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Interactions of tagatose with the sugar metabolism are responsible for *Phytophthora infestans* growth inhibition

Paola Elisa Corneo [a,b], Andrea Nesler [c], Cesare Lotti [b], Abdessalem Chahed [b,c,d], Urska Vrhovsek [b], Ilaria Pertot [a,b], Michele Perazzolli [a,b,*]

[a] Center Agriculture Food Environment (C3A), University of Trento, via E. Mach 1, 38089 San Michele all'Adige, Italy
[b] Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38089 San Michele all'Adige, Italy
[c] Bi-PA nv, Technologielaan 7, 1840 Londerzeel, Belgium
[d] Department of Plant Induced Resistance and Bioprotection, University of Reims, Moulin de la Housse, 51687 Reims, France

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ABSTRACT

Tagatose is a rare sugar metabolised by a limited number of microorganisms that inhibits a large spectrum of phytopathogens. In particular, tagatose inhibited *Phytophthora infestans* growth and negatively affected mitochondrial processes. However, the possible effects of tagatose on *P. infestans* metabolism have not yet been investigated. The aim of this study was to analyse the impact of this rare sugar on the sugar metabolism in *P. infestans*, in order to better understand its mode of action. Tagatose inhibited the growth of *P. infestans* with a precise reprogramming of the carbohydrate metabolism that involved a decrease of glucose, glucose-1-phosphate and mannose content and β-glucosidase activity. The combination of tagatose with common sugars led to three different responses and highlighted antagonistic interactions. In particular, glucose partially attenuated the inhibitory effects of tagatose, while fructose fully impaired tagatose-mediated growth inhibition and metabolite changes. Moreover, sucrose did not attenuate tagatose effects, suggesting that the inhibition of sucrose catabolism and the alteration of glucose-related pathways contributed to the growth inhibition caused by tagatose to *P. infestans*. The interactions of tagatose with the common sugar metabolism were found to be a key mode of action against *P. infestans* growth, which may represent the basis for the further development of tagatose as an eco-friendly fungicide.

1. Introduction

Rare sugars have been defined as monosaccharides and their derivatives that rarely exist in nature (Granström et al., 2004) and they include hexoses (e.g., tagatose, allose, gulose, and sorbose) and pentoses (e.g., lyxose, xylulose, and xylitol) (Ahmed, 2001; Jayamuthunagai et al., 2017). Conversely, common sugars are frequently found in the environment and they include well characterised sugars, such as glucose, fructose and their derivatives (Zhang et al., 2017). Although rare sugars are mainly known as low-calorie sweeteners (Levin, 2002; Matsuo et al., 2002), they acquired medical interest as anticancer (Beerens et al., 2012), anti-hyperglycaemic (Hossain et al., 2015; Lu et al., 2008), anticariogenic (Wong, 2000; Yun et al., 2017) and prebiotic molecules (Bertelsen et al., 1999; Vastenavond et al., 2012). Moreover, the development of novel enzymatic and microbial processes lowered the production costs of rare sugars and extended their application to numerous disciplines, environmental and agricultural science included (Izumori, 2006; Li et al., 2013).

Among the rare sugars, tagatose is a ketohexose, C-4 epimer of fructose and isomer of galactose, and it is used as a low-calorie sweetener, substitute of sucrose (Kim, 2004). Tagatose can be added to beverages, health foods and dietary products since it was generally recognised as safe by the Food and Drug Administration (Kim, 2004; Levin, 2002). Only about 20 % of ingested tagatose is absorbed and metabolised in the human gut (Bertelsen et al., 1999; Vastenavond et al., 2012) and the unabsorbed fraction is fermented by the gut microbiota (Bertelsen et al., 2001). However, tagatose can be used as source of carbohydrates by a restricted range of microbial taxa and it can be used only by some bacterial species as phosphorylated intermediate of the tagatose-6-phosphate pathway in the lactose, galactose and galactitol catabolism (Van der Heiden et al., 2013), indicating selective nutritional or antinutritional effects on specific microbial taxa. Tagatose is

* Corresponding author at: Center Agriculture Food Environment (C3A), University of Trento, via E. Mach 1, 38089 San Michele all'Adige, Italy.
E-mail address: michele.perazzolli@unitn.it (M. Perazzolli).

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fermented only by specific gut microorganisms (e.g. *Enterococcus faecalis* and *Lactobacillus* spp.) (Bertelsen et al., 2001) and inhibits the growth of numerous human pathogenic bacteria (e.g. *Campylobacter* spp., *Salmonella typhi* and *Escherichia coli*) (Bertelsen et al., 2001; Lobete et al., 2017). Thus, tagatose can act as a human prebiotic product, enhancing the growth of beneficial microorganisms and inhibiting that of bacterial pathogens (Bertelsen et al., 2001; Vastenavond et al., 2012).

A similar prebiotic effect was observed in plants, where tagatose increased the relative abundance of some plant beneficial microorganisms associated with the grapevine phyllosphere (*Alternaria* spp., *Aureobasidium* spp., *Exiguobacterium* spp. and *Exophiala* spp.) (Perazzolli et al., 2020). Moreover, the main beneficial effect of tagatose on grapevines was linked to the inhibition of oomycete and fungal phytopathogens, such as *Plasmopara viticola* and *Erysiphe necator* (Perazzolli et al., 2020). Tagatose can inhibit the growth of a wide range of phytopathogens and it was patented for the control of numerous crop diseases, including tomato and potato late blight (Ohara et al., 2008). Late blight, caused by the oomycete pathogen *Phytophthora infestans*, is one of the most devastating plant diseases (Agrios, 2005) and it represents a major threat to global food security, costing billions of dollars every year in terms of control efforts and production losses (Fry, 2008). As the control of late blight requires the use of chemical fungicides with potential harmful effects on the environment and human health (Fry, 2008), the demand for eco-friendly alternatives has increased (Axel et al., 2012). Tagatose could represent a valid alternative to chemical fungicides, thanks to the absence of negative impacts on human health (Ohara et al., 2008). However, further studies are needed on tagatose mode of action against phytopathogens and on the mechanisms underlying its growth inhibitory effects, in order to further develop efficient biofungicides. For instance, tagatose was able to inhibit the growth of *P. infestans in vitro* by decreasing the respiration processes and ATP content (Chahed et al., 2020), but further inhibitory effects on the metabolism of *P. infestans* have not yet been unravelled. It is known that rare sugars (e.g. psicose, sorbose and arabinose) are able to inhibit the activity of some enzymes (e.g. glucose-6-phosphate-dehydrogenase and disaccharidase) involved in the common sugar metabolism of mammalian cells (Matsuo et al., 2001; Oku et al., 2014; Seri et al., 1996). In particular, tagatose inhibited the activity of intestinal disaccharidases responsible for the sucrose and maltose metabolism (Seri et al., 1995), and one of its derivatives (tagatose-1-phosphate) was involved in the regulation of glycogen synthesis and catabolism (Agius, 1994; Lu et al., 2008). Likewise, another tagatose derivative (tagatose 6-phosphate) inhibited the transaldolase of *E. coli* (Stellmacher et al., 2016). Therefore, it is of crucial importance to investigate possible interactions between the metabolism of rare and common sugars, in order to clarify the growth inhibition mechanisms. For example, tagatose caused a growth retardation of *Streptococcus mutans* and the inhibitory effect was attenuated by the presence of fructose, but not of glucose (Hasibul et al., 2018), suggesting the presence of antagonistic interactions between rare and common sugar metabolism. However, interactions between rare and common sugars have never been studied in the case of environmentally and agriculturally relevant microorganisms. The objective of this study was to investigate the interactions between tagatose and the metabolism of common sugars in *P. infestans*, with particular attention to the first intermediates of the glycolysis and some intermediates of the tricarboxylic acid cycle, in order to elucidate the mode of action of tagatose and to identify possible attenuation effects of common sugars on the growth inhibitory properties of tagatose.

2. Materials and methods

2.1. Phytophthora infestans sugar treatment, growth conditions and radial growth assessment

Phytophthora infestans strain VB3 was stored in glycerol at -80 °C in the fungal collection of the Fondazione Edmund Mach (Italy) and it was maintained in Petri dishes on pea agar medium (PAM, 12.5 % frozen peas and 1.2 % agar in distilled water) at 18 °C (Chahed et al., 2020).

Phytophthora infestans plugs (5 mm diameter) were cut from the edge of 10-day-old colonies and a plug was placed at the centre of each Petri dish (90 mm diameter) on PAM covered by a cellophane layer (90 mm diameter) to facilitate subsequent mycelium collection. Eight treatments were analysed, PAM as control (Control-incubated), PAM augmented with 5 g L^{-1} tagatose (Tag-incubated), 5 g L^{-1} glucose (Glu-incubated), 5 g L^{-1} fructose (Fru-incubated, 5 g L^{-1}) or 5 g L^{-1} sucrose (Suc-incubated) and PAM augmented with the combination of tagatose (5 g L^{-1}) and a common sugar (5 g L^{-1}), such as tagatose and glucose (Tag-Glu-incubated), tagatose and fructose (Tag-Fru-incubated), tagatose and sucrose (Tag-Suc-incubated). Dishes were incubated at 18 °C and *P. infestans* radial growth was assessed four and ten days after incubation (DAI), as the average of the two perpendicular diameters, subtracted the plug diameter and divided by two. Five replicates were analysed for each treatment and the experiment was carried out twice.

2.2. Quantification of Phytophthora infestans metabolites

Mycelium plugs (200 mg) were collected at 4 and 10 DAI with a sterile scalpel, frozen in liquid-N_2 and kept at −80 °C and ground using a mixer mill disruptor (MM200, Retsch, Haan, Germany) at 25 Hz for 45 s with sterile steel jars and beads refrigerated in liquid-N_2. The metabolite extraction was carried out as previously described (Chitarrini et al., 2017) with slight modifications and the content of tagatose, glucose, fructose, galactose, mannose, sucrose, glucose-1-phosphate (glucose-1-P), glucose-6-phosphate (glucose-6-P), citric acid, succinic acid, malic acid, α-ketoglutaric acid, 3-hydroxy-3-methylglutaric acid (HMG), ribose and myo-inositol was measured by gas chromatography-mass spectrometry (GC–MS) analysis according to Chitarrini et al. (2017). Briefly, 100 mg of ground mycelium was subjected to extraction by adding 900 µL of cool (−20 °C) extraction solvent (isopropanol/acetonitrile/water, 3:3:2 v/v/v), a 10 µL aliquot of a solution containing nicotinic-D4, and glucose-D7 (1000 mg L^{-1}) was added as an internal standard. The extraction mixture was vortexed for 10 s, shaken at 4 °C for 5 min and centrifuged at 12,000 × g for 2 min at 5 °C. A second round of extraction was carried out following the same procedure. The two supernatants were merged and re-suspended in a final volume of 2 mL using the extraction solvent. A total of 200 µL of supernatant was placed in a 1.5 mL tube and evaporated to dryness under N_2. The residue was re-suspended in 500 mL of acetonitrile/water (50:50 v/v), vortexed for 10 s, sonicated, vortexed again 10 s and centrifuged at 12,000 × g for 2 min. The supernatant was then transferred into a 2 mL high recovery vial and dried out under N_2. The dried extract was subjected to online derivatisation performed by the autosampler just before the injection of the sample. The derivatization procedure can be divided in two steps: first 20 µL of methoxamine hydrochloride in pyridine (20 mg mL^{-1}) was added to inhibit cyclisation of reducing sugars and shaken at 30 °C for 1 h; then 80 µL of N-methyl-Ntrimethylsilyl-trifluoroacetamide with 1% trimethylchlorosilane was added for the trimethylsilylation of acidic protons and shaken at 37 °C for 30 min. Finally, 5 µL of a solution containing decane (1000 mg L^{-1}) and heptadecane (1000 mg L^{-1}) were added in order to monitor the chromatographic analysis and the instrumental conditions. One microliter of derivatised extract was injected for GC–MS analysis. Analyses were performed using a Trace GC Ultra combined with a mass spectrometer TSQ Quantum GC and an autosampler Triplus RSH equipped with automatic tool change (Thermo Electron Corporation, Waltham, MA, United States). An RXI-5-Sil MS w-Integra-GuardR (fused silica) (30 m × 0.25 mm × 0.25 mm) column was used for compound separation. Helium was used as the carrier gas at 1.2 mL min^{-1} and the injector split ratio was set to 1:10. The injector, transfer line and source temperature were set to 250 °C. The initial oven temperature was kept at 65 °C for 2 min, increased by 5.2 °C min^{-1} to 270 °C and held at 270 °C for 4 min. The mass spectrometer was operated in electron ionisation mode. Data

acquisition was performed in full scan mode from 50 to 700 *m/z*. Data processing was performed using XCALIBUR 4.0 software (Thermo Fisher Scientific, San Jose, CA, USA).

Each metabolite was quantified as mg kg^{-1} of mycelium fresh weight, using a calibration curve of each pure compound (Sigma-Aldrich, Merc, Kenilworth, NJ, USA) dissolved in methanol:water 80:20 mix within a range of 0.01 mg L^{-1} and 1200 mg L^{-1}. Compound identification was obtained using one or, in the case of a few compounds, two specific mass to charge ratios (*m/z* ratio) characteristics for the individual metabolites (extracted ion monitoring) and excluding saturated fragments. Three replicates were analysed for each treatment. In order to obtain sufficient mycelium powder, each replicate was a pool of 35 and 24 plugs in the case of Tag- and Tag-Suc-incubated samples at 4 and 10 DAI, 20 and eight plugs for the other treatments at 4 and 10 DAI, respectively.

2.3. 1,3-β-Glucan quantification

Mycelium plugs were collected with a sterile scalpel at 10 DAI. The content of 1,3-β-glucan (β-glucan content) was assessed using the aniline blue assay, as previously described (Fortwendel et al., 2009). Briefly, frozen mycelium of *P. infestans* was lyophilised for 24 h in a 1.5 mL-tube using LyoLab 3000 (Heto, Allerød, Denmark). The lyophilised mycelium (5 mg) was re-suspended in 500 µL of 1 M NaOH, sonicated 30 s and incubated at 52 °C for 30 min as solubilisation process and two technical replicates of each sample (50 µL) were aliquoted in a clear 96-well plate (Corning, New York, NY, USA) for β-glucan quantification and the remaining volume (100 µL) was used for protein quantification.

For β-glucan quantification, 185 µL of aniline blue mix (40 mL of 0.1 % aniline blue in sterile distilled water, 21 mL of 1 N HCl and 59 mL of 1 M glycine in NaOH buffer, pH 9.5) was added to each well. Dilutions of curdlan (Carbosynth, Berkshire, UK) were prepared as standard curve (0, 1, 5, 10, 25, 50, 100 and 200 µg mL^{-1}), from a stock solution of 1 mg mL^{-1} prepared dissolving curdlan in 1 M NaOH at 80 °C for 30 min (Shedletzky et al., 1997). The plate was incubated in the dark for 30 min at 52 °C and cooled at room temperature until fully decolorised. Fluorescence was acquired at 405 nm excitation and 460 emission using a Synergy 2 Multi-Mode Microplate Reader (Biotek, Winooski, VT, USA).

For protein quantification, 100 µL of each sample was mixed with 100 µL of Bradford reagent (Pierce Coomassie Plus, Thermo Fisher Scientific) in a 96-well clear microplate (Corning). Samples were incubated for 10 min at room temperature and the absorbance was measured at 595 nm (A_{595}) with a Synergy 2 Multi-Mode Microplate Reader (Biotek). A standard curve of bovine serum albumin (Sigma-Aldrich; 0, 0.001, 0.005, 0.01, 0.025, 0.05 and 0.1 mg mL^{-1}) was used as reference to determine the protein concentration and the samples were diluted in sterile distilled water (appropriated dilutions to A_{595} <1.5). β-glucan content was expressed as β-glucan per unit of total proteins (mg mg^{-1}). Five replicates were used for each treatment and the experiment was carried out twice. In order to obtain sufficient mycelium powder, each replicate was a pool of 20 plugs in the case of Tag- and Tag-Suc-incubated samples and three plugs for the other treatments.

2.4. β-glucosidase activity assessment

Mycelium plugs (500 mg) were collected with a sterile scalpel at 10 DAI, washed twice in 50 mM phosphate buffer pH 7 and frozen at −20 °C until subsequent analysis. The frozen mycelium was ground in 1 mL of 50 mM phosphate buffer pH 7 in stainless-steel jars (pre-cooled at -80 °C) at 20 Hz for 1 min using a mixer mill disruptor (MM200, Retsch, Haan, Germany) and centrifuged at 21,000 × g for 15 min at 4 °C. The supernatant was immediately processed using the β-glucosidase Activity Assay Kit (Sigma-Aldrich, Merc, Kenilworth, NJ, USA), following the manufacturer instructions, and it was used for the β-glucosidase activity assay and for protein quantification.

For the β-glucosidase activity assay, two technical replicates (20 µL) of each sample were aliquoted in a clear 96-well plate (Corning) and 200 µL master mix (192.3 µL of Assay Buffer and 7.7 µL of β-NPG Substrate) were added to each sample. As blank, 220 µL of sterile distilled water was added to a well of the 96-well plate. In a different well, 20 µL of sterile distilled water was mixed with 200 µL of the calibrator provided in the kit. The initial absorbance of each sample was measured at 405 nm (A_{405}) on a Synergy 2 Multi-Mode Microplate Reader (Biotek). The plate was then incubated in the dark at 37 °C for 20 min and the final A_{405} was measured. The enzymatic activity (Units mL^{-1}) was calculated according to the following formula: β-glucosidase activity = $[A_{405(final)} - A_{405(initial)}] / [A_{405(calibrator)} - A_{405(blank)}] \times 250$; where $A_{405(calibrator)}$ and $A_{405(blank)}$ were the A_{405} measured at 20 min.

For protein quantification, 100 µL of each sample was mixed with 100 µL of Bradford reagent (Pierce Coomassie Plus, Thermo Fisher Scientific) in a 96-well clear microplate (Corning). Samples were incubated for 10 min at room temperature and the A_{595} was measured with a Synergy 2 Multi-Mode Microplate Reader (Biotek). A standard curve of bovine serum albumin (Sigma-Aldrich; 0, 0.001, 0.005, 0.01, 0.025, 0.05 and 0.1 mg mL^{-1}) was used as reference to determine the protein concentration of each sample. The β-glucosidase activity was then expressed as unit of β-glucosidase per unit of total proteins (U mg^{-1}) as reported in Cordero Otero et al. (2003). Three replicates were used for each treatment and the experiment was carried out twice. In order to obtain sufficient mycelium powder, each replicate was a pool of 24 plugs in the case of Tag- and Tag-Suc-incubated samples and 10 plugs for the other treatments.

2.5. Statistical analysis

Data were analysed with PAST 2.17c (Hammer et al., 2001). For radial growth, β-glucosidase activity and β-glucan content the Kruskal-Wallis test was used to demonstrate non-significant differences between the two experimental repetitions ($P > 0.05$) and the data from the two experimental repetitions were pooled. For radial growth and β-glucosidase activity, a Kruskal-Wallis test was used on untransformed data to detect significant differences among treatments ($P \leq 0.05$) based on Mann-Whitney Bonferroni corrected *P*-values ($P \leq 0.05$) (Zar, 1996). For metabolite data, the missing values were imputed with a random value between zero and the limit of quantification (LOQ) (Chitarrini et al., 2017). Data of metabolite and β-glucan content were Log_{10} transformed, the normal distribution was validated by the Shapiro-Wilk test ($P > 0.05$), variance homogeneity was assessed with the F-test ($P > 0.05$) and significant differences between sugar-incubated (Glu-, Fru-, Suc-, Tag-, Tag-Glu-, Tag-Fru- and Tag-Suc-incubated sample) and control (Control-incubated) samples were assessed for each metabolite using a parametric two-sample test (Student's *t*-test, $P \leq 0.05$) (Maridueña-Zavala et al., 2017). If variance homogeneity (F-test, $P \leq 0.05$) or normal distribution of the data ($P \leq 0.05$) were not satisfied, the Welch *t*-test or the Mann-Whitney test were used as non-parametric two-sample tests ($P \leq 0.05$), respectively (Xi et al., 2014). Moreover, the Kruskal-Wallis test was used to detect significant differences among treatments ($P \leq 0.05$) based on Mann-Whitney Bonferroni corrected *P*-values ($P \leq 0.05$) (Zar, 1996) on Log_{10}-transformed β-glucan content data. Changes in metabolite and β-glucan content were expressed as Log_2-transformed fold change values between sugar-incubated (Glu-, Fru-, Suc-, Tag-, Tag-Glu-, Tag-Fru- and Tag-Suc-incubated sample) and control (Control-incubated) samples, as reported by Maridueña-Zavala et al. (2017), and visualised according to pathway maps of the Kyoto Encyclopedia of Genes and Genomes (KEGG) for *P. infestans* T30−4 (T01333 KEGG). Significant differences in tagatose content between 4 and 10 DAI were assessed in Tag-, Tag-Glu-, Tag-Fru-, and Tag-Suc-incubated samples using a Student's *t*-test ($P \leq 0.05$). The Kendall non-parametric correlation analysis was run between the mean values of *P. infestans* radial growth, β-glucan content, β-glucosidase activity and metabolite content measured at 10 DAI.

3. Results

3.1. Tagatose effects on Phytophthora infestans growth and metabolite content are modulated by common sugars

Phytophthora infestans growth was lower in Tag-incubated samples compared to Control-, Glu-, Fru- and Suc-incubated samples at 4 and 10 DAI (Fig. 1). Mycelial growth in Glu-, Fru- and Suc-incubated samples was comparable to the growth of Control-incubated samples at 4 and 10 DAI. The combination of tagatose with a common sugar led to three different outcomes: i) radial growth in Tag-Glu-incubated samples was higher compared to that in Tag-incubated samples, but lower compared to that in Control-incubated samples at 4 and 10 DAI (partial growth inhibition of Tag-Glu incubation); ii) radial growth in Tag-Fru-incubated samples was comparable to that in Control-incubated samples at 4 DAI and higher compared to that in Tag-incubated samples at 4 and 10 DAI (restored growth of Tag-Fru incubation); iii) radial growth in Tag-Suc-incubated samples was comparable to that in Tag-incubated samples and lower compared to that in Control-incubated samples at 4 and 10 DAI (growth inhibition of Tag-Suc incubation).

In order to investigate the effects of tagatose on the common sugar metabolism, the metabolite content of *P. infestans* was assessed by GC–MS analysis. Glucose and glucose-1-P content was lower in Tag-incubated samples compared to Control-incubated samples at 4 and 10 DAI, while that of fructose and galactose was higher (Fig. 2A, Table S1). At 10 DAI, the content of sucrose and mannose was higher and lower in Tag-incubated samples compared to Control-incubated samples, respectively.

In Tag-Glu-incubated samples, glucose-1-P content was lower compared to Control-incubated samples at both time points (Fig. 2B). Glucose and galactose content was higher in Tag-Glu-incubated samples compared to Control-incubated samples at 4 DAI, while sucrose, fructose and mannose content was higher at 10 DAI (Fig. 2B). Comparing metabolite changes in Tag-Glu-and Tag-incubated samples, it was found that the presence of glucose partially attenuated metabolite changes caused by tagatose and impaired the tagatose-mediated decrease in glucose content (Fig. 2A and B).

In Tag-Fru-incubated samples, fructose and galactose content was higher compared to Control-incubated samples at both time points, mannose and sucrose content was higher at 4 DAI at 10 DAI, respectively (Fig. 2C). Overall, the presence of fructose in Tag-Fru-incubated samples (Fig. 2C) impaired the decrease in glucose, glucose-1-P and mannose content found in Tag-incubated samples (Fig. 2A).

In Tag-Suc-incubated samples, glucose and glucose-1-P content was lower compared to Control-incubated samples at 4 and 10 DAI, while fructose and galactose content was higher at both time points (Fig. 2D). Sucrose content was higher at 10 DAI in Tag-Suc-incubated samples compared to Control-incubated samples and to a greater extent compared to fold changes found in Tag-Glu- and Tag-Fru-incubated samples (Student's *t*-test, $P \leq 0.05$), in agreement with the higher availability of sucrose in the media (Table S1). Metabolite changes found in Tag-Suc-incubated samples were analogous to those in Tag-incubated samples (Fig. 2A and D).

The content of glucose, glucose-1-P, glucose-6-P and galactose was comparable in Glu-, Fru-, Suc- and Control-incubated samples, with the exception of glucose and glucose-1-P in Glu-incubated samples at 4 and 10 DAI, respectively (Fig. S1). The content of one (sucrose) and two (fructose and mannose) sugars was higher in Glu-, Fru- and Suc-incubated samples compared to Control-incubated samples at one (10 DAI) and two (4 and 10 DAI) time points, respectively.

The content of succinic and malic acid was lower in Tag- and Tag-Suc-incubated samples compared to Control-incubated samples at 4 and 10 DAI, as well as that of α-ketoglutaric acid and HMG at 10 DAI (Table S1). Likewise, succinic acid content at 4 DAI and malic acid content at 4 and 10 DAI were lower in Glu- and Tag-Glu-incubated samples compared to Control-incubated samples. Myo-inositol content was higher in all sugar-incubated samples compared to Control-incubated samples at 10 DAI, except Suc- and Tag-Fru-incubated samples. As expected, tagatose was detected only in Tag-, Tag-Glu-, Tag-Fru- and Tag-Suc-incubated samples, but not in Control-, Glu-, Fru- and Suc-incubated samples. Moreover, tagatose content in Tag-, Tag-Glu-, Tag-Fru- and Tag-Suc-incubated samples was comparable between 4 and 10 DAI (Student's *t*-test, $P > 0.05$).

3.2. Tagatose effects on β-glucan content and β-glucosidase activity of Phytophthora infestans are modulated by common sugars

Phytophthora infestans β-glucan content was higher in Tag-incubated samples compared to Control-incubated samples at 10 DAI (Fig. 3). β-glucan content was higher in Glu- and Suc-incubated samples compared to Control-incubated samples, while it was comparable in Fru- and Control-incubated samples. β-glucan content was higher in Tag-Glu-, Tag-Fru- and Tag-Suc-incubated samples compared to Control-incubated samples and it was comparable to Tag-incubated samples and samples incubated with the respective common sugar (Glu-, Fru- or Suc-incubated samples).

Phytophthora infestans β-glucosidase activity was lower in Tag-incubated samples compared to Control-incubated samples (Fig. 4). In Glu-, Fru- and Suc-incubated samples, β-glucosidase activity was comparable to Control-incubated samples. The combination of tagatose with a common sugar led to three different outcomes: i) β-glucosidase activity in Tag-Glu-incubated samples was comparable to that in Tag- and Control-incubated samples (β-glucosidase inhibition of Tag-Glu incubation); ii) β-glucosidase activity in Tag-Fru-incubated samples was higher compared to that in Tag-incubated samples and it was comparable to that in Control- and Fru-incubated samples (restored β-glucosidase activity of Tag-Fru incubation); iii) β-glucosidase activity in Tag-Suc-incubated samples was comparable to that in Tag-incubated

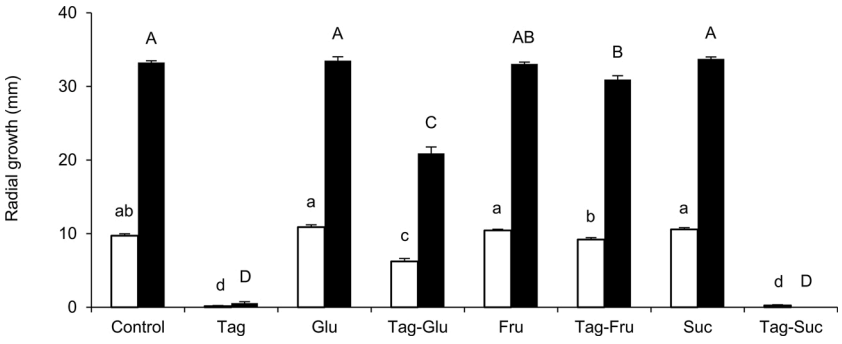

Fig. 1. Effect of tagatose and common sugars on *Phytophthora infestans* growth. *Phytophthora infestans* growth (mm) was assessed four (white) and ten (black) days after incubation (DAI) on pea agar medium in the absence (Control) and presence of 5 g L^{-1} tagatose (Tag), 5 g L^{-1} glucose (Glu), 5 g L^{-1} fructose (Fru) or 5 g L^{-1} sucrose (Suc) and in the combination of 5 g L^{-1} tagatose with 5 g L^{-1} glucose (Tag-Glu), 5 g L^{-1} fructose (Tag-Fru) or 5 g L^{-1} sucrose (Tag-Suc). The radial growth was calculated as average of the two perpendicular diameters subtracted by the plug diameter and divided by two. The Kruskal-Wallis test showed no significant differences between the two experimental repetitions ($P > 0.05$) and data from the two experiments were pooled. Bars represent mean and standard error values of ten replicates from the two experiments for each treatment. Different lowercase and uppercase letters indicate significant differences among treatments at 4 and 10 DAI respectively, according to Kruskal-Wallis test ($P \leq 0.05$).

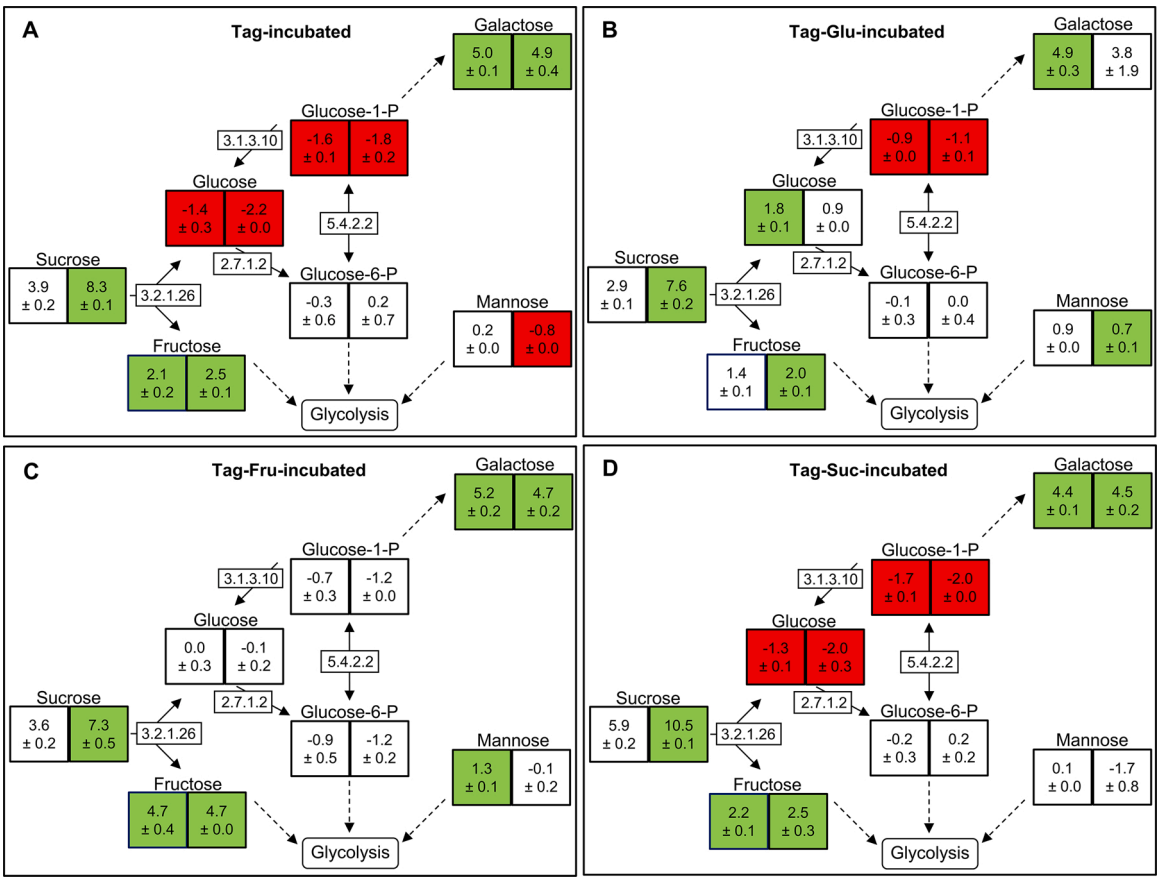

Fig. 2. Metabolite changes in *Phytophthora infestans* caused by tagatose alone and combined with common sugars. *Phytophthora infestans* metabolite content was assessed four and ten days after incubation (DAI) on pea agar medium in the absence (Control-incubated) and presence of 5 g L^{-1} tagatose (Tag-incubated), 5 g L^{-1} glucose (Glu-incubated), 5 g L^{-1} fructose (Fru-incubated) or 5 g L^{-1} sucrose (Suc-incubated) and in the combination of 5 g L^{-1} tagatose with 5 g L^{-1} glucose (Tag-Glu-incubated), 5 g L^{-1} fructose (Tag-Fru-incubated) or 5 g L^{-1} sucrose (Tag-Suc-incubated). Metabolite changes in Tag-incubated (A), Tag-Glu-incubated (B), Tag-Fru-incubated (C) and Tag-Suc-incubated (D) samples were calculated as Log_2 (fold change) values as compared with the Control-incubated samples. For each metabolite, Log_2 (fold change) mean and standard error values of three replicates at 4 DAI (left box of each metabolite) and 10 DAI (right box of each metabolite) are shown. Significantly higher (green) or lower (red) metabolite content compared to the control are highlighted, according to parametric or non-parametric two-sample tests ($P \leq 0.05$) on Log_{10}-transformed metabolite content (mg kg^{-1}). Enzyme that catalyse the key chemical reactions are reported according to the KEGG pathway maps of *P. infestans*: 3.2.1.26, invertase; 2.7.1.2, glucokinase; EC 3.1.3.10, glucose-1-phosphatase; 5.4.2.2, phosphoglucomutase. Metabolite changes caused by the common sugars are reported in Fig. S1 and the complete results of metabolite quantification are reported in Table S1. Abbreviations: Glucose-6-P, glucose-6-phosphate; Glucose-1-P, glucose-1-phosphate.

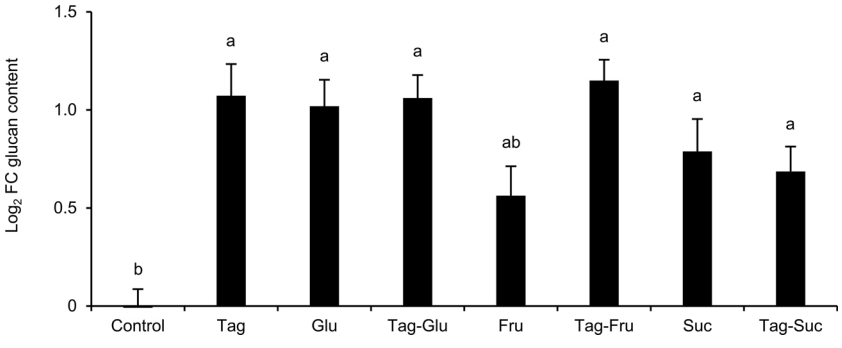

Fig. 3. Effect of tagatose and common sugars on β-glucan content of *Phytophthora infestans*. *Phytophthora infestans* β-glucan content was assessed ten days after incubation (DAI) on pea agar medium in the absence (Control) and presence of 5 g L^{-1} tagatose (Tag), 5 g L^{-1} glucose (Glu), 5 g L^{-1} fructose (Fru) or 5 g L^{-1} sucrose (Suc) and in the combination of 5 g L^{-1} tagatose with 5 g L^{-1} glucose (Tag-Glu), 5 g L^{-1} fructose (Tag-Fru) or 5 g L^{-1} sucrose (Tag-Suc). The Kruskal-Wallis test showed no significant differences between the two experimental repetitions ($P > 0.05$) and data from the two experiments were pooled. Changes in β-glucan content were calculated as Log_2 (fold change) values between sugar-incubated (Glu-, Fru-, Suc-, Tag-, Tag-Glu-, Tag-Fru- and Tag-Suc-incubated sample) and Control-incubated samples. Bars represent mean and standard error values of ten replicates from the two experiments for each treatment. Different letters indicate significant differences among treatments according to Kruskal-Wallis ($P \leq 0.05$) on Log_{10}-transformed β-glucan content. β-glucan content, expressed per unit of total proteins (mg mg^{-1}), is reported in Table S1.

samples and it was lower compared to that in Control-incubated-samples (β-glucosidase inhibition of Tag-Suc incubation).

Overall, correlations revealed that the radial growth of *P. infestans* was positively correlated with glucose-1-P, mannose content, succinic acid, malic acid and β-glucosidase activity at 10 DAI (Table 1). β-glucosidase activity was negatively correlated with galactose content and positively correlated with ribose content, while β-glucan content was negatively correlated with glucose-1-P content, α-ketoglutaric acid,

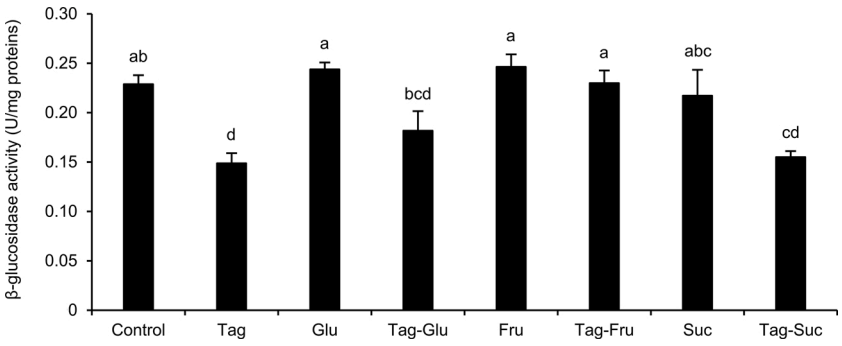

Fig. 4. Effect of tagatose and common sugars on β-glucosidase activity of *Phytophthora infestans*. *Phytophthora infestans* β-glucosidase activity was assessed ten days after incubation (DAI) on pea agar medium in the absence (Control) and presence of 5 g L^{-1} tagatose (Tag), 5 g L^{-1} glucose (Glu), 5 g L^{-1} fructose (Fru) or 5 g L^{-1} sucrose (Suc) and in the combination of 5 g L^{-1} tagatose with 5 g L^{-1} glucose (Tag-Glu), 5 g L^{-1} fructose (Tag-Fru) or 5 g L^{-1} sucrose (Tag-Suc). The Kruskal-Wallis test showed no significant differences between the two experimental repetitions ($P > 0.05$) and data from the two experiments were pooled. The β-glucosidase activity units were expressed per unit of total proteins (U mg^{-1}). Bars represent mean and standard error values of six replicates from the two experiments for each treatment. Different letters indicate significant differences among treatments according to Kruskal-Wallis ($P \leq 0.05$).

succinic acid and HMG content.

4. Discussion

In this study, the interactions between tagatose and the metabolism of common sugars were investigated, in order to better understand its mode of action against *P. infestans*. The inhibitory effect of tagatose on *P. infestans* growth was associated with changes in the content of common sugars, such as a decrease in glucose and mannose content and an increase in sucrose, fructose and galactose content. An increase in β-glucan content and a decrease in glucose-1-P content and β-glucosidase activity were also observed in Tag-incubated samples, indicating the complex effects of tagatose on the sugar metabolism of *P. infestans*. In particular, the decrease in glucose and glucose-1-P content suggested that tagatose could increase glucose catabolism or inhibit the metabolic pathways responsible for glucose synthesis. For example, the reduced β-glucosidase activity in Tag-incubated samples possibly contributed to the decrease in glucose content, since β-glucosidases are responsible for polysaccharide (e.g. cellulose and cellobiose) (Saha et al., 1994) and disaccharide (Dhake and Patil, 2005; Saha et al., 1994) hydrolysis. The positive correlations of radial growth with β-glucosidase activity and glucose-1-P content in the different incubation conditions tested, suggested that glucose-related pathways are key metabolic targets of tagatose in *P. infestans*.

The combination of tagatose with common sugars led to three different *P. infestans* responses and highlighted antagonistic interactions between tagatose and common sugars in the growth inhibition processes. For example, *P. infestans* growth inhibition and metabolite changes caused by tagatose were partially attenuated by the presence of glucose. In particular, the decrease of glucose and mannose found in Tag-incubated samples was impaired in Tag-Glu-incubated samples. However, the decrease of β-glucosidase activity, glucose-1-P and the increase of β-glucan content were comparable in Tag-Glu- and Tag-incubated samples, highlighting that some tagatose effects were not affected by the presence of glucose. In Tag-Fru-incubated samples, *P. infestans* growth and β-glucosidase activity were restored and the metabolite changes caused by tagatose were impaired. In particular, glucose, glucose-1-P and mannose content was comparable in Tag-Fru- and Control-incubated samples, indicating that the presence of fructose impaired the effects of tagatose on *P. infestans* growth and sugar metabolism. Fructose is an epimer of tagatose and these sugars may compete for enzymatic catalytic sites through their similar chemical structures, as in the case of the mammalian fructokinase (Lu et al., 2008). Conversely, the presence of sucrose did not attenuate the effects of tagatose. In particular, *P. infestans* growth, β-glucosidase activity and changes in glucose, glucose-1-P and β-glucan content were comparable in Tag-Suc- and Tag-incubated samples. Similar interactions between rare and common sugar metabolism were also described in *S. mutans*, where the tagatose-mediated growth retardation was attenuated by the presence of fructose, but not glucose, in a growth medium containing sucrose, indicating that tagatose inhibits sucrose catabolism and that fructose mitigates tagatose effects (Hasibul et al., 2018). Similarly, when tagatose was supplied alone, or combined with sucrose, there was an inhibition of the postprandial rise in blood glucose levels in mammals (Guerrero-Wyss et al., 2018; Lu et al., 2008), suggesting that tagatose inhibited sucrose hydrolysis. Since sucrose did not restore *P. infestans* growth and sugar metabolism, tagatose possibly inhibited sucrose transport or hydrolysis, reducing the availability of energy required for *P. infestans* growth. Tagatose can inhibit the activity of disaccharidases, such as the mammalian sucrase responsible for sucrose hydrolysis (Oku et al., 2014; Seri et al., 1995). Likewise, sorbose and arabinose inhibited the sucrase activity in mammal cells (Oku et al., 2014), suggesting common inhibitory effects of rare sugars on sucrose hydrolysis. However, tagatose effects were partially attenuated by the presence of glucose, suggesting that additional pathways were also affected downstream the sucrose hydrolysis. In particular, enzymes involved in the metabolism of glucose and its derivatives were possibly inhibited by tagatose, such as the β-glucosidase activity. However, further enzymatic processes may be involved in the inhibitory properties of tagatose on *P. infestans*, as found on the recombinant fructokinase and phosphomannose isomerase of *Hyaloperonospora arabidopsidis* (Mochizuki et al., 2020). Some metabolic pathways were affected by tagatose independently of the presence of common sugars. In particular, galactose and β-glucan content increased when tagatose was supplied alone or combined with a common sugar. β-glucan is one of the main components of the cell wall in oomycetes (Bartnicki-Garcia and Wang, 1983) and 1,3-1, 6 β-glucan acts as intracellular storage of carbohydrates (Bulone, 2009). Tagatose possibly affected the activity of the enzymes involved in β-glucan synthesis and/or degradation, leading to an accumulation of stored polysaccharides, as found for tagatose-1-phosphate and the glycogen metabolism in mammalian cells (Agius, 1994; Lu et al., 2008). Likewise, the UDP-glucose pyrophosphorylase activity, which is implicated in β-glucan biosynthesis, can be increased by phosphonate treatment in *P. citrophthora* (Barchietto et al., 1992). In addition to sugar metabolism, the content of tricarboxylic acid cycle intermediates was affected by the presence of tagatose. In particular, malic acid, succinic acid and α-ketoglutaric acid content was lower in Tag- and Tag-Suc-incubated samples compared to Control-incubated samples, suggesting that alterations of sugar metabolism (e.g. decrease of glucose and glucose-1-P content) were accompanied by a decrease in some key organic acids for *P. infestans* growth (Rodenburg et al., 2019). In particular, malic acid and succinic acid can be metabolised as an alternative carbon source in *P. infestans* when sugars are not available (Savory et al., 2018), suggesting that their catabolism can be affected by tagatose.

The supply of a common sugar alone did not affect *P. infestans* growth, β-glucosidase activity, glucose-6-P content and, with some exception, glucose and glucose-1-P. However, an increase in sucrose, fructose and mannose content was found in Glu-, Fru- and Suc-incubated samples compared to Control-incubated samples. *Phytophthora infestans*

Table 1
Correlations among *Phytophthora infestans* radial growth, β-glucan content, β-glucosidase activity and metabolite content.

	Radial growth	β-glucan	β-glucosidase	Glucose-1-P	Glucose	Sucrose	Mannose	Galactose	Ketoglutaric acid	Succinic acid	Malic acid	HMG	Myo-inositol	Ribose
Radial growth														
β-glucan	−0.21													
β-glucosidase	**0.62***	−0.11												
Glucose-1-P	**0.64***	**−0.57***	0.25											
Glucose	0.43	0.07	0.40	0.21										
Sucrose	−0.57	0.21	−0.33	**−0.64***	−0.29									
Mannose	**0.71***	−0.07	0.55	0.50	**0.71***	−0.43								
Galactose	−0.56	0.48	**−0.57***	−0.48	−0.24	0.40	−0.40							
Ketoglutaric acid	0.50	**−0.57***	0.18	**0.86***	0.21	−0.50	0.50	−0.40						
Succinic acid	**0.64***	**−0.57***	0.40	**0.71***	0.36	−0.50	0.50	**−0.64***	**0.57***					
Malic acid	**0.64***	−0.43	0.33	**0.86***	0.21	**−0.64***	0.50	**−0.56***	**0.86***	**0.57***				
HMG	0.54	**−0.69***	0.24	**0.93***	0.23	−0.54	0.46	**−0.57***	**0.93***	**0.77***	**0.85***			
Myo-inositol	−0.29	0.36	−0.33	−0.21	0.14	0.14	0.00	**0.56***	−0.21	−0.50	−0.21	−0.31		
Ribose	0.55	−0.11	**0.59***	0.47	**0.62***	−0.40	**0.84***	−0.37	0.47	0.40	0.47	0.47	0.11	

Kendall's τ correlation coefficients are based on *Phytophthora infestans* radial growth, β-glucan content, β-glucosidase activity and metabolite content assessed at ten days after incubation on pea agar medium in the absence (Control-incubated) and presence of 5 g L^{-1} tagatose (Tag-incubated), glucose (Glu-incubated), fructose (Fru-incubated) or sucrose (Suc-incubated) or in the combination of 5 g L^{-1} tagatose with 5 g L^{-1} of glucose (Tag-Glu-incubated), fructose (Tag-Fru-incubated) or sucrose (Tag-Suc-incubated). Significant correlations are marked in bold and by asterisks ($P \leq 0.05$). Metabolite abbreviations: Glucose-1-P, glucose-1-phosphate; Ketoglutaric acid, α-ketoglutaric acid; HMG, 3-hydroxy-3-methylglutaric acid. No significant correlations were found for glucose-6-phosphate, fructose and citric acid and data were omitted.

can grow on a minimal medium augmented with a range of different carbon sources and it grows more efficiently in the presence of glucose, sucrose and fructose compared to mannose (Brouwer et al., 2014). Thus, *P. infestans* can modulate the metabolic pathways depending on the carbon source available and host colonisation (Rodenburg et al., 2019). For example, the addition of sucrose increased the expression levels of sucrase genes (Judelson et al., 2009) possibly to reinforce sucrose hydrolysis and limit common sugar accumulation in the cell. The β-glucan content was higher in Glu- and Suc-incubated samples compared to Control-incubated samples, suggesting that common sugar availability increased the carbohydrate storage in *P. infestans*. Likewise, *P. cinnamomi* grown on a glucose-rich substrate accumulated β-glucans (Zevenhuizen and Bartnicki-Garcia, 1970), to cope with possible periods of starvation, as described in other microorganisms(Wilson et al., 2010).

5. Conclusions

The interactions between tagatose and the common sugar metabolism provided a better understanding of tagatose mode of action against *P. infestans* and revealed that growth inhibition was associated with a precise reprogramming of the carbohydrate metabolism with a decrease in glucose, glucose-1-phosphate and mannose content and β-glucosidase activity. The tagatose effects were partially attenuated by the presence of glucose, fully impaired by the presence of fructose and not influenced by the presence of sucrose. Thus, tagatose possibly inhibited sucrose catabolism, altered glucose-and organic acid-related pathways leading to *P. infestans* growth inhibition. Furthermore, this study provided useful information for the tagatose formulation as biofungicide, indicating that the presence of fructose, or other common sugars, could interfere with its efficacy. The assessment of tagatose effects of on plant metabolism represents a further step in the efficacy evaluation, in order to verify possible implications on plant growth or plant resistance induction. Moreover, further transcriptomic and enzymatic studies are required to better identify cellular processes affected by this rare sugar.

6. Author contributions

PEC conceived and carried out the experiments, data analysis, result interpretation and manuscript drafting. AN contributed to the microbiological experiments. CL carried out the metabolomic analysis. AC helped in the experimental setup. UV contributed with metabolomic data interpretation. IP conceived the study and revised the manuscript. MP conceived the study, coordinated the project and helped in manuscript drafting. All authors read and approved the final manuscript.

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Declaration of Competing Interest

AC and AN were employed by Bi-PA nv. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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infestans strain VB3.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.micres.2021.126724.

References

Agius, L., 1994. Control of glucokinase translocation in rat hepatocytes by sorbitol and the cytosolic redox state. Biochem. J. 298, 237–243.

Agrios, G.N., 2005. Plant Pathology. Elsevier, San Diego, CA.

Ahmed, Z., 2001. Production of natural and rare pentoses using microorganisms and their enzymes. Electron. J. Biotechnol. 4, 13–14.

Axel, C., Zannini, E., Coffey, A., Guo, J., Waters, D.M., Arendt, E.K., 2012. Ecofriendly control of potato late blight causative agent and the potential role of lactic acid bacteria: a review. Appl. Microbiol. Biotechnol. 96, 37–48.

Barchietto, T., Saindrenan, P., Bompeix, G., 1992. Physiological responses of *Phytophthora citrophthora* to a subinhibitory concentration of phosphonate. Pestic. Biochem. Physiol. 42, 151–166.

Bartnicki-Garcia, S., Wang, M.C., 1983. Biochemical aspects of morphogenesis in *Phytophthora*. In: Erwin, D.C., Bartnicki-Garcia, S., Tsao, P.H. (Eds.), *Phytophthora*: Its Biology, Taxonomy, Ecology, and Pathology. American Phytopathological Society Press, St. Paul, MN, U.S.A, pp. 121–137.

Beerens, K., Desmet, T., Soetaert, W., 2012. Enzymes for the biocatalytic production of rare sugars. J. Ind. Microbiol. Biotechnol. 39, 823–834.

Bertelsen, H., Jensen, B.B., Buemann, B., 1999. D-Tagatose-a novel low-calorie bulk sweetener with prebiotic properties. World Rev. Nutr. Diet. 85, 98–109.

Bertelsen, H., Andersen, H., Tvede, M., 2001. Fermentation of D-tagatose by human intestinal bacteria and dairy lactic acid bacteria. Microb. Ecol. Health Dis. 13, 87–95.

Brouwer, H., Coutinho, P.M., Henrissat, B., de Vries, R.P., 2014. Carbohydrate-related enzymes of important *Phytophthora* plant pathogens. Fungal Genet. Biol. 72, 192–200.

Bulone, V., 2009. Biosynthetic enzymes for (1, 3)-β-glucans and (1, 3; 1, 6)-β-glucans in protozoans and chromistans: biochemical characterization and molecular biology. In: Bacic, A., Stone, B.A., Fincher, G.B. (Eds.), Chemistry, Biochemistry, and Biology of 1-3 Beta Glucans and Related Polysaccharides. Elsevier, Academic Press, San Diego, pp. 233–258.

Chahed, A., Nesler, A., Navazio, L., Baldan, B., Busato, I., Ait Barka, E., Pertot, I., Puopolo, G., Perazzolli, M., 2020. The rare sugar tagatose differentially inhibits the growth of *Phytophthora infestans* and *Phytophthora cinnamomi* by interfering with mitochondrial processes. Front. Microbiol. 11, 128.

Chitarrini, G., Soini, E., Riccadonna, S., Franceschi, P., Zulini, L., Masuero, D., Vecchione, A., Stefanini, M., Di Gaspero, G., Mattivi, F., 2017. Identification of biomarkers for defense response to *Plasmopara viticola* in a resistant grape variety. Front. Plant Sci. 8, 1524.

Cordero Otero, R., Ubeda Iranzo, J., Briones-Perez, A., Potgieter, N., Villena, M., Pretorius, I., Rensburg, P.v., 2003. Characterization of the β-Glucosidase activity produced by enological strains of non-*Saccharomyces* yeasts. J. Food Sci. 68, 2564–2569.

Dhake, A., Patil, M., 2005. Production of ß-glucosidase by *Penicillium purpurogenum*. Braz. J. Microbiol. 36, 170–176.

Fortwendel, J.R., Juvvadi, P.R., Pinchai, N., Perfect, B.Z., Alspaugh, J.A., Perfect, J.R., Steinbach, W.J., 2009. Differential effects of inhibiting chitin and 1, 3-β-D-glucan synthesis in ras and calcineurin mutants of *Aspergillus fumigatus*. Antimicrob. Agents. 476–482. Ch. 53.

Fry, W., 2008. *Phytophthora infestans*: the plant (and R gene) destroyer. Mol. Plant Pathol. 9, 385–402.

Granström, T.B., Takata, G., Tokuda, M., Izumori, K., 2004. Izumoring: a novel and complete strategy for bioproduction of rare sugars. J. Biosci. Bioeng. 97, 89–94.

Guerrero-Wyss, M., Durán Agüero, S., Angarita Dávila, L., 2018. D-tagatose is a promising sweetener to control glycaemia: a new functional food. Biomed Res. Int. 2018, 8718053.

Hammer, Ø., Harper, D.A., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 9.

Hasibul, K., Nakayama-Imaohji, H., Hashimoto, M., Yamasaki, H., Ogawa, T., Waki, J., Tada, A., Yoneda, S., Tokuda, M., Miyake, M., 2018. D-Tagatose inhibits the growth and biofilm formation of *Streptococcus mutans*. Mol. Med. Rep. 17, 843–851.

Hossain, A., Yamaguchi, F., Hirose, K., Matsunaga, T., Sui, L., Hirata, Y., Noguchi, C., Katagi, A., Kamitori, K., Dong, Y., 2015. Rare sugar D-psicose prevents progression and development of diabetes in T2DM model Otsuka Long-Evans Tokushima Fatty rats. Drug. Des. Dev. Ther. 9, 525–535.

Izumori, K., 2006. Izumoring: a strategy for bioproduction of all hexoses. J. Biotechnol. 124, 717–722.

Jayamuthunagai, J., Gautam, P., Srisowmeya, G., Chakravarthy, M., 2017. Biocatalytic production of D-tagatose: a potential rare sugar with versatile applications. Crit. Rev. Food Sci. Nutr. 57, 3430–3437.

Judelson, H.S., Tani, S., Narayan, R.D., 2009. Metabolic adaptation of *Phytophthora infestans* during growth on leaves, tubers and artificial media. Mol. Plant Pathol. 10, 843–855.

Kim, P., 2004. Current studies on biological tagatose production using L-arabinose isomerase: a review and future perspective. Appl. Microbiol. Biotechnol. 65, 243–249.

Levin, G.V., 2002. Tagatose, the new GRAS sweetener and health product. J. Med. Food 5, 23–36.

Li, Z., Gao, Y., Nakanishi, H., Gao, X., Cai, L., 2013. Biosynthesis of rare hexoses using microorganisms and related enzymes. Beilstein J. Org. Chem. 9, 2434–2445.

Lobete, M.M., Noriega, E., Batalha, M.A., de Beurme, S., Van de Voorde, I., Van Impe, J. F., 2017. Effect of tagatose on growth dynamics of*Salmonella Typhimurium* and *Listeria monocytogenes* in media with different levels of structural complexity and in UHT skimmed milk. Food Control 73, 31–42.

Lu, Y., Levin, G., Donner, T.W., 2008. Tagatose, a new antidiabetic and obesity control drug. Diabetes Obes. Metab. 10, 109–134.

Maridueña-Zavala, M.G., Freire-Peñaherrera, A., Cevallos-Cevallos, J.M., Peralta, E.L., 2017. GC-MS metabolite profiling of *Phytophthora infestans* resistant to metalaxyl. Eur. J. Plant Pathol. 149, 563–574.

Matsuo, T., Baba, Y., Hashiguchi, M., Takeshita, K., Izumori, K., Suzuki, H., 2001. Dietary D-psicose, a C-3 epimer of D-fructose, suppresses the activity of hepatic lipogenic enzymes in rats. Asia Pac. J. Clin. Nutr. 10, 233–237.

Matsuo, T., Suzuki, H., Hashiguchi, M., Izumori, K., 2002. D-Psicose is a rare sugar that provides no energy to growing rats. J. Nutr. Sci. Vitaminol. 48, 77–80.

Mochizuki, S., Fukumoto, T., Ohara, T., Ohtani, K., Yoshihara, A., Shigematsu, Y., Tanaka, K., Ebihara, K., Tajima, S., Gomi, K., Ichimura, K., Izumori, K., Akimitsu, K., 2020. The rare sugar d-tagatose protects plants from downy mildews and is a safe fungicidal agrochemical. Commun. Biol. 3, 423.

Ohara, T., Ishida, Y., Kudou, R., Kakibuchi, K., Akimitsu, K., Izumori, K., Tajima, S., 2008. Plant Disease Control Agent Comprising D-tagatose As Active Ingredient, and Plant Disease Control Method. Patent No.: EP 2 329 713 A1, 1-46. Kagawa University, Takamatsu.

Oku, T., Murata-Takenoshita, Y., Yamazaki, Y., Shimura, F., Nakamura, S., 2014. D-sorbose inhibits disaccharidase activity and demonstrates suppressive action on postprandial blood levels of glucose and insulin in the rat. Nutr. Res. 34, 961–967.

Perazzolli, M., Nesler, A., Giovannini, O., Antonielli, L., Puopolo, G., Pertot, I., 2020. Ecological impact of a rare sugar on grapevine phyllosphere microbial communities. Microbiol. Res. 232, 126387.

Rodenburg, S.Y.A., Seidl, M.F., Judelson, H.S., Vu, A.L., Govers, F., de Ridder, D., 2019. Metabolic model of the *Phytophthora infestans*-tomato interaction reveals metabolic switches during host colonization. mBio 10, e00454–19.

Saha, B.C., Freer, S.N., Bothast, R.J., 1994. Production, purification, and properties of a thermostable β-glucosidase from a color variant strain of *Aureobasidium pullulans*. Appl. Environ. Microbiol. 60, 3774–3780.

Savory, F.R., Milner, D.S., Miles, D.C., Richards, T.A., 2018. Ancestral function and diversification of a horizontally acquired oomycete carboxylic acid transporter. Mol. Biol. Evol. 35, 1887.

Seri, K., Sanai, K., Negishi, S., Akino, T., 1995. Prophylactic and Remedial Preparation for Diseases Attendant on Hyperglycemia, and Wholesome Food. Godo Shusei Co Ltd. (US Patent 5468734), Tokyo, JP.

Seri, K., Sanai, K., Matsuo, N., Kawakubo, K., Xue, C., Inoue, S., 1996. L-arabinose selectively inhibits intestinal sucrase in an uncompetitive manner and suppresses glycemic response after sucrose ingestion in animals. Metabolism 45, 1368–1374.

Shedletzky, E., Unger, C., Delmer, D.P., 1997. A microtiter-based fluorescence assay for (1, 3)-β-glucan synthases. Anal. Biochem. 249, 88–93.

Stellmacher, L., Sandalova, T., Schneider, S., Schneider, G., Sprenger, G.A., Samland, A. K., 2016. Novel mode of inhibition by D-tagatose 6-phosphate through a Heyns rearrangement in the active site of transaldolase B variants. Acta Crystallogr. D Struct. Biol. 72, 467–476.

Van der Heiden, E., Delmarcelle, M., Lebrun, S., Freichels, R., Brans, A., Vastenavond, C. M., Galleni, M., Joris, B., 2013. A pathway closely related to the D-tagatose pathway of gram-negative Enterobacteria identified in the gram-positive bacterium *Bacillus licheniformis*. Appl. Environ. Microbiol. 79, 3511–3515.

Vastenavond, C.M., Bertelsen, H., Hansen, S.J., Laursen, R.S., Saunders, J., Eriknauer, K., 2012. Tagatose (D-tagatose). Alternative Sweeteners, fourth edition. CRC Press, pp. 197–222.

Wilson, W.A., Roach, P.J., Montero, M., Baroja-Fernández, E., Muñoz, F.J., Eydallin, G., Viale, A.M., Pozueta-Romero, J., 2010. Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbiol. Rev. 34, 952–985.

Wong, D., 2000. Sweetener determined safe in drugs, mouthwashes, and toothpastes. Dent. Today 19, 34–35.

Xi, B., Gu, H., Baniasadi, H., Raftery, D., 2014. Statistical analysis and modeling of mass spectrometry-based metabolomics data. In: Raftery, D. (Ed.), Mass Spectrometry in Metabolomics. Methods in Molecular Biology (Methods and Protocols), 1198. Springer, Humana Press, New York, NY, pp. 333–353.

Yun, E.J., Lee, A.R., Kim, J.H., Cho, K.M., Kim, K.H., 2017. 3, 6-Anhydro-l-galactose, a rare sugar from agar, a new anticariogenic sugar to replace xylitol. Food Chem. 221, 976–983.

Zar, J.H., 1996. Biostatistical Analysis, 3rd ed. Prentice Hall.

Zevenhuizen, L., Bartnicki-Garcia, S., 1970. Structure and role of a soluble cytoplasmic glucan from *Phytophthora cinnamomi*. J. Gen. Microbiol. 61, 183–188.

Zhang, W., Zhang, T., Jiang, B., Mu, W., 2017. Enzymatic approaches to rare sugar production. Biotechnol. Adv. 35, 267–274.