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Contribution of fruit microbiome to raspberry volatile organic compounds emission

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A R T I C L E I N F O

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A B S T R A C T

Raspberry fruit (*Rubus idaeus* L.) is highly appreciated by consumers for its quality characteristics and aromatic profile, determined by nearly 300 volatile organic compounds (VOCs). Although several microbes produce VOCs, their direct involvement in fruit aroma determination has been largely overlooked. In this study, the contribution of fruit-associated microbiota to fruit volatile emissions was evaluated by performing an untargeted GC–MS analysis of VOCs occurring in control (C), sterile (S) and artificially reinoculated berries (R). C and R bacterial fruit microbiomes were characterised by next generation sequencing (NGS). The treatments significantly affected the fruit volatilomes, thus confirming the role of bacteria in fruit aroma construction. In particular, aldehydes, monoterpenes, norisoprenoids, and other aroma-active compounds were significantly lower in S raspberries, and recolonisation could only partially restore the emission of terpenoid compounds. Significant correlations were found among NGS data and volatile emissions, including a positive correlation between *Lactobacillus* and *Pae- nibacillus* spp. and norisoprenoids, and a negative correlation between Enterobacteriaceae and monoterpenes. Several VOC-emitting bacterial taxa (including *Bacillus*, *Lactobacillus, Methylobacterium, Paenibacillus*, *Pseudo- monas* spp.) are recurrently found in the raspberry-associated microbiome, suggesting that future applications aimed at the control of microbial colonisation may enhance fruit aroma.

1. Introduction

Small berry fruit include several species characterised by remarkable nutraceutical and aromatic properties, harvested at physiological maturity and consumed within a few days. Among these fruit, raspberry (*Rubus idaeus* L.) is widely cultivated in America and Europe. Besides being appreciated for its high content in health-beneficial compounds, such as anthocyanins, flavonols, catechins, ascorbic and ellagic acid derivatives (Schulz and Chim, 2019), raspberries are well recognised by consumers for their characteristic flavour and aromatic profile. Nearly 300 volatile organic compounds (VOCs) have been reported to contribute to raspberry sensory quality (Aprea et al., 2015). Abundance and composition of volatile compounds in fruit is highly dependent on several factors, such as cultivar, maturity, pre- and postharvest fruit handling (El Hadi and Ahmed, 2013).

Plants are naturally colonised by diverse bacterial and fungal communities, referred to as microbiota (Bulgarelli et al., 2013), which may vary according to organ, genotype (Morella et al., 2020) and environmental conditions. The microbiota can influence the host plant phenotype, and convey resistance to stress and biotic factors (Van Wees et al., 2008; Berlec, 2012; Finkel et al., 2017; Purahong et al., 2018). Long-known effects of plant-associated microbes on their host plants include nutritional enhancement, growth promotion and induction of resistance/tolerance to stress (Bailly and Weisskopf, 2017; Sharifi and Ryu, 2018), and induction or the control of postharvest diseases in fruit (Mari et al., 2016; Zhang et al., 2020). More recently, microbial com- munities have been held responsible for the production of VOCs with physiological and ecological roles (Cellini et al., 2021; Weisskopf et al., 2021), for instance, in inducing plant resistance responses (Cellini et al., 2018) or modulating floral emissions (Ponzoni et al., 2008; Pen˜uelas et al., 2014; Cellini et al., 2019). In contrast, the direct involvement of the fruit-associated microbiota in the determination of fruit quality has

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been largely overlooked.

Raspberry has been the subject of microbiome studies (i.e., the ge- netic characterisation of the microbiota) on soil (Oszust and Frac, 2021) and fruit (Perpetuini et al., 2019), highlighting the functions expressed by the plant-associated microflora. Although genotype-specific bacterial communities have been found on the surface of raspberry fruit (Per- petuini et al., 2019), their contribution to VOC emission has not been investigated. The aim of this study, therefore, was to investigate the actual contribution of fruit-associated microbiota to fruit aromatic properties by analysing volatilomes of control and artificially reinocu- lated berries.

2. Material and methods

2.1. Sample origin and treatment

Raspberry plants of cultivar 'Enrosadira' were grown at Ponte di Pietra (FC, Italy) during summer 2019 following standard agriculture practices. Harvest was performed by hand-picking berries at full matu- rity stage. Harvested berries were put in cold boxes and immediately brought to the laboratory.

The experimental design included three replicates, made up of six berries each, for the three different treatments (Control, C; Sterile; S; Recolonised, R). For the C samples, berries were dipped for 10 s in 0.01 M $MgSO_4$ supplemented with cycloheximide (0.05 g L^{-1}), to eliminate epiphytic fungi but at the same time avoiding the washing out of the bacterial microbiome. Another batch of fruit was washed 5 min under agitation (70 rpm) in 0.01 M $MgSO_4$ plus cycloheximide, and the wash was collected. Berries were then surface-sterilised by sequential rinsing with 1.5% NaClO, 70% ethanol, and three times with sterile deionised water; S samples were taken from this fruit batch. The wash collected before sterilisation was concentrated by centrifugation for 15 min at 5000 × g, resuspended in 0.002 L, and used to reinoculate previously sterilised berries (R samples) with 0.0001 L of concentrated suspension.

They were then let dry in sterility, put in a glass pot and closed with a paper lid, to allow aeration. After 24 h, three berries for each replicate and treatment were collected, the pot was closed with an air-tight pierceable lid, maintained 24 h at room temperature and subsequently stored at —80 °C. The remaining berries were washed 15 min at 100 rpm in $MgSO_4$ solution in order to resuspend the epiphytic microbial popu- lation. C and R washings were stored at —80 °C for subsequent DNA extraction, whereas S washings were plated on Luria-Bertani agar me- dium (Sigma Aldrich, St. Louis, MO, USA) to check sterility.

2.2. Fruit volatile analysis

Each replicate (n = 3) consisted of a pool of three berries. Raspberry fruit volatiles were analysed by solid phase microextraction-gas chro- matography-mass spectrometry (SPME-GC–MS) as described in San- giorgio et al. (2021). Briefly, samples were taken out from —80 °C and put at —20 °C for 20 min before equilibration. 1-octanol (52.5 ng per sample) was used as internal standard for each sample and equilibration was carried out at 40 °C for 20 min. Samples were then exposed to a DVB Carboxen PDMS Stable Flex SPME fibre (Supelco, Bellefonte, PA, USA) for 40 min, which was desorbed in the injector of a Shimadzu GC–MS-QP2010 Plus (Shimadzu, Tokyo, Japan) at 250 °C for 10 min in the split mode. The chromatographic separation of VOCs was performed on an RTX-WAX fused-silica capillary column (Restek, Bellefonte, PA, USA). GC–MS heating program and conditions were the same as those reported by Sangiorgio et al. (2021). VOCs were identified based on their on mass spectra and linear retention indices, which were compared with the NIST/EPA/NIH Mass Spectral Database (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, USA) and the ChemSpider information resource (http://www.chemspider.com).

2.3. DNA extraction and next generation sequencing for microbiome analysis

To perform metagenomic analysis, DNA was extracted from washing suspensions according to the CTAB protocol (Maguire et al., 1994), using the pellet obtained from centrifuging the washing solutions at 13,000 × g for 10 min. DNA quality and quantity were measured by spectrophotometric quantification with a NanoDrop ND-8000 V1.1.1 spectrop hotometer (Thermo Fisher Scientific, Dreieich, Germany). BacterialV3-V4 regions were amplified with 16S Amplicon PCR Forward = 5'TCGTCGGCAGCGTCAGATGTGT ATAAGAGACAGCCTACGGGNGGCWGCAG and Reverse = 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHV GGGTATCTAATCC primers according to Illumina protocols and subjected to automated sequencing by BioFab research (Rome, Italy).

2.4. Bioinformatic analysis

BBMap version 38.79 was used to remove low-quality reads using a quality-trim left and right ends before mapping with an average phred score ≥25 (Bushnell, 2014). Sequences shorter than 170 bases were discarded. The sequences were analysed using the Qiime2 v2020.2.0 (Bolyen et al., 2019). Qiime2 dada2 plugin was used to length trimming, denoising, chimera and PhiX removal. SILVA 16S rRNA sequences database, release 312 (Quast, 2012) with a 97% identity criterion, was used to assign taxonomy to features. Visualisation of microbiome composition was performed using https://sankeymatic.com/build/. Bacterial genera were considered to be part of the core microbiome if they were present in at least three out of four cultivars, as long as their family was present in the excluded genotype.

2.5. Statistical analysis

Past software (Version 4.0) (Hammer et al., 2001)was used for basic statistical functions. One-way ANOVA was computed to investigate whether single VOCs and VOCs classes were significantly different among treatments. The significance level of all analysis was $p = 0.05$. R (Version 1.1.463), together with the external package "mix- Omics"(Rohart, 2017), was used for PCA analysis and visualisation employed in this work. Multiple Factor Analysis was performed with the external package "FactoMineR" (Lˆe and Husson, 2008). Pearsons' and Spearmans' correlations were analysed with "Hmisc" (Harrell, 2004) and predicted-R^2 with "olsrr" package (Hebbali, 2020). R-squared and Predicted R-squared were computed for VOC/NGS pairs when both Pearsons' and Spearmans' R were significant. Pearsons' R value between NGS and VOC data was calculated using raw NGS data, as well as transformed NGS data calculated as follows: Transformed OTU abundance = -1/(1 + OTU abundance). When the difference between R-squared and predicted R-squared was lower than 0.25, the association model between VOC and NGS was considered acceptable for prediction.

3. Results

3.1. Effects of surface sterilisation and recolonisation on the fruit volatilome

Fruit samples of the cultivar 'Enrosadira' were washed, surface-sterilised, and re-inoculated with the wash suspension to recolonise the fruit with its original microbiota. VOC emission from control (C), sterilised (S) and recolonised (R) samples was investigated by GC–MS. PCA shows a clear separation of sample classes based on their VOC profiles (Fig. 1a). 2-pentanone, *o*-cymene, limonene, β-myrcene and β-pinene contributed to the differentiation of sterile and recolonised berries from the control ones (Fig. 1b). Surface-sterilised berries evi- denced a generalised reduction of VOC emissions. In particular,

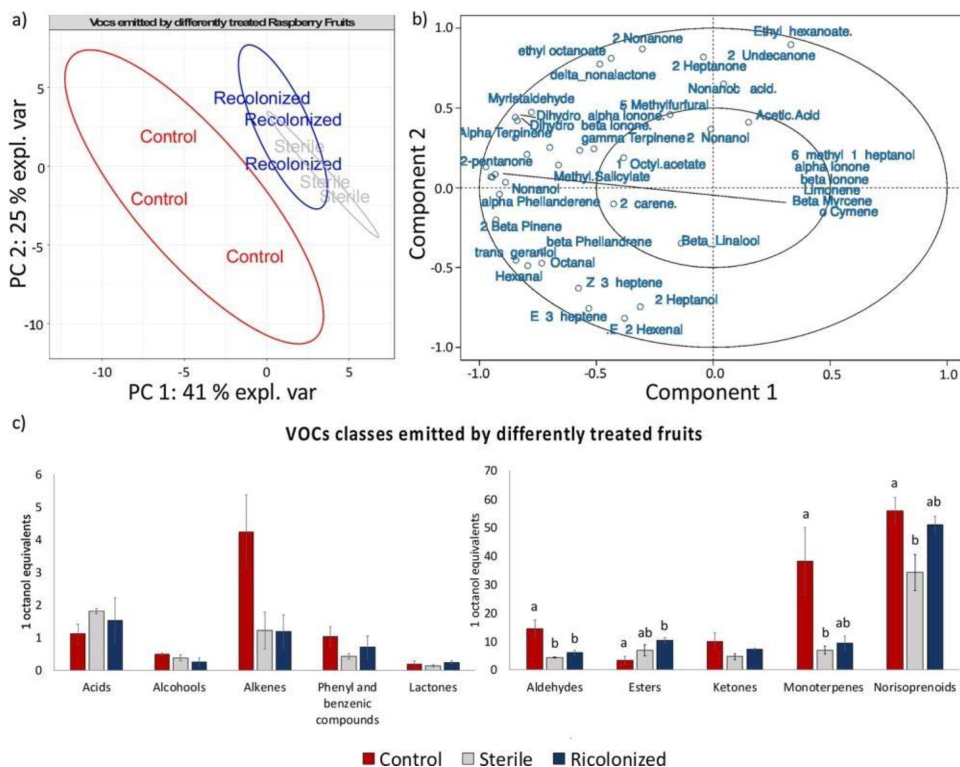

Fig. 1. (**a**) PCA of VOCs emitted by untreated (Control), sterile and recolonised raspberries ('Enrosadira') analysed by GC–MS. (**b**) Correlation circle plot relative to components 1 and 2. (**c**) VOC emissions (divided in chemical classes) from differently treated raspberries. Data are expressed as mean ± SE. One-way ANOVA was performed. Different letters indicate significant differences between treatments ($p \leq 0.05$) for each VOC class, according to Tukey's post-hoc test. Values are expressed as 1-octanol equivalents. 1-octanol equivalent (TICs) corresponds to 52.5 ng 1-octanol per sample under the present experimental conditions.

aldehydes, monoterpenes and norisoprenoids significantly decreased with sterilisation treatment, and recolonisation could partly restore the emission of terpenoid compounds (Fig. 1c). Specifically, hexanal, octa- nal, 1-nonanol and β-pinene emissions significantly decreased in S and R treatments (the latter two compounds being detectable only in the control). Limonene, *o*-cymene and α-ionone were reduced in S, but R emissions were similar to C. In R berries, β-phellandrene could not be detected (Table 1).

3.2. *Bacterial microbiome of differently treated raspberries*

Next Generation Sequencing (NGS) analysis revealed that control raspberries were colonised by a wide variety of bacterial taxa, while R berries mainly hosted taxa belonging to Enterobacteriaceae family (Fig. 2). Indeed, C berries showed a higher biodiversity (Shannon's diversity index $H = 2.18$) with respect to R ones ($H = 0.61$). C berries were mainly colonised by *Brevibacillus* (31%), *Rosenbergiella* (27%) and *Methylobacterium* (13%) genera. After sterilisation, S berries were washed and a wash aliquot was plated on LB agar. The absence of bacterial growth confirmed the efficacy of the sterilisation treatment.

3.3. *Determination of raspberry core microbiome and volatilome*

Volatiles of raspberry cultivars 'Imara', 'Regina', 'Anne' and 'Enrosadira' were combined to construct a core volatilome (i.e. VOCs present in all genotypes analysed), which consisted of seventeen VOCs (Fig. 3a). It included the main classes found in fruit from different genotypes with the exception of alkenes, phenyl and benzenic compounds and lactones which were not present in all berries. Similarly, microbiome data of the four raspberry cultivars included in this and in previous work (Perpetuini et al., 2019; Sangiorgio et al., 2021) were combined to construct a core microbiome (Fig. 3b), which included 2 genera of Alphaproteo- bacteria (*Methylobacterium*, *Sphingomonas*), 1 of Betaproteobacteria (*Burkholderia*) and 3 of Gammaproteobacteria (*Acinetobacter*, *Pantoea*, *Pseudomonas*). Moreover, it contained 4 genera of Bacilli (*Bacillus*, *Lactobacillus*, *Paenibacillus* and *Vagococcus*) (Fig. 3b). All compounds of the core volatilome have been found to be produced by at least one bacterium belonging to the core microbiome, except for γ-terpinene, *o*-cymene, β-ionone, dihydro-α-ionone, dihydro-β-ionone (Lemfack et al., 2018; Sangiorgio et al., 2021).

3.4. *Fruit volatiles correlation with bacterial microbiome*

MFA was performed to assess the correlation between microbiome composition (at genus level) (NGS) and VOCs emission by control and recolonised berries (Fig. 4). MFA model explained a variance of 42.2% on dimension 1 and of 30% on dimension 2. In the graph, variables that are positively correlated are grouped together, whereas those that are negatively correlated are positioned at opposite sides (Fig. 4a). NGS and VOCs driving the differences between differently treated berries observed on dimension 1 are shown (Fig. 4b). The variables with larger values, contribute the most to the definition of the dimension, being the most important in explaining the variability existing in the dataset. RV coefficient of MFA evaluates the relationship between two sets of vari- ables analysed for the same samples. In this work, NGS and VOCs data were associated with a RV score of 0.78 ($p = 0.02$).

To better understand specific interactions between volatiles and fruit-associated microbiome, a correlation analysis between bacterial genera and volatile emission was performed. Spearman's rank correlation matrix was computed and results were further screened considering only significant ($p < 0.05$) correlations (Table 2). 42 positive and 52 negative correlations were identified between VOCs and bacterial genera.

To validate the correlations found among VOCs and NGS data, predicted R-squared was calculated for those correlations that were significant both by applying Pearson's and Spearman's method (Table 2). Pearson's R value was calculated both using original and transformed NGS data (Transformed OTU abundance = -1/(1 + OTU abundance)) and the highest value was reported. Data transformation was included to account for asymptotic correlations. Twelve predicted R-squared, calculated with original NGS data, appeared to be coherent with their respective R-squared value: *Streptomyces*-nonanoic acid, *Fictibacillus*-(*E*)-

Table 1
VOCs detected by GC–MS in control, sterile and recolonised raspberries (‘Enrosadira’). Data are expressed as mean ± SE of 1-octanol equivalents. 1-octanol equivalent (TICs) corresponds to 52.5 ng 1-octanol per sample under the present experimental conditions. n.d. = not detected. One-way ANOVA was performed. Different letters indicate significant differences between treatments ($p \leq 0.05$) for each VOC, according to Tukey’s post-hoc test.

		Control (1-octanol equivalents)	Sterile	Recolonised
Acids	Acetic acid	1.02 ± 0.22	0.05	1.3 ± 0.66
	Nonanoic acid	0.08 ± 0.08	0.07 ± 0.04	0.23 ± 0.07
Alcohols	2-heptanol	0.43 ± 0.08	0.31 ± 0.07	0.16 ± 0.11
	2-nonanol	0.05 ± 0.05	0.06 ± 0.03	0.08 ± 0.04
	Nonanol	0.18 ± 0.09	n.d.	n.d.
	6-methyl-1-heptanol	0.03 ± 0.03	n.d.	n.d.
Aldehydes	Hexanal	5.65 ± 0.75 **a**	0.82 ± 0.16 **b**	1.57 ± 0.31 **b**
	(*E*)-2-hexenal	6.82 ± 2.35	2.51 ± 0.17	2.9 ± 0.78
	Octanal	0.76 ± 0.11 **a**	0.15 ± 0.08 **b**	0.16 ± 0.08 **b**
	5-methyl furfural	0.81 ± 0.03	0.63 ± 0.06	1.17 ± 0.19
	Tetradecanal	0.44 ± 0.22	0.15 ± 0.04	0.29 ± 0.1
Alkenes	(*E*)-3-heptene	1.89 ± 0.56	0.54 ± 0.17	0.49 ± 0.23
	(*Z*)-3-heptene	2.34 ± 0.58	0.67 ± 0.4	0.69 ± 0.28
Esters	Ethyl hexanoate	2.26 ± 1.13 **b**	5.96 ± 1.88 **ab**	9.14 ± 0.81 **a**
	1-octyl acetate	0.75 ± 0.15	0.73 ± 0.08	0.65 ± 0.02
	Ethyl octanoate	0.35 ± 0.18	0.17 ± 0.1	0.61 ± 0.15
Ketones	2-pentanone	7.35 ± 2.35	1.41 ± 0.3	2.83 ± 0.15
	2-heptanone	0.45 ± 0.16	0.55 ± 0.12	0.73 ± 0.08
	2-nonanone	1.08 ± 0.27	0.92 ± 0.25	1.63 ± 0.31
	2-undecanone	1.15 ± 0.24	1.77 ± 0.47	2.05 ± 0.05
Monoterpenes	β-pinene	0.54 ± 0.16	n.d.	n.d.
	α-phellandrene	15.33 ± 4.72	2.93 ± 0.63	4.7 ± 1.78
	β-myrcene	4.91 ± 1.9	0.81 ± 0.28	1.21 ± 0.59
	α-terpinene	0.86 ± 0.43	0.1 ± 0.06	0.28 ± 0.14
	Limonene	2.54 ± 0.79 **a**	0.4 ± 0.09 **b**	0.68 ± 0.26 **ab**
	β-phellandrene	9.65 ± 2.96	1.8 ± 0.39	n.d.
	γ-terpinene	0.67 ± 0.37	0.06 ± 0.06	0.82 ± 0.32
	o-cymene	2.69 ± 0.9 **a**	0.4 ± 0.06 **b**	0.96 ± 0.03 **ab**
	2-carene	0.38 ± 0.24	0.12 ± 0.07	0.31 ± 0.05
	(*E*)- geraniol	0.67 ± 0.09	0.35 ± 0.03	0.39 ± 0.03
Norisoprenoids	Dihydro-α-ionone	0.96 ± 0.24	0.4 ± 0.14	0.68 ± 0.17
	Dihydro-β-ionone	6.53 ± 1.33	3.17 ± 0.91	5.45 ± 0.8
	α-ionone	13.35 ± 0.8 **a**	6.65 ± 1.32 **b**	11.16 ± 1.34 **ab**
	β-ionone	35 ± 2.49	24.01 ± 4.18	

Table 1 (*continued*)

		Control (1-octanol equivalents)	Sterile	Recolonised
Phenyl and benzenic compounds	Methyl salicylate	1.02 ± 0.31	0.42 ± 0.09	0.7 ± 0.35
Lactones	δ-nonalactone	0.18 ± 0.09	0.14 ± 0.03	0.24 ± 0.05

1.73 ±

3 3. 7 ± 1. 04

2-hexenal, *Sphyngopyxis*-octanal, *Fictibacillus*-(*E*)-3-heptene, *Sediminibacterium*-(*E*)-3-heptene, *Sediminibacterium*-(*Z*)-3-heptene, *Acetobacter*-ethyl octanoate, *Brevibacillus*-2-heptanone, *Finegoldia*-2-carene, *Escherichia-Shigella*-(*E*)-geraniol, *Lactobacillus*-β-ionone and *Acetobacter*- δ-nonalactone. Additionally, 10 cases were identified correlating VOC emissions with transformed NGS data: *Corynebacterium*-nonanoic acid, *Cutibacterium*-octanal, *Staphylococcys*-5-methylfurfural, and *Enterobacter* with five monoterpene compounds (β-pinene, β-myrcene, α-phellandrene, β-phellanderene and limonene), nonanol and 2-pentanone.

4. Discussion

4.1. Sterilisation and recolonisation treatments modify fruit volatile emissions

While microbial communities have traditionally received more attention for their roles in the soil and rhizosphere, their involvement in fruit quality has raised interest only recently (Leff and Fierer, 2013). With regard to fruit aroma, for instance, the contribution of *Methylobacterium* spp. to the VOC profile of strawberries has been suggested in different studies (Verginer et al., 2010; Nasopoulou et al., 2014). The ability of *Bacillus* spp. to produce 4-(4-hydroxyphenyl)-butan-2-one (also referred to as raspberry ketone or frambinone) has also been pointed out (Feron et al., 2007). Nonetheless, all these researches focused on single species (e.g. *Methylobacterium* or *Bacillus*) and targeted specific volatiles (e.g. furanones). Moreover, they were performed by experimentally enriching the microbial community with selected bacteria.

In this work, the contribution of fruit-associated microbiota to fruit volatile emissions was evaluated by performing an untargeted analysis of VOCs in control (C), sterile (S) and artificially reinoculated raspberries (R). Sterilisation and recolonisation significantly affected the volatilome profile of fruit, thus confirming the role of bacteria in fruit aroma construction. Although efficacy of the sterilisation treatment was verified by assessing the absence of growth of viable and culturable (VC) cells on LB agar, the possible persistence on sterile fruits of bacterial cells in viable but not culturable (VBNC) state cannot be excluded. VBNC state is a survival strategy that takes place when microorganisms are exposed to stressful conditions (Dong et al., 2020), which might be the case of the sterilisation procedure. Nonetheless, the contribution to fruit volatilome by bacteria in VBNC state was probably of minor entity. In fact, under favourable conditions, VBNC cells could return to a cultur- able state in a time frame ranging between 10 days and 3 months (Pinto et al., 2015), which is longer than the time intercurrent, in our experi- ments, between sterilization and VOC analysis.

To further dissect the influence of different bacterial genera on fruit aroma, the whole microbiome was characterised by NGS. Aldehydes, monoterpenes, norisoprenoids, and other aroma-active compounds (octanal, nonanol) were significantly lower in sterile berries, and recolonisation partially restored the emission of terpenoid compounds (Fig. 1c). Interestingly, hexanal, octanal and limonene have been found to be produced by several bacterial genera such as *Lactobacillus*, *Pseu- domonas*, *Staphylococcus* and *Streptomyces* (Lemfack et al., 2018), abundantly found on recolonised fruits.

Only for one compound (ethyl hexanoate, characterised by overripe fruit aroma) the emission from recolonised fruit was higher than from control, as a possible result of plant tissue degradation. The absence of

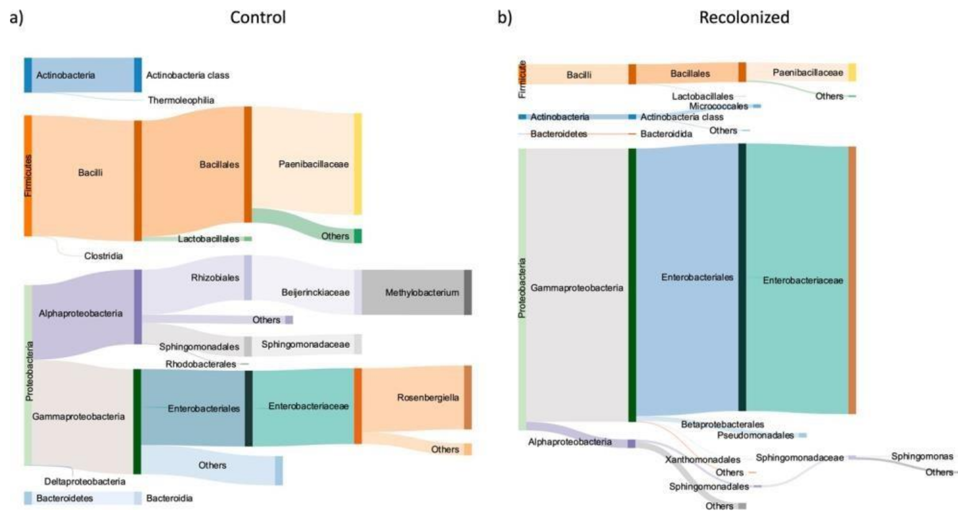

Fig. 2. Sankey's diagram showing (**a**) Control and (**b**) Recolonised fruit microbiome composition.

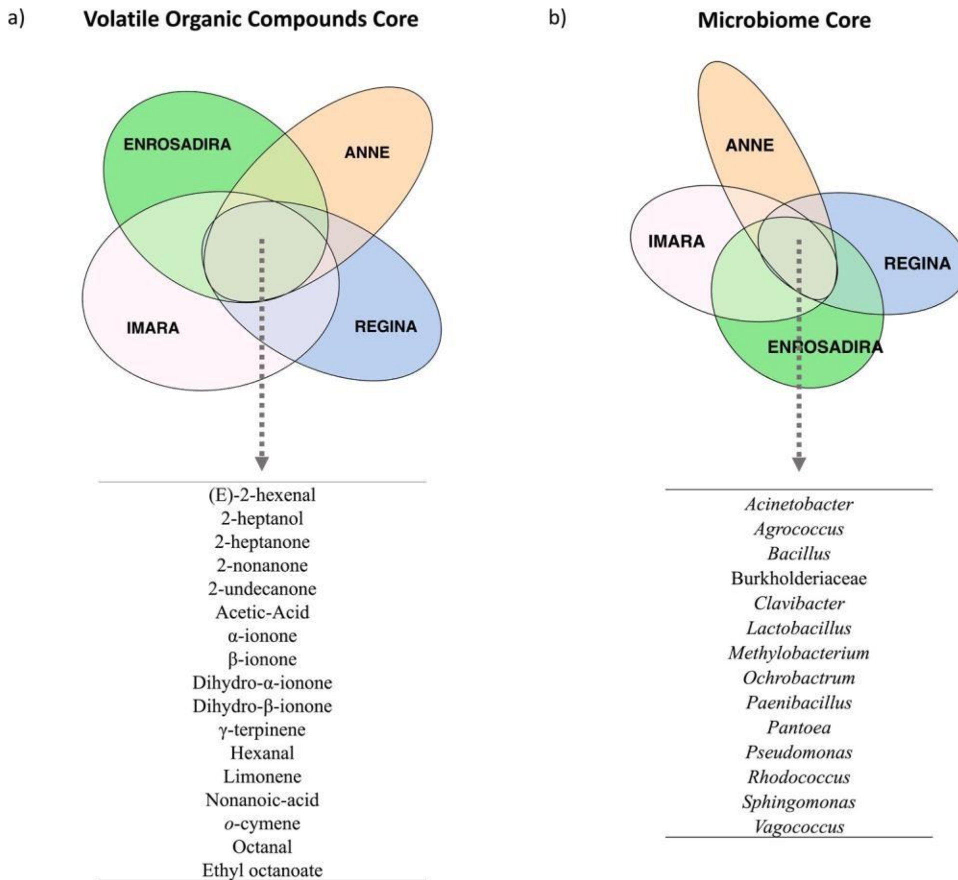

Fig. 3. Core volatilome (**a**) and microbiome (**b**) of raspberries ('Imara', 'Regina', 'Anne' and 'Enrosadira').

β-pinene and nonanol in sterile and recolonised berries, suggests a crucial influence of the microbiome in their emissions. In this view, the alteration of the microbiome structure observed in recolonised fruit may have not allowed to fully restore their emission. However, an effect of the sterilisation treatment on the emission of these two compounds cannot be fully ruled out, despite the fact that compounds with similar chemical properties and concentrations, such as 2-carene and 2-nonanol, were not significantly affected by the sterilisation treatment.

Interestingly, β-pinene has been observed to be produced by several bacterial genera found on raspberry, such as *Paenibacillus* spp., which is part of the core microbiome, *Pedobacter* spp. and *Streptococcus* spp. (Lemfack et al., 2018). Similarly, nonanol has been found to be produced by several bacteria isolated from raspberry, such as *Pseudomonas* (Ercolini et al., 2010), *Streptococcus* (Hertel et al., 2016) and *Bacillus* (Guo, 2020; Reese et al., 2020). The strong reduction of these genera in recolonised berries might partly explain the absence of β-pinene and nonanol when fruit microbiome was manipulated. VOCs belonging to the class of furfurals have not yet been reported to be produced by

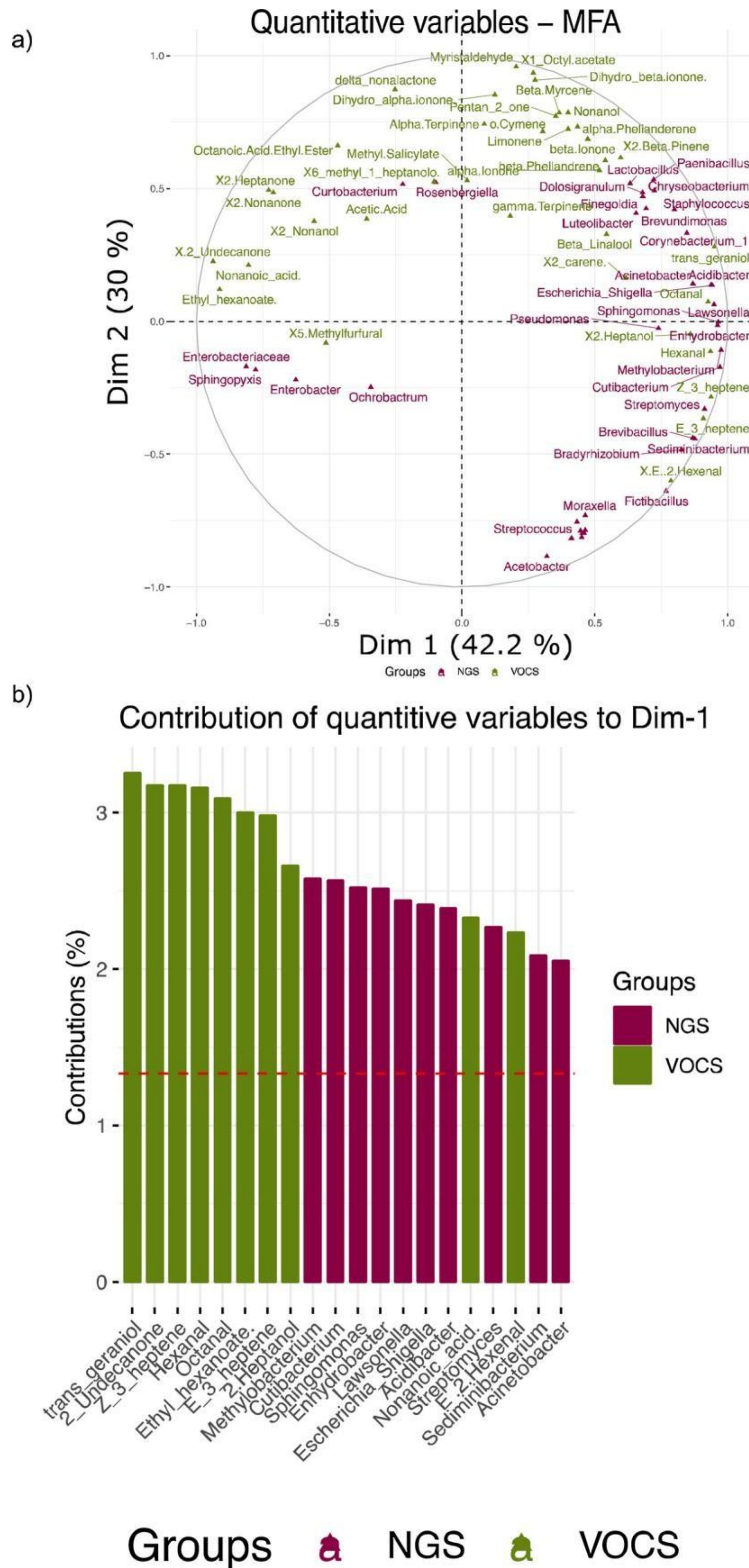

Fig. 4. Multiple Factor Analysis of VOCs and bacterial genera identified by NGS analysis of raspberries. (**a**) Diagram plotting quantitative VOC and NGS variables in a bidimensional space. (**b**) Diagram showing contribution of qualitative variables to Dimension 1. In both panels, VOCs are indicated in green and NGS variables are indicated in purple. (For interpretation of the references to colour in this figure legend, the reader should refer to the web version of this article).

Table 2

Significant correlations between VOCs and NGS, as determined by Spearman's test ($p < 0.05$) and Pearsons' correlation. For the latter, R value was calculated, either using original or transformed (Transformed OTU abundance = -1/(1+OTU abundance) NGS data. The highest values are reported if significant. R-squared and predicted R-squared are shown; * symbol indicates that R-squared and predicted R-squared were calculated with transformed NGS data.

VOC class	VOC	Bacterial Genus	Spearman's R value	Pearsons' R value	R-squared	Predicted R-squared
		Bradyrhizobium	−0.88			
		Corynebacterium	−0.99	0.93	0.86	0.64*
		Enhydrobacter	−0.81			
		Escherichia_Shigella	−0.82	0.83	0.68	0.437*
Acids	Nonanoic acid	*Lawsonella*	−0.99	−0.87	0.75	−1.832*
		Methylobacterium	−0.81	−0.81	0.66	0.36
		Sediminibacterium	−0.99	−0.84	0.70	0.27
		Sphingomonas	−0.93	−0.84	0.70	0.22
		Streptomyces	−0.97	−0.90	0.81	0.63
		Acidibacter	−0.89	−0.82	0.68	0.414*
	2-nonanol	*Brevibacillus*	−0.83			
		Curtobacterium	0.94			
Alcohols	Nonanol	*Enterobacter*	−0.82	−0.98	0.96	0.863*
		Corynebacterium	0.83			
	2-heptanol	*Escherichia_Shigella*	0.85			
		Lawsonella	0.83			
		Pseudomonas	0.89	0.85	0.73	0.43
	(*E*)-2-hexenal	*Fictibacillus*	0.90	0.99	0.98	0.97
	Hexanal	*Staphylococcus*	0.89			
		Cutibacterium	0.94	0.96	0.92	0.865*
	Octanal	*Escherichia_Shigella*	0.85	0.88	0.77	0.586*
		Sphingopyxis	−0.94	−0.94	0.88	0.74
		Staphylococcus	0.94			
Aldehydes		Enterobacteriaceae	0.94			
	5-methyl furfural	*Sphingopyxis*	0.88			
		Staphylococcus	−0.83	−0.97	0.94	0.875*
		Acetobacter	−0.85	−0.87	0.75	0.525*
	Tetradecanal		−0.82			
		Paenibacillus	0.89			
		Pedobacter	−0.85	−0.87	0.76	0.557*
	(*E*)-3-heptene	*Fictibacillus*	0.81	0.93	0.87	0.68
Alkenes		*Sediminibacterium*	0.83	0.96	0.91	0.84
	(*Z*)-3-heptene	*Fictibacillus*	0.81	0.90	0.82	0.44
		Sediminibacterium	0.83	0.93	0.87	0.73
	1-octyl-acetate		−0.85			
Esters		*Rothia*	−0.85			
	Ethyl octanoate		−0.85	−0.91	0.83	0.71
		Pedobacter	−0.85	−0.86	0.75	0.346*
		Enterobacter	−0.87	−0.96	0.93	0.718*
	2-pentanone	*Ochrobactrum*	−0.85			
		Rosenbergiella	0.83	0.81	0.66	−886.79
		Acidibacter	−0.84			
		Brevibacillus	−0.94	−0.95	0.91	0.78
Ketones	2-heptanone		0.81	0.85	0.72	0.171*
		Cutibacterium	−0.94	−0.90	0.81	0.423*
		Sphingopyxis	0.82			
		Staphylococcus	−0.83			
	2-nonanone	*Acinetobacter*	−0.89	−0.69	0.48	−0.70
		Fictibacillus	−0.99	−0.86	0.75	0.29
	2-undecanone	*Staphylococcus*	−0.89			
		Enterobacter	−0.92	−0.98	0.95	0.892*
	β-pinene	Enterobacteriaceae	−0.88	0.87	0.76	0.45
		Sphingopyxis	−0.87	−0.86	0.74	0.42*
	α-phellanderene	*Enterobacter*	−0.93	−0.96	0.91	0.767*
	β-myrcene	*Enterobacter*	−0.93	−0.95	0.91	0.718*
	α-terpinene	*Kocuria*	−0.83			
		Enterobacter	−0.92	−0.96	0.92	0.776*
		Enterobacteriaceae	−0.88			
	β-phellandrene	*Rosenbergiella*	0.88			
		Sphingopyxis	−0.87	−0.85	0.72	0.381*
Monoterpenes		*Enterobacter*	−0.93	−0.94	0.89	0.653*
	Limonene	*Ochrobactrum*	−0.85			
		Rosenbergiella	0.94			
		Sphingopyxis	−0.88			
		Acidibacter	0.81	0.85	0.72	0.12
	2-carene	*Dolosigranulum*	0.94	0.91	0.83	−0.53
		Finegoldia	0.85	0.90	0.81	0.57
		Enterobacteriaceae	−0.93			
	o-cymene	*Sphingopyxis*	−0.83			
		Staphylococcus	0.81	0.45	0.20	−0.61
	(*E*)-geraniol	*Enterobacter*	−0.84	−0.75	0.57	−0.33

(*continued on next page*)

Table 2 (*continued*)

VOC class	VOC	Bacterial Genus	Spearman's R value	Pearsons' R value	R-squared	Predicted R-squared
		Escherichia_Shigella	0.85	0.93	0.86	0.77
		Sphingomonas	0.83	0.90	0.81	0.54
		Staphylococcus	0.83	0.88	0.78	−17.41
	α-ionone	*Paenibacillus*	0.94	0.98	0.97	0.933*
		Blastomonas	−0.85			
		Chryseobacterium	0.85	0.92	0.84	0.467*
	β-ionone	*Lactobacillus*	0.85	0.96	0.93	0.73
		Paenibacillus	0.83	0.92	0.95	0.27
Norisoprenoids		*Rothia*	−0.85			
	Dihydro-α-ionone	*Paenibacillus*	0.83			
		Acetobacter	−0.85	−0.86	0.73	0.526*
	Dihydro-β-ionone	*Kocuria*	−0.82			
		Paenibacillus	0.89			
		Pedobacter	−0.85	−0.86	0.73	0.522*
		Acetobacter	−0.86	−0.98	0.97	0.95
		Chryseobacterium	0.82			
		Dolosigranulum	0.83			
Lactones	δ-nonalactone	*Lactobacillus*	0.82			
		Paenibacillus	0.87			
		Pedobacter	−0.86	−0.92	0.84	0.429*

plants, whereas several studies highlight both production and degra- dation of these compounds by microorganisms, such as *Pseudomonas* spp. and *Cupriavidus* spp., respectively (Koopman et al., 2010; Crigler and Altman, 2020). 5-methyl furfural has been found to be produced by several bacterial species isolated from raspberry fruit and grown on its juice (Sangiorgio et al., 2021), in particular by bacteria belonging to the Enterobacteriaceae family, which is in accordance with correlation identified in this work. This might justify emission increase of this vol- atile observed in artificially recolonised berries.

In addition to hedonic properties, several compounds reported in this work may play a role in defence against pathogens. Hexanal, for instance, is an inducer of systemic resistance in plants and an inhibitor of bacterial quorum-sensing (Zhang et al., 2018; Anusha et al., 2021), and has been reported to extend fruit shelf-life (Song et al., 1996). Methyl salicylate is also one of the main regulators of plant defences. Terpenes are natural antimicrobial compounds, also effective on postharvest fruit preservation (Sivakumar and Bautista-Baños., 2014).

Taken together, the data presented here suggest that the fruit- associated microbiota probably contributes to a more intense and complex raspberry aroma, and its perturbation during fruit handling and storage might affect final fruit quality and shelf-life duration. In this work, it was hypothesised that bacteria contributed to fruit aroma for- mation either by directly synthetising specific VOCs (Veselova et al., 2019) or by degrading/consuming plant VOCs as carbon sources (Junker and Tholl, 2013). However, bacteria can have also modified fruit vola- tilome by inducing VOCs emission by the host plant. In fact, several studies demonstrated that plant bacterisation may induce a systemic alteration of the emission of specific VOCs by the host (Farré-Armengol et al., 2016; Sharifi et al., 2018).

4.2. Commonalities of VOCs and fruit-associated bacteria in different raspberry cultivars

To evaluate the robustness of the findings of this work, both the microbiota and the VOC emissions of four raspberry cultivars were compared. Twenty-one VOCs were present in all the cultivars, including monoterpenes, norisoprenoids, and medium-length ketones and fatty acids. Microbiome data show the occurrence of genera potentially contributing to fruit aroma, such as *Paenibacillus*, Enterobacteriaceae, *Lactobacillus*, *Bacillus* (for which the emission of C5-C9 alcohols, alde- hydes, ketones and acids was reported; Lemfack et al., 2018; Sangiorgio et al., 2021), and *Methylobacterium*, known for producing strawberry flavour components such as 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one (Verginer et al., 2010; Nasopoulou et al., 2014).

Moreover, *Bacillus*, *Lactobacillus* and *Sphingomonas* spp. include specialised plant symbionts, modulating processes such as fruit quality and defence from pests and pathogens (Sansinenea, 2019; Daranas et al., 2019; Asaf et al., 2020). The presence of microbes conserved among cultivars, highly integrated with plant metabolism, and potentially coevolved with their host, suggests that these species may have a sig- nificant role in determining the fruit phenotype.

4.3. Investigating association between volatiles and bacterial microbiome

To further dissect the influence of different bacterial genera on fruit aroma, after characterising 'Enrosadira' volatilome on the same fruit, microbiome was characterised by NGS. This analysis revealed that remarkable differences in the proportion of OTUs emerged after the recolonisation process (Fig. 2). The qualitative differences in the microbiota were reflected by the change in aroma profiles, as shown by the significant MFA association. However, the association of micro- biome data resulting from NGS (characterised by high dimensionality, discrete values and sparsity) with other -omics data is not straightfor- ward (Xia et al., 2018; Xia, 2020). Thus, Spearman's correlation test seemed the most adequate tool in absence of direct biological informa- tion on VOC relation to bacteria (Table 2).

For some compounds, such as nonanoic acid, monoterpene family and medium-chain fatty acid esters, the previously described antimi- crobial properties can explain their negative correlations with a variety of OTUs (Sahin et al., 2006; Nakayama et al., 2015; Álvarez-Martínez et al., 2021). On the other hand, correlations between OTUs and VOCs may indicate direct production or utilisation by bacteria, enhanced release by the plant tissues, selective effects of VOCs on specific bacterial taxa, or even indirect, non-causal relations. Thus, the overall aroma profile probably results from the complex interaction of all these effects and from the proportion of bacterial populations at their ecologic equilibrium.

4.4. OTU-specific correlations with VOCs

The possibility to gain further insight in the role of specific OTUs was explored by assessing the predictive power of their linear or asymptotic correlation with VOCs. Asymptotic correlation may fit several biological processes like substrate limitation, volatilisation from liquid phase, or microbial interspecies interactions.

Some bacterial taxa (*Fictibacillus*, *Lactobacillus*, *Paenibacillus*, *Sed- iminibacterium*, but also potential or opportunistic human pathogens such as *Escherichia* and *Cutibacterium*) show a significant positive

correlation with VOCs, such as (*E*)-2-hexenal, octanal, heptene isomers and norisoprenoids. The latter compounds are key components of raspberry aroma, and the observed correlation with *Lactobacillus* and *Paenibacillus* might indicate a positive effect of these genera on fruit quality. *Lactobacillus* spp. have been associated to an increased pro- duction of norisoprenoids from carotenoid-rich media (Mapelli-Brahm et al., 2020). However, norisoprenoid synthesis by *Lactobacillus* and *Paenibacillus* spp. has not been reported, and the contribution of these microbes to raspberry aroma requires further investigation.

The most striking effect of recolonisation of sterilized fruit was the enrichment of Enterobacteriaceae populations. This group, and most prominently the genus *Enterobacter*, showed a negative correlation with several monoterpenes (limonene, β-pinene, α- and β-phellandrene, β-myrcene). An explanation may be the monoterpene subtraction by the increased bacterial population (Harder, 2000; Park et al., 2003; Yang et al., 2007). Alternatively, the observation of similar monoterpene emission levels from sterile (Enterobacteriaceae-free) and recolonised (Enterobacteriaceae-enriched) raspberries would rather suggest that monoterpenes, emitted by the whole microbiota, are highly effective in antagonising Enterobacteriaceae colonisation. Since this group includes potential human pathogens, this aspect of VOCs relations with the microbiota has a relevance for fruit safety.

5. Conclusions and future perspectives

The untargeted approach used in this research shed a new light on the contribution of the bacterial microbiota to raspberry aroma. Corre- lation analysis highlighted several significant associations between bacterial genera and volatile emissions, which might be better explained analysing bacterial metabolism and the complexity of interactions occurring at fruit-niche level. This research provides the first charac- terisation of raspberry core microbiome and, based on our results, bacteria naturally resident on raspberry fruit could be selected to improve fruit quality, aroma, shelf-life and safety in an ecological and sustainable way. Future work will aim at clarifying the mechanisms of interaction with the fruit, as well as the optimal conditions for the enhancement of raspberry aroma, safety and overall fruit quality.

Author statement

All authors agree with the content of the manuscript and its sub- mission to the journal.

Daniela Sangiorgio: Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - editing. **Antonio Cel- lini**: Methodology, Investigation, Visualization, Writing - original draft, Writing - editing. **Francesco Spinelli**: Experimental designs and plan- ning, Methodology, Writing – original draft, Writing - review & editing, Project administration, Supervision. **Chiara Pastore**: Methodology, Investigation. **Brian Farneti:** Investigation. **Stefano Savioli**: Method- ology, Investigation. **Maria Teresa Rodriguez-Estrada**: Methodology, Investigation, Writing - review & editing. **Irene Donati**: Experimental design and planning, Investigation, Writing - review & editing.

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References

A´lvarez-Martínez, F.J., et al., 2021. Antibacterial plant compounds, extracts and essential oils: an updated review on their effects and putative mechanisms of action. Phytomedicine. https://doi.org/10.1016/j.phymed.2021.153626, 153626.

Anusha, B., Thiribhuvanamala, G., Prabakar, K., 2021. Induction of defense isoforms by hexanal and bacterial antagonists in mango. J. Pharmacogn. Phytochem. 10, 558–561, 2.

Aprea, E., Franco, B., Gasperi, F., 2015. Volatile compounds of raspberry fruit: from analytical methods to biological role and sensory impact. Molecules 20, 2445–2474. https://doi.org/10.3390/molecules20022445, 2.

Asaf, S., et al., 2020. Sphingomonas: from diversity and genomics to functional role in environmental remediation and plant growth. Crit. Rev. Biotechnol. 40, 138–152. https://doi.org/10.1080/07388551.2019.1709793, 2.

Bailly, A., Weisskopf, L., 2017. Mining the volatilomes of plant-associated microbiota for new biocontrol solutions. Front. Microbiol. 8 https://doi.org/10.3389/fmicb.2017.01638, 1638.

Berlec, A., 2012. Novel techniques and findings in the study of plant microbiota: search for plant probiotics. Plant Sci. 193, 96–102. https://doi.org/10.1016/j.plantsci.2012.05.010.

Bolyen, E., Rideout, J., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 848–857. https://doi.org/10.1038/s41587-019-0209-9.https://doi.org/10.1038/s41586-019-1689-y.

Bulgarelli, D., et al., 2013. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 64, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106.

Bushnell, B., 2014. BBMap: A Fast, Accurate, Splice-aware Aligner (No. LBNL-7065E). Lawrence Berkeley National Lab.(LBNL), Berkeley, CA (United States). DE-AC02-05CH11231.

Cellini, A., et al., 2018. Biological relevance of volatile organic compounds emitted during the pathogenic interactions between apple plants and Erwinia amylovora. Mol. Plant Pathol. 19.1, 158–168. https://doi.org/10.1111/mpp.12509.

Cellini, A., et al., 2019. Pathogen-induced changes in floral scent may increase honeybee-mediated dispersal of Erwinia amylovora. ISME J. 13 (4), 847–859. https://doi.org/10.1038/s41396-018-0319-2.

Cellini, A., et al., 2021. Bacterial volatile compound-based tools for crop management and quality. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2021.05.006.

Crigler, Mark A.Eiteman, Altman, E., 2020. Characterization of the furfural and 5-hydroxymethylfurfural (HMF) metabolic pathway in the novel isolate Pseudomonas putida ALS1267. Appl. Biochem. Biotechnol. 190, 918–930. https://doi.org/10.1007/s12010-019-03130-x, 3.

Daranas, N., et al., 2019. Biological control of bacterial plant diseases with Lactobacillus plantarum strains selected for their broad-spectrum activity. Ann. Appl. Biol. 174, 92–105. https://doi.org/10.1111/aab.12476, 1.

Dong, K., et al., 2020. Induction, detection, formation, and resuscitation of viable but non-culturable state microorganisms. Compr. Rev. Food Sci. Food Saf. 19 (1), 149–183. https://doi.org/10.1111/1541-4337.12513.

El Hadi, M., Ahmed, M., et al., 2013. Advances in fruit aroma volatile research. Molecules 18 (7), 8200–8229. https://doi.org/10.3390/molecules18078200.

Ercolini, D., et al., 2010. Different molecular types of Pseudomonas fragi have the same overall behaviour as meat spoilers. Int. J. Food Microbiol. 142 (1–2), 120–131. https://doi.org/10.1016/j.ijfoodmicro.2010.06.012.

Farre´-Armengol, G., et al., 2016. Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. Trends Plant Sci. 21 (10), 854–860. https://doi.org/10.1016/j.tplants.2016.06.005.

Feron, G., et al., 2007. Microbial production of 4-hydroxybenzylidene acetone, the direct precursor of raspberry ketone. Lett. Appl. Microbiol. 45 (1), 29–35. https://doi.org/10.1111/j.1472-765X.2007.02147.x.

Finkel, O.M., et al., 2017. Understanding and exploiting plant beneficial microbes. Curr. Opin. Plant Biol. 38, 155–163. https://doi.org/10.1016/j.pbi.2017.04.018.

Guo, J., et al., 2020. Antifungal activity of volatile organic compounds from Bacillus amyloliquefaciens XJ5 and control effect against Monilinia fructigena. Chinese Journal of Biological Control 36 (4). https://doi.org/10.16409/j.cnki.2095-039x.2020.04.018, 575.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST-palaeontological statistics, ver. 1.89. Palaeontol. Electron.

Harder, J., 2000. Anaerobic utilization of essential oils bydenitrifying bacteria. Biodegradation 55–63. https://doi.org/10.1023/A:1026552724696.

Harrell, F.E., 2004. Hmisc S Function Library. Programs available from. http://biostat.mc.vanderbilt.edu/s/Hmisc.

Hebbali, A., 2020. Package ‘olsrr’.

Hertel, M., et al., 2016. Detection of signature volatiles for cariogenic microorganisms. Eur. J. Clin. Microbiol. Infect. Dis. 35, 235–244. https://doi.org/10.1007/s10096-015-2536-1, 2.

Junker, R., Tholl, D., 2013. Volatile organic compound mediated interactions at the plant-microbe interface. J. Chem. Ecol. 39, 810–825. https://doi.org/10.1007/s10886-013-0325-9, 7.

Koopman, F., et al., 2010. Identification and characterization of the furfural and 5 (hydroxymethyl) furfural degradation pathways of Cupriavidus basilensis HMF14. Proc. Natl. Acad. Sci. 107, 4919–4924. https://doi.org/10.1073/pnas.0913039107, 11.

Lˆe, S.J.J., Husson, F., 2008. FactoMineR: an r package for multivariate analysis. J. Stat. Softw. 25 (1), 1–18. https://doi.org/10.18637/jss.v025.i01.

Leff, J.W., Fierer, N., 2013. Bacterial communities associated with the surfaces of fresh fruits and vegetables. PLoS One. https://doi.org/10.1371/journal.pone.0059310.

Lemfack, M.C., et al., 2018. mVOC 2.0: a database of microbial volatiles. Nucleic Acids Res. 46, D1261–D1265. https://doi.org/10.1093/nar/gkx1016. D1.
Maguire, T.L., Collins, G., Sedgley, M., 1994. A modified CTAB DNA extraction procedure for plants belonging to the family Proteaceae. Plant Mol. Biol. Rep. 2, 106–109, 12.
Mapelli-Brahm, P., et al., 2020. The impact of fermentation processes on the production, retention and bioavailability of carotenoids: an overview. Trends Food Sci. Technol. 99, 389–401. https://doi.org/10.1016/j.tifs.2020.03.013.
Mari, M., Bautista-Banos, S., Sivakumar, D., 2016. Decay control in the postharvest system: role of microbial and plant volatile organic compounds. Postharvest Biol. Technol. 122, 70–81. https://doi.org/10.1016/j.postharvbio.2016.04.014.
Morella, N.M., et al., 2020. Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection. Proc. Natl. Acad. Sci. 117, 1148–1159. https://doi.org/10.1073/pnas.1908600116, 2.
Nakayama, M., et al., 2015. Antibacterial effects of monoglycerol fatty acid esters and sucrose fatty acid esters on Bacillus spp. Food Sci. Technol. Res. 21, 431–437. https://doi.org/10.3136/fstr.21.431, 3.
Nasopoulou, C., et al., 2014. Localization of strawberry (Fragaria x ananassa) and Methylobacterium extorquens genes of strawberry flavor biosynthesis in strawberry tissue by in situ hybridization. J. Plant Physiol. 171, 1099–1105. https://doi.org/10.1016/j.jplph.2014.03.018, 13.
Oszust, K., Frac, M., 2021. First report on the microbial communities of the wild and planted raspberry rhizosphere – A statement on the taxa, processes and a new indicator of functional diversity. Ecol. Indicators 121. https://doi.org/10.1016/j.ecolind.2020.107117.
Park, Y., In-Cheol, K., Chang, H., 2003. Microbial conversion of (+)-limonene by an Enterobacter agglomerans isolate. J. Microbiol. Biotechnol. 4, 636–639, 13.
Pen˜uelas, J., et al., 2014. Removal of floral microbiota reduces floral terpene emissions. Sci. Rep. 4 https://doi.org/10.1038/srep06727, 6727.
Perpetuini, G., et al., 2019. Genetic and functional characterization of the bacterial community on fruit of three raspberry (Rubus idaeus) cultivars. J. Berry Res. 9, 227–247. https://doi.org/10.3233/JBR-180340, 2.
Pinto, D., Santos, M., Chambel, L., 2015. Thirty years of viable but nonculturable state research: unsolved molecular mechanisms. Crit. Rev. Microbiol. 41.1, 61–76. https://doi.org/10.3109/1040841X.2013.794127.
Ponzoni, C., et al., 2008. Biotransformation of acyclic monoterpenoids by Debaryomyces sp., Kluyveromyces sp., and Pichia sp. Strains of environmental origin. Chem. Biodivers. 5, 471–483. https://doi.org/10.1002/cbdv.200890046.
Purahong, W., et al., 2018. Plant microbiome and its link to plant health: host species, organs and Pseudomonas syringae pv. Actinidiae infection shaping bacterial phyllosphere communities of kiwifruit plants. Front. Plant Sci. 871 https://doi.org/10.3389/fpls.2018.01563 art. no. 1563.
Quast, C., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41.D1, 590–596. https://doi.org/10.1093/nar/gks1219.
Reese, K.L., et al., 2020. Metabolic profiling of volatile organic compounds (VOCs) emitted by the pathogens Francisella tularensis and Bacillus anthracis in liquid culture. Sci. Rep. 10, 1–17. https://doi.org/10.1038/s41598-020-66136-0, 1.
Rohart, F., et al., 2017. mixOmics: An R package for 'omics feature selection and multiple data integration. PLoS Comput. Biol. 13.11 https://doi.org/10.1371/journal.pcbi.1005752.
Sahin, N., Ibrahim, K., Erdogan, Y., 2006. Investigation of antimicrobial activities of nonanoic acid derivatives. Fresenius Environ. Bull. 15, 141–143, 2.
Sangiorgio, D., et al., 2021. Does organic farming increase raspberry quality, aroma and beneficial bacteria biodiversity. Microorganisms. https://doi.org/10.3390/microorganisms9081617.
Sansinenea, E., 2019. Bacillus spp.: As Plant Growth-Promoting Bacteria Secondary Metabolites of Plant Growth Promoting Rhizomicroorganisms, pp. 225–237. https://doi.org/10.1007/978-981-13-5862-3_11.
Schulz, M., Chim, J., 2019. Nutritional and bioactive value of Rubus berries. Food Biosci. 31 https://doi.org/10.1016/j.fbio.2019.100438, 100438.
Sharifi, R., Ryu, C.M., 2018. Revisiting bacterial volatile-mediated plant growth promotion: lessons from the past and objectives for the future. Ann. Bot. 122, 349. https://doi.org/10.1093/aob/mcy108, 3.
Sharifi, R., Sang-Moo, L., Ryu, C.M., 2018. Microbe-induced plant volatiles. New Phytol. 220, 684–691. https://doi.org/10.1111/nph.14955, 3.
Sivakumar, D., Bautista-Ban˜os, S., 2014. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. Crop. Prot. 64, 27–37. https://doi.org/10.1016/j.cropro.2014.05.012.
Song, J., et al., 1996. Hexanal vapor is a natural, metabolizable fungicide: inhibition of fungal activity and enhancement of aroma biosynthesis in apple slices. J. Am. Soc. Hortic. Sci. 121, 937–942. https://doi.org/10.21273/JASHS.121.5.937, 5.
Van Wees, S.C.M., Sjoerd, V., Pieterse, C.M.J., 2008. Plant immune responses triggered by beneficial microbes. Curr. Opin. Plant Biol. 11, 443–448. https://doi.org/10.1016/j.pbi.2008.05.005, 4.
Verginer, M., et al., 2010. Monitoring the plant epiphyte Methylobacterium extorquens DSM 21961 by real-time PCR and its influence on the strawberry flavor. FEMS Microbiol. Ecol. 74, 136–145. https://doi.org/10.1111/j.1574-6941.2010.00942.x, 1.
Veselova, M.A., Plyuta, V.A., Khmel, I.A., 2019. Volatile compounds of bacterial origin: structure, biosynthesis, and biological activity. Microbiology 88, 261–274. https://doi.org/10.1134/S0026261719030160.
Weisskopf, L., Stefan, S., Garbeva, P., 2021. Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. Nat. Rev. Microbiol. 19, 391–404. https://doi.org/10.1038/s41579-020-00508-1, 6.
Xia, Y., 2020. Correlation and association analyses in microbiome study integrating multiomics in health and disease. Prog. Mol. Biol. Transl. Sci. 171, 309–491.
Xia, Y., Sun, J., Chen, D.G., 2018. Introductory overview of statistical analysis of microbiome data. Stat. Anal. Microbiome Data R 43–75. https://doi.org/10.1007/978-981-13-1534-3_3.
Yang, E., Yeon-Jin, P., Chang, H., 2007. Cloning of four genes involved in limonene hydroxylation from Enterobacter cowanii 6L. J. Microbiol. Biotechnol. 17, 1169–1176, 7.
Zhang, Y., et al., 2018. Hexanal as a QS inhibitor of extracellular enzyme activity of Erwinia carotovora and Pseudomonas fluorescens and its application in vegetables. Food Chem. 255, 1–7. https://doi.org/10.1016/j.foodchem.2018.02.038.
Zhang, X., et al., 2020. Control effects of Bacillus siamensis G-3 volatile compounds on raspberry postharvest diseases caused by Botrytis cinerea and Rhizopus stolonifer. Biol. Control. 141 https://doi.org/10.1016/j.biocontrol.2019.104135, 104135.