccharides. Sucrose and raffinose, which exist only as cyclic were effective for the hyperinduction. Their cleavage may lead to the above acyclic hexose with the same configuration at C-3 and C-5 hydroxyl groups. In the case of melibiose, though it possesses same C-3 and C-5 hydroxyl configuration and can perform an acyclic form of its monosaccharide chain, it linked to other monosaccharide at C-6.

**Figure 11.** Chemical structure of non-effective oligosaccharides. D-cellobiose, lactose, maltose, and maltotriose which share common configuration at C-3 and C-5 linked to other monosaccharide at position C-4. Trehalose was also found to be only in cyclic form.
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