RESEARCH ARTICLE

Metaproteomic characterization of the *Vitis vinifera* rhizosphere

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One sentence summary: The first study reporting the rhizosphere proteome of *V. vinifera*, describing the bacterial community structure and activity of an important ecosystem for the Italian landscape, agriculture and economy.

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ABSTRACT

The rhizosphere is a hotspot of microbial activity where the release of root exudates stimulates bacterial density and diversity. The majority of the bacterial cells in soil are viable, unculturable, but active. Proteomic tools could be useful in gaining information about microbial community activity and to better understand the real interactions between roots and soil. The aim of this work was to characterize the bacterial community associated with *Vitis vinifera* cv. Pinot Noir roots using a metaproteome approach. Our results confirmed the large potential of proteomics in describing the environmental microbial communities and their activities: in particular, we showed that bacteria belonging to *Streptomyces, Bacillus, Bradyrhizobium, Burkholderia* and *Pseudomonas* genera are the most active in protein expression. Concerning the biological activity of these genera in the rhizosphere, we observed the exclusive presence of the phosphorus metabolic process and the regulation of primary metabolic processes. To our knowledge, this is the first study reporting the rhizosphere proteome of *V. vinifera*, describing the bacterial community structure and activity of an important ecosystem for the Italian landscape, agriculture and economy.

Keywords: rhizosphere; metaproteome; grapevine; *Streptomyces*; *Bacillus*; *Pseudomonas*

INTRODUCTION

The rhizosphere is a soil hotspot of microbial activity (Hrynkiewicz and Baum 2011) where the release of plant root exudates modulates the density and diversity of microbial communities and shapes the associated microbiota (Mendes, Garbeva and Raaijmakers 2013; Philippot *et al.* 2013). Mendes and co-workers (2013) identified three groups of microorganisms in the rhizosphere microbiome: 'the good', organisms with beneficial effects on plants; 'the bad', plant pathogenic microorganisms; and 'the ugly', human pathogenic microorganisms. Microbial densities in the rhizosphere are two to three orders of

magnitude higher than those recorded in the bulk soil (BS) (Watt *et al.* 2006) and the majority of these bacterial cells are viable and active but unculturable; the percentage of unculturable bacteria also changes according to the host plant (Hugenholtz and Pace 1996).

Moreover, not all bacterial populations are equally responsive to root exudates: specific microbial groups can be typically favored by the plant roots (Mavingui *et al.* 1992; Lemanceau *et al.* 1995; Edel *et al.* 1997) and these populations, which are selected according to the different root exudation and rhizodeposition, show a higher fitness in this environment (Dias *et al.* 2013; Philippot *et al.* 2013). Furthermore, agronomic practices (such as tillage or application of phytosanitary treatments), soil types, plant growth stages, genotypes and other environmental factors also affect the composition of the microbial community in the rhizosphere (Smalla *et al.* 2001; Wu *et al.* 2008; Dini-Andreote *et al.* 2010; Hardoim *et al.* 2011).

Thanks to culture-independent methods, especially to recent advances in next-generation sequencing strategies, the complexity of the soil/rhizosphere microbial community has been explored in depth. However, metagenomics does not provide information on the metabolic activity of the identified bacteria nor about the molecular (secreted proteins) interactions occurring between bacterial communities and plant roots. In fact, proteins are direct expressions of cellular functions and are the drivers of cellular activities encoded in the genome (Stres and Tiedje 2006). Therefore, the identification, by proteomic tools, of the repertoire of proteins that microorganisms secrete and use to compete and cooperate could be one of the best ways to gain information about the activity of the microbial community. This could also result in a better understanding of the actual interaction pathways occurring between roots and bacterial communities in the soil.

The aim of the present study was to characterize the microbial community associated with the roots of *Vitis vinifera* cv. Pinot Noir not only from a taxonomic perspective, but also from a functional point of view. In recent years, the microbiome associated with *V. vinifera* has received considerable attention using genomic approaches: the biodiversity of arbuscular mycorrhizal fungi (AMF) colonizing *V. vinifera* has been described by Holland *et al.* (2014), who recorded *Funnelliformis* and *Rhizophagus* AMF genera as mainly associated with vine roots. Regarding bacterial taxa associated to grapevine, both endophyte and epiphyte bacterial communities have been investigated in a number of studies. For example, Canfora *et al.* (2018) describe microbiome variations during different fertilization treatments while Manici *et al.* (2017) report the rhizosphere microbiome modifications induced by long-term grapevine cultivation and agro-environmental conditions. To our knowledge, few papers have described the rhizosphere metaproteome of agricultural plants (Wang *et al.* 2011a,b; Knief *et al.* 2012; Moretti *et al.* 2012; Lin *et al.* 2013) and no data are available in the literature regarding the rhizosphere proteome of *V. vinifera*. Our interest in grapevines is justified by both economic and historical reasons. In fact, in 2016 the International Organization of Vine and Wine (OIV) declared that in Italy, 690 000 ha of agricultural land are cultivated with grapevines resulting in 7.9 million tonnes of fruit and 50.9 million hl of wine; and in Piedmont (Italy), 43 500 ha of agricultural land are cultivated with grapevines leading to the production of 2.5 million hl of high quality wine (OIV 2017). Since 2014, the hills of the Piedmont area covering The Langhe, Roero and Monferrato have been included on the UNESCO World Heritage list (http://whc.unesco.org/en/list/1390). Moreover, this work provides important indications regarding the metabolism of the grapevine rhizosphere and represents the first step in attaining knowledge of the mechanisms underlying the crop improvement obtained by an integrated vineyard management. This metaproteome approach can be considered as a first example to be applied to other important grapevine cultivars in order to better understand the impact of the genetic structure of the plant on the modulation of the composition and the activity of associated microbial communities.

MATERIALS AND METHODS

Soil sampling

Soil sampling was performed at flowering time, in an integrated pest management (IPM) vineyard located close to Carpeneto (Italy) and belonging to Agrion Fondazione per la Ricerca, l'Innovazione e lo Sviluppo Tecnologico dell'Agricoltura Piemontese (Fig. 1A,B). This vineyard was planted in 1988 with *Vitis vinifera* cv. Pinot Noir, a very important cultivar in the Piedmont region for the production of Piemonte Denominazione di Origine Controllata (DOC) Pinot Black wine. DOC is one of the recognitions which guarantee the geographical origin and quality of Italian wine. Soil sampling was performed in May 2014 during the flowering stage of the plants; this period was chosen because, during this phase, plants are metabolically active and their impact on the rhizosphere bacterial communities, in the form of root exudates, is more evident (Ondreičková *et al.* 2016). Samples were collected by means of three soil probes per plant (N = 7) obtained with a soil corer (Fig. 1D), and the roots entrapped in the soil cores collected close to the stem were considered for the sampling of rhizosphere soil. The soil adhering to these roots was removed using sterile gloves. The BS was sampled in an area without grapevines, just outside the borders of the vineyard (N = 7, three replicates), at a depth of 30 cm, after removing the surface layer (Fig. 1E). Soil samples for proteomic analysis were stored at -80°C.

Phytosanitary treatments were performed according to Annex III of Directive 2009/128/EC: (i) soil-borne disease suppression and prevention should be based on crop rotation, use of resistant cultivar and adequate fertilization and irrigation; (ii) sustainable biological methods are preferred to pesticides for the control of plant pathogens; and (iii) if the use of pesticides is necessary, the most selective and the least dangerous for both organisms and the environment should be chosen and distributed in low amounts at low frequencies. IPM aims to grow healthy crops with the least possible disruption to agroecosystems and encourages natural pest control mechanisms (Matyjaszczyk 2015). Chemical treatments performed during vine growth were: (i) weeding with glyphosate, in April, among the plants, but not between the lines; (ii) fungicide treatment against *Peronospora* spp. (Metalaxil-m + Mancozeb) and against *Oidium* spp. (Ciflufenamid), each month from April to the end of fruiting; (iii) fungicide treatment against *Botrytis cinerea* (Cyprodinil + Fludioxonil), in July; and (iv) two insecticide (Thiamethoxam + Chlorpyrifos-metile) treatments, in July. The sampling was performed 20 days after chemical treatment. Physical/chemical analyses were performed on each soil sample according to D.M. 13/09/99; detailed soil analysis methods are reported in the footnotes of the supporting information (S1). Data regarding temperature, humidity and rainfall are also reported in the supporting information (S2).
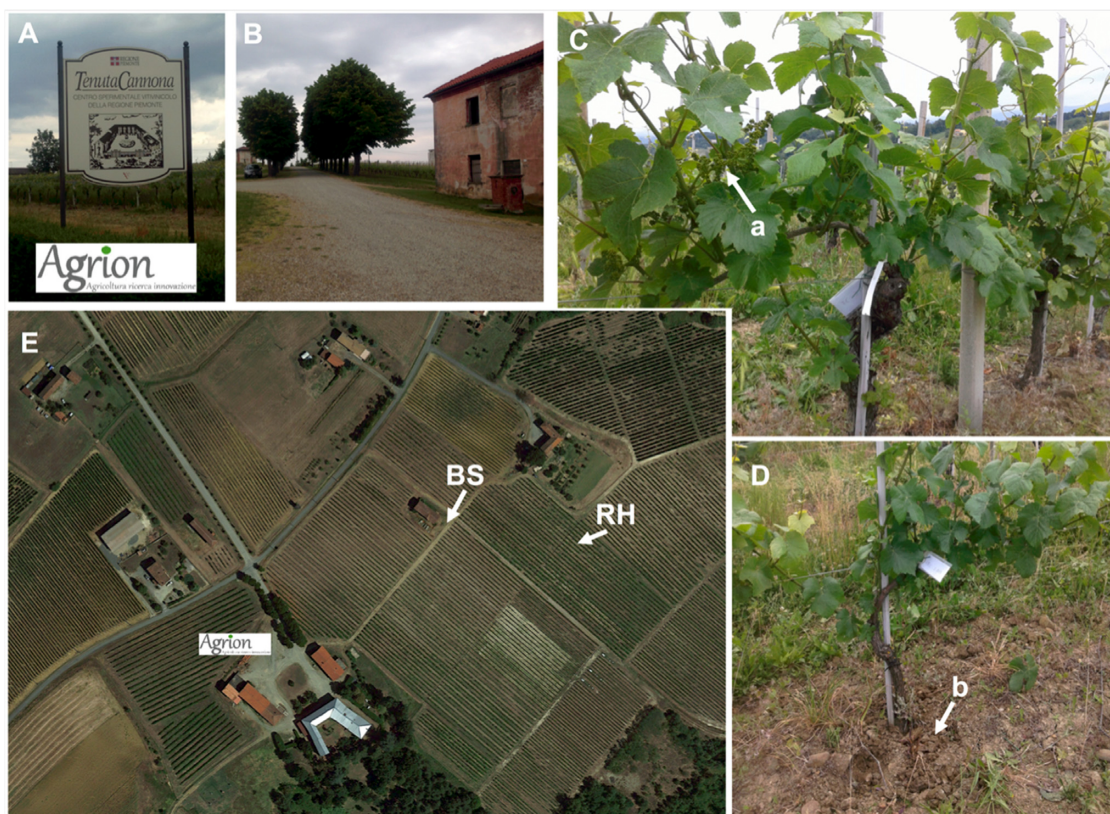
Figure 1. Vineyard location and sampling map: (**A**) and (**B**) represent the vineyard in Tenuta Cannona, Agrion Fondazione per la ricerca, l'innovazione e lo sviluppo tecnologico dell'agricoltura piemontese; (**C**) plants in the phenological stage at sampling time, the a arrow indicates the flowers; (**D**) positions of the three holes (b arrow) made in order to reach the plant root apparatus and hence rhizosphere soil; and (**E**) GIS map of the two sampling sites, one in an area without grapevines, just outside the borders of the vineyard (bulk soil: BS), and one inside the vineyard (rhizosphere soil: RH). The image was produced by the author using QGIS v. 2.10 Pisa (QGIS Development Team, 2015. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org). The permission to use the Agrion logo in Fig. 1 was available on the journal website.

Protein extraction, digestion and MS/MS analysis

Soil proteins were extracted using a NoviPure™ Soil Protein extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The obtained protein pellet was resuspended in 400 μl of 50 mM ammonium bicarbonate and quantified by the Bradford method (Bradford 1976). Proteins were digested with Trypsin (Roche, Segrate, Milano, Italy), resuspended in 50 mM ammonium bicarbonate (37°C overnight) after a reduction step (DTT to a final concentration of 10 mM, 30 min at 60°C) and an alkylation step (iodoacetamide to a final concentration of 20 mM, 30 min at room temperature in the dark). After digestion, protein peptides were purified using Solid Phase Extraction cartridge C18 (Supelco, USA) and finally eluted with 100% Acetonitrile.

The mass spectrometry analyses were performed using a micro-LC Eksigent Technologies (Dublin, USA) system with a Halo Fused C18 column (0.5 × 100 mm, 2.7 μm) as a stationary phase. The injection volume was 4.0 μL and the oven temperature was set at 40°C. The mobile phase was a mixture of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B), eluting at a flow rate of 15.0 μL min^{-1} at an increasing concentration of solvent B from 2% to 40% in 30 min. The LC system was interfaced with a 5600+ TripleTOF system (AB Sciex, Concord, Canada) equipped with a DuoSpray Ion Source. The samples were subjected to the traditional data-dependent acquisition (DDA): mass spectrometer analysis was performed using a mass range of 100–1500 Da, followed by a MS/MS product

ion scan from 200 to 1250 Da with the abundance threshold set at 30 cps (35 candidate ions can be monitored during every cycle). The ion source parameters in electrospray positive mode were set as follows: curtain gas (N$_2$) at 25 psig, nebulizer gas GAS1 at 25 psig, and GAS2 at 20 psig, ionspray floating voltage (ISFV) at 5000 V, source temperature at 450°C and declustering potential at 25 V. The MS data were acquired with Analyst TF 1.7 (AB Sciex).

Protein identification

Based on the 16S rDNA sequences presented in Novello *et al.* (2017), a protein sequence database was created. In particular, for each taxonomic unit, all protein sequences present in NCBInr were downloaded and used to create an in-house protein database to perform Mascot analysis (see below). The genomic sequences were included in the BioProject PRJNA394211 available at NCBI. The BioProject contains four BioSamples with the following IDs: SAMN07350830, SAMN07350831, SAMN07350832 and SAMN07350833. A total of 27 237 reads were obtained and resulted in a protein database including a total of 11 788 243 sequences and 3831 487 183 residues, useful for the identification of proteins and the corresponding bacterial genera (Novello *et al.* 2017).

Mass data were analyzed using Mascot (Matrix Science Inc., Boston, MA, USA) against the in-house protein sequence database prepared as described above. The search was performed on Mascot v. 2.3.0; the digestion enzyme selected was

trypsin, with three maximum missed cleavages; a search tolerance of 120 ppm was specified for the peptide mass tolerance, and 0.6 Da for the MS/MS tolerance. The charges of the peptides to search for were set to 2+, 3+ and 4+, and the search was set on monoisotopic mass. The following modifications were used: oxidized methionine and deamidation (NQ) as variable modifications. Proteins with at least one peptide with an ion score higher than the homology or identity ion score value, were considered as significant.

Biodiversity and statistical analysis

A table with absolute abundance (number of proteins per species) for each sample was used as the input for analysis with the RAM package of R (R Core Team 2018) to obtain Shannon-Wiener and Simpson biodiversity indexes.

Statistical analyses were performed with StatView 4.5 (Abacus Concepts). To assess differences in soil characteristics, microbial protein production and biodiversity indexes between BS and RH soils, data were statistically analyzed by one-way ANOVA, followed by Fisher's probable least-squares difference test with cut-off significance at p <0.05. Mean phyla frequency was calculated as the mean of the percentage ratio between the number of identified proteins (in each soil replica) expressed by the considered phyla and the total number of identified proteins (in each soil replica).

Blast2GO analysis

To perform the Blast2GO analysis (http://www.blast2go.com/b2ghome) we downloaded the protein FASTA sequences from http://www.ncbi.nlm.nih.gov. Data analysis was performed with Blast2GO standard parameters (Conesa *et al.* 2015). The evidence code (EC) annotations, obtained by mapping equivalent GO annotations, were visualized by reconstructing the structure of the Gene Ontology relationships and ECs on Kyoto Encyclopedia of Genes and Genomes (KEGG) maps (http://www.genome.jp/kegg). The data of biological processes and molecular functions were recorded.

RESULTS

Soil characterization

Detailed soil analyses are reported in the supporting information (S1). The two soils were clay loam, with a neutral pH. RH soil presented higher values of organic matter (N, C/N ratio, P_2O_5) compared with BS soil.

Protein identification

Protein identification is reported in detail in tables S3 and S4 (supporting information) and summarized in Fig. 2. Raw data are available via ProteomeXchange with identifier PXD007670. Using MS/MS analysis, a total of 579 proteins by 150 bacterial genera were identified in the two soils. In particular, 259 and 300 proteins were exclusively detected in BS and RH soils, respectively, while 20 proteins were expressed by the same 16 genera in both soils, as shown in the Venn diagram (Fig. 2, proteins). Table 1 lists the 20 commonly expressed proteins that are mainly involved in transport (eg. MFS and ABC transporters).

Specific protein expression occurred in the two soils involving mutually exclusive genera: 56 proteins by 49 genera were exclusive of BS [Fig. 2 (genera) and supporting information S3,
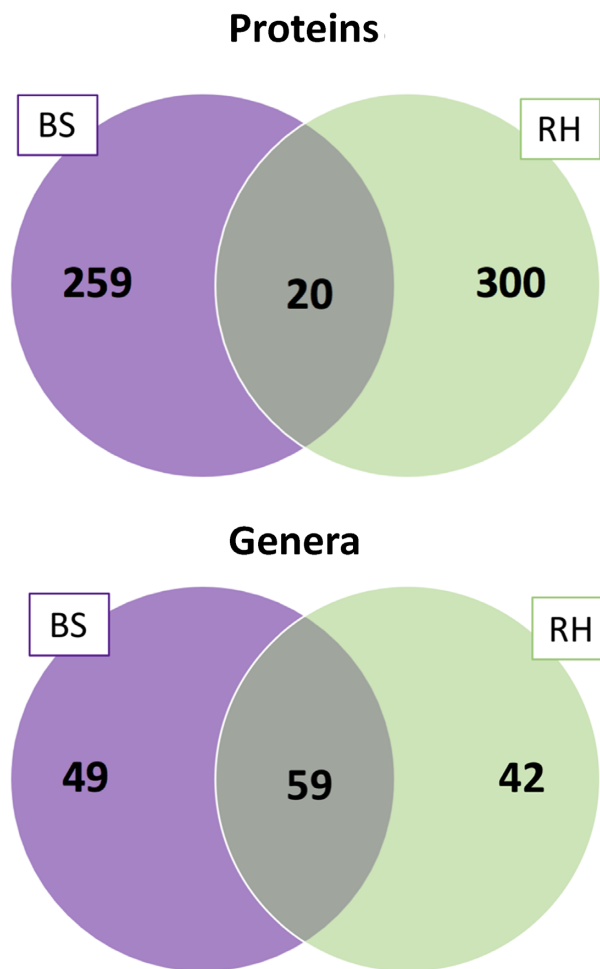

Figure 2. Venn diagrams of the number of identified proteins and bacterial genera in the two samples. BS: bulk soil; RH: rhizosphere soil associated with the roots of *Vitis vinifera* cv. Pinot Noir.

white lines], while in RH 54 proteins were expressed by 42 genera [Fig. 2 (genera) and supporting information S4, white lines]. Finally, 59 genera were shared between the two soils [Fig. 2 (genera) and supporting information, S3 and S4, yellow lines]: among these, 43 genera expressed 203 proteins in BS and 246 different proteins in RH soils.

The identified genera belonged to 12 phyla in BS and 11 in RH, as shown in Fig. 3. The most active phyla, in terms of the number of proteins detected and identified, were *Proteobacteria*, *Actinobacteria* and *Firmicutes* in both soils. In RH, *Actinobacteria* expressed a higher number of proteins than in BS (30.7% vs. 27.6%, respectively); conversely, the proportion of proteins released by *Proteobacteria* was lower in RH than in BS (41% vs. 50.3%, respectively) (Fig. 3). Moreover, proteins originated by the phylum *Chloroflexi* occurred only in BS (Fig. 3).

In Table 2, all the proteins expressed by 42 different genera exclusively present in RH soil are listed.

The biodiversity analysis based on identified protein data revealed that the Shannon-Wiener's index was 3.17 ± 0.42 in BS and 3.56 ± 0.07 in RH while the Simpson's index was 0.88 ± 0.07 in BS and 0.94 ± 0.01 in RH soil. Differences between the biodiversity indices in the two soils were not statistically significant.

Table 1. Proteins identified both in bulk (BS) and in rhizosphere soil (RH).

NCBI accession number	Protein name	Blast results	Reference organism	Genus	Phylum	Protein score	Protein Molecular Weight (MW) (kDa)	Protein Iso-electric point (pI)
gi|947758754	MFS transporter		*Acidovorax* sp.	*Acidovorax*	Proteobacteria	47	41 015	10.65
gi|491317843	outer membrane protein omp38		*Acinetobacter* sp. CIP 53.82	*Acinetobacter*	Proteobacteria	155	37 963	5.02
gi|488805021	phosphate ABC transporter substrate-binding protein PstS		*Afipia felis*	*Afipia*	Proteobacteria	118	35 802	8.7
gi|492876585	phosphate ABC transporter substrate-binding protein PstS		*Afipia* sp.	*Afipia*	Proteobacteria	201	35 846	8.86
gi|639257240	membrane protein		*Afipia* sp. OHSU_II-C1	*Afipia*	Proteobacteria	84	25 041	5.85
gi|504766130	protein-export membrane protein SecF		alpha proteobacterium HIMB5	alpha proteobacterium HIMB5	Proteobacteria	44	33 177	6.64
gi|924342542	pyridine nucleotide-disulfide oxidoreductase		*Bacillus* sp. FJAT-21 945	*Bacillus*	Firmicutes	60	19 763	6.07
gi|653555505	hypothetical protein	porin	*Bradyrhizobium* sp. Ai1a-2	*Bradyrhizobium*	Proteobacteria	87	56 373	8.18
gi|493661417	hypothetical protein	decarboxylase	*Bradyrhizobium* sp. ORS 285	*Bradyrhizobium*	Proteobacteria	48	58 134	6.86
gi|503259067	23S rRNA (guanosine(2251)-2∼-O)-methyltransferase RlmB		*Intrasporangium calvum*	*Intrasporangium*	Actinobacteria	56	33 582	10.07
gi|563076641	hypothetical protein X755_06985	NF (Not Found)	*Mesorhizobium* sp. LNJC405B00	*Mesorhizobium*	Proteobacteria	38	22 028	6.75
gi|494134540	hypothetical protein	NF	*Micromonospora* sp. ATCC 39 149	*Micromonospora*	Actinobacteria	47	17 936	10.25
gi|521714005	PPE family protein		*Mycobacterium* sp. 01 2931	*Mycobacterium*	Actinobacteria	44	14 251	5.26
gi|519305603	glycosyl transferase family protein		*Pseudomonas syringae* pv. *actinidiae* ICMP 19 096	*Pseudomonas*	Proteobacteria	85	34 069	5.9
gi|739344918	hypothetical protein	NF	*Rhizobium* sp. YR295	*Rhizobium*	Proteobacteria	38	80 516	7.67
gi|948029843	TonB-dependent receptor		*Sphingobium* sp. Leaf26	*Sphingobium*	Proteobacteria	45	108 109	4.82
gi|639146534	apolipoprotein N-acyltransferase		*Streptomyces* sp. AW19M42	*Streptomyces*	Actinobacteria	44	57 137	9.5
gi|973384776	cytoplasmic protein		*Streptomyces* sp. NRRL F-5122	*Streptomyces*	Actinobacteria	61	44 596	9.01
gi|495108259	peptidase M23*		*Variovorax*	*Variovorax*	Proteobacteria	38	17 952	9.6
gi|961355845	late control protein		*Xanthomonas translucens*	*Xanthomonas*	Proteobacteria	38	35 945	8.96

*This protein is a Gly-Gly endopeptidase, it has no assigned GO term, and therefore it is not present in the Blast2GO results.

Different genera of potential beneficial microorganisms ('the good') were active in the two soils; specifically, the good represented 36.5% and 32.5% in BS and RH, respectively. The distribution of the identified proteins in each genus are described by the pie charts shown in Fig. 4A: the more active good genera involved in rhizosphere metabolism were *Streptomyces*, *Bacillus* and *Pseudomonas*.

Regarding 'the bad' and 'the ugly', different plant and human potential pathogen genera were identified as protein producers in the *V. vinifra* rhizosphere (Fig. 4B and C): specifically, the bad represented 1.8% in the two soils and the ugly represented 8.6% in BS and 10% in RH. In the rhizosphere, the bad were represented by *Xanthomonas* sp., *Pseudomonas syringae* and *Agrobacterium* sp., while the ugly were represented by *Clostridium* sp., *Acinetobacter* sp., *Mycobacterium* sp., *Nocardia* sp., *Staphylococcus* sp., *Streptococcus* sp., *Bacillus cereus* and *Burkholderia cepacia*.

Biological classification of the identified proteins

The percentages of proteins with Blast2GO assignment were 52% and 46% for BS and RH soils, respectively. Blast2GO analysis results are shown in Figs 5 and 6. Biological processes involved in BS (Fig. 5) were rather different from those occurring in RH

(Fig. 6). In particular, despite substantial maintenance of the different biological processes involved in cell metabolism, in RH we observed a higher number of proteins involved in the macromolecule, cellular macromolecule, cellular nitrogen compound, cellular aromatic compound, heterocycle, nucleobase-containing compound and organic cyclic compound metabolic processes, compared with BS soil. Specifically, considering the aforementioned biological processes, the more active genera were *Streptomyces*, *Bacillus*, *Bradyrhizobium*, *Burkholderia* and *Pseudomonas*.

Moreover, in RH we observed the exclusive presence of the phosphorus metabolic process (supporting information, S4, blue entries) and the regulation of biosynthetic, cellular, macromolecule, nitrogen compound (supporting information, S4, orange entries) and primary metabolic processes (Fig. 6 and Table 3). Table 3 shows the list of proteins responsible for the specific RH biological processes with the corresponding genera. A total of 36 proteins involved in biological processes that were specific for RH soil resulted, 89% of them expressed by genera which were found in both BS and RH soils.

Regarding the phosphorus metabolic process, as shown in Fig. 6 and in Table 3, the active genera were: *Streptomyces* (expressing a putative molybdopterin biosynthesis protein, a

Table 2. Proteins identified in soil associated with the roots of *Vitis vinifera* cv. Pinot Noir (RH), secreted by genera only present in RH soil.

NCBI accession number	Protein name	Blast2GO results	Reference organism	Genus	Phylum	Protein score	Protein Mr (kDa)	Protein pI
gi|117648342	hypothetical protein Acel_0671	NF	*Acidothermus cellulolyticus 11B*	*Acidothermus*	Actinobacteria	50	11 383	11.8
gi|506282604	non-ribosomal peptide synthetase		*Actinosynnema mirum*	*Actinosynnema*	Actinobacteria	66	869 268	5.5
gi|502426901	ABC transporter		*Actinosynnema mirum*	*Actinosynnema*	Actinobacteria	81	35 370	6.34
gi|219953757	conserved hypothetical protein		*Anaeromyxobacter dehalogenans 2CP-1*	*Anaeromyxobacter*	Proteobacteria	69	40 543	12.21
gi|557821538	hypothetical protein	tetratrico-peptide repeat family protein	*Asticcacaulis* sp. AC402	*Asticcacaulis*	Proteobacteria	71	102 731	6.06
gi|910018990	hypothetical protein	NF	*Azospirillum* sp. B4	*Azospirillum*	Proteobacteria	63	30 706	4.94
gi|757147818	NADPH:quinone reductase		*Azospirillum* sp. B506	*Azospirillum*	Proteobacteria	63	36 157	6.01
gi|1000278704	cytochrome c class I		*Bacteroidetes bacterium OLB9*	*Bacteroidetes*	Bacteroidetes	34	52 034	7.24
gi|647376848	glucose-methanol-choline oxidoreductase		*Brevibacterium* sp. VCM10	*Brevibacterium*	Actinobacteria	60	55 564	4.67
gi|328845914	short chain dehydrogenase family protein		*Brevundimonas diminuta ATCC 11568*	*Brevundimonas*	Proteobacteria	35	30 322	5.41
gi|946727154	hypothetical protein	NF	*Brevundimonas* sp. Leaf280	*Brevundimonas*	Proteobacteria	61	15 152	11.16
gi|947787639	alanine acetyltransferase		*Brevundimonas* sp. Root1279	*Brevundimonas*	Proteobacteria	71	21 810	8.18
gi|818891013	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase, YrbI family		*candidate division Kazan bacterium GW2011_GWC1_52_13*	*candidate division Kazan bacterium GW2011_GWC1_52_13*	Bacteria candidate phyla	54	43 109	9.68
gi|952349132	bifunctional 5		*candidate division NC10 bacterium CSP1-5*	*candidate division NC10 bacterium CSP1-5*	Bacteria candidate phyla	74	30 467	9.17
gi|931379683	hypothetical protein AMJ44_00255	DNA polymerase I	*candidate division WOR_1 bacterium DG_54_3*	*candidate division WOR_1 bacterium DG_54_3*	Bacteria candidate phyla	43	101 779	8.9
gi|931375769	hypothetical protein AMJ44_08340	riboflavin biosynthesis protein RibF	*candidate division WOR_1 bacterium DG_54_3*	*candidate division WOR_1 bacterium DG_54_3*	Bacteria candidate phyla	60	33 509	10.66
gi|973121479	cell division protein FtsX, partial		*candidate division WS6 bacterium 34_10*	*candidate division WS6 bacterium 34_10*	Bacteria candidate phyla	74	74 001	4.73
gi|530551546	3-deoxy-manno-octulosonate-8-phosphatase		*candidate division Zixibacteria bacterium RBG-1*	*candidate division Zixibacteria bacterium RBG-1*	Bacteria candidate phyla	52	18 871	7.68
gi|909617018	hypothetical protein	CoA-substrate-specific enzyme activase	*Candidatus Solibacter usitatus*	*Candidatus Solibacter usitatus*	Acidobacteria	70	111 483	6.21
gi|759622522	two-component sensor histidine kinase		*Comamonas* sp. B-9	*Comamonas*	Proteobacteria	60	47 414	6.02
gi|947635400	hypothetical Protein	NF	*Deinococcus* sp. Leaf326	*Deinococcus*	Deinococcus-thermus	77	339 004	9.9
gi|505144868	DUF1446 domain-containing Protein		*Fibrella aestuarina*	*Fibrella*	Bacteroidetes	77	46 497	5.66

Table 2. Continued.

gi|947391936	NDP-hexose 4-ketoreductase		*Frigoribacterium* sp. Leaf164	*Frigoribacterium*	Actinobacteria	68	91 108	5.47
gi|946878616	XRE family transcriptional regulator		*Frigoribacterium* sp. Leaf263	*Frigoribacterium*	Actinobacteria	41	32 104	6.19
gi|663115269	hypothetical protein	NF	*Glycomyces* sp. NRRL B-16210	*Glycomyces*	Actinobacteria	41	22 173	4.77
gi|972944152	RNA-splicing ligase RtcB		*Hymenobacter* sp. DG5B	*Hymenobacter*	Bacteroidetes	49	51 187	8.27
gi|808084406	RNA-splicing ligase RtcB		*Hymenobacter* sp. MIMtkLc17	*Hymenobacter*	Bacteroidetes	46	51 248	7.8
gi|551361186	malic enzyme		*Ideonella* sp. B508-1	*Ideonella*	Proteobacteria	66	82 548	5.91
gi|551359555	multidrug transporter		*Ideonella* sp. B508-1	*Ideonella*	Proteobacteria	35	109 882	5.94
gi|501035236	twitching motility protein PilT		*Kineococcus radiotolerans*	*Kineococcus*	Actinobacteria	38	41 862	6.44
gi|946896370	hypothetical protein	NF	*Leifsonia* sp.	*Leifsonia*	Actinobacteria	103	36 244	6.27
gi|947506795	haloacid dehalogenase		*Leifsonia* sp. Leaf325	*Leifsonia*	Actinobacteria	65	29 654	4.58
gi|702088295	hypothetical protein LF41_1176	NF	*Lysobacter dokdonensis DS-58*	*Lysobacter*	Proteobacteria	32	7 067	4.83
gi|835625928	AMP-binding protein		*Methylibium* sp. YR605	*Methylibium*	Proteobacteria	62	62 617	6.3
gi|495482518	hypothetical protein	TPR repeat-containing protein	*Microcystis* sp. T1-4	*Microcystis*	Cyanobacteria	78	44 375	4.35
gi|499875781	histidine kinase		*Myxococcus xanthus*	*Myxococcus*	Proteobacteria	83	95 265	5.87
gi|503988718	sulfatase		*Niastella koreensis*	*Niastella*	Bacteroidetes	37	79 975	9.25
gi|943675027	glycosyl transferase family 1		*Nonomuraea* sp. NBRC 110462	*Nonomuraea*	Actinobacteria	75	44 448	10.04
gi|943865890	hypothetical protein	NF	*Nonomuraea* sp. NBRC 110462	*Nonomuraea*	Actinobacteria	74	24 499	10.88
gi|500777924	osmotically inducible protein C		*Parvibaculum lavamentivorans*	*Parvibaculum*	Proteobacteria	74	43 312	5.98
gi|948224817	sodium:proton antiporter		*Phycicoccus* sp. Soil748	*Phycicoccus*	Actinobacteria	66	67 561	5.75
gi|495147384	aldo/keto reductase		*Polaromonas* sp. CF318	*Polaromonas*	Proteobacteria	87	36 128	6.8
gi|490462327	hypothetical protein	NF	*Porphyromonas endodontalis*	*Porphyromonas*	Bacteroidetes	50	52 693	9.58
gi|948221616	citryl-CoA lyase		*Pseudoxanthomonas* sp. Root630	*Pseudoxanthomonas*	Proteobacteria	66	30 618	6.55
gi|947808185	hypothetical protein	NF	*Rhizobacter* sp. Root404	*Rhizobacter*	Proteobacteria	61	38 693	6.33
gi|504239320	hypothetical protein	NF	*Rubrivivax gelatinosus*	*Rubrivivax*	Proteobacteria	63	49 998	5.98
gi|1001989170	hypothetical protein TH61_05675	NF	*Rufibacter* sp. DG15C	*Rufibacter*	Bacteroidetes	35	30 451	5.09
gi|772725198	hypothetical protein	NF	*Saccharothrix* sp. ST-888	*Saccharothrix*	Actinobacteria	37	18 344	11.44
gi|919126200	type I polyketide synthase		*Saccharothrix* sp. ST-888	*Saccharothrix*	Actinobacteria	97	287 674	5.29
gi|764627440	hypothetical protein	NF	*Skermanella aerolata*	*Skermanella*	Proteobacteria	49	15 829	5.13
gi|495543735	thiol reductant ABC exporter subunit CydD		*Stenotrophomonas* sp. SKA14	*Stenotrophomonas*	Proteobacteria	75	61 736	7.18
gi|51855300	ComE-like competence protein		*Symbiobacterium thermophilum IAM 14863*	*Symbiobacterium*	Firmicutes	68	86 420	10.39
gi|921079705	sodium:proton antiporter		*Tetrasphaera japonica*	*Tetrasphaera*	Actinobacteria	72	67 600	6.03
gi|946887613	glycerophosphodiester phosphodiesterase		*Tetrasphaera* sp. Soil756	*Tetrasphaera*	Actinobacteria	63	65 817	5.45

⬛ Proteins involved in the phosphorus metabolic process
🟩 Proteins without Blast2GO assignment

two-component sensor histidine kinase, a phosphoenolpyruvate synthase and a histidine kinase), *Burkholderia* (expressing a sensor histidine kinase, a pantetheine-phosphate adenylyltransferase, a hypothetical protein, a polyphosphate kinase 2), *Bacillus* (expressing a carbamate kinase and a thymidylate synthase), *Arthrobacter* (expressing a glycosyl hydrolase from family 15), *candidate division NC10 bacterium CSP1–5* (expressing bifunctional 5), *candidate division Zixibacteria bacterium RBG-1* (3-deoxy-manno-octulosonate-8-phosphatase), *Comamonas* (two-component sensor histidine kinase), *Methylobacterium* (ATPase), *Myxococcus* (histidine kinase), *Pseudomonas* (NADH-quinone oxidoreductase subunit G), *Rhizobium* (PAS domain-containing sensor histidine kinase) and *Stigmatella* (histidine kinase).

The other five RH-specific regulatory processes, mentioned above and reported in Table 3, mainly concerned

Table 3. Proteins involved in specific RH biological processes.

NCBI accession number	Protein name	Genus	phosphorus metabolic process	regulation of biosynthetic process	regulation of cellular metabolic process	regulation of macromolecule metabolic process	regulation of nitrogen compound metabolic process	regulation of primary metabolic process
gi|359836986	yhcZ-like uncharacterized transcriptional regulatory protein	*Actinoplanes*		x	x	x	x	x
gi|521090623	MerR family transcriptional regulator	*Amycolatopsis*		x	x	x	x	x
gi|947969229	glycosyl hydrolase family 15	*Arthrobacter*	x					
gi|515721482	carbamate kinase	*Bacillus*	x					
gi|651536048	transcriptional regulator	*Bacillus*		x	x	x	x	x
gi|921233208	thymidylate synthase	*Bacillus*	x					
gi|493664379	transcriptional regulator	*Bradyrhizobium*		x	x	x	x	x
gi|500951593	transcriptional regulator	*Bradyrhizobium*		x	x	x	x	x
gi|492898323	sensor histidine kinase	*Burkholderia*	x					
gi|495627132	pantetheine-phosphate adenylyltransferase	*Burkholderia*	x					
gi|740962859	hypothetical protein	*Burkholderia*	x	x	x	x	x	x
gi|747210628	polyphosphate kinase 2	*Burkholderia*	x					
gi|952349132	bifunctional 5	candidate division NC10 bacterium CSP1-5	x					
gi|530551546	3-deoxy-manno-octulosonate-8-phosphatase	candidate division Zixibacteria bacterium RBG-1	x					
gi|494944039	IclR family transcriptional regulator	*Caulobacter*		x	x	x	x	x
gi|759622522	two-component sensor histidine kinase	*Comamonas*	x					
gi|503661011	valine--tRNA ligase	*Mesorhizobium*		x	x	x	x	x
gi|501287217	ATPase	*Methylobacterium*	x					
gi|759608906	MerR family transcriptional regulator	*Micromonospora*		x	x	x	x	x
gi|499875781	histidine kinase	*Myxococcus*	x					
gi|926406132	MerR family transcriptional regulator	*Nocardia*		x	x	x	x	x
gi|953984713	LysR family transcriptional regulator	*Pseudomonas*		x	x	x	x	x
gi|953991462	NADH-quinone oxidoreductase subunit G	*Pseudomonas*	x					
gi|489644364	PAS domain-containing sensor histidine kinase	*Rhizobium*	x					
gi|501190326	AraC family transcriptional regulator	*Sorangium*		x	x	x	x	x
gi|488695855	histidine kinase	*Stigmatella*	x					
gi|328881659	MerR family transcriptional regulator	*Streptomyces*		x	x	x	x	x
gi|332745525	putative molybdopterin biosynthesis protein	*Streptomyces*	x					
gi|499342207	transcriptional regulator	*Streptomyces*		x	x	x	x	x
gi|529244478	two-component sensor histidine kinase	*Streptomyces*	x					
gi|648478522	phosphoenolpyruvate synthase	*Streptomyces*	x					
gi|663311700	helix-turn-helix transcriptional regulator	*Streptomyces*		x	x	x	x	x
gi|664258558	histidine kinase	*Streptomyces*	x	x	x	x	x	x
gi|664481143	helix-turn-helix transcriptional regulator	*Streptomyces*		x	x	x	x	x
gi|917163475	helix-turn-helix transcriptional regulator	*Streptomyces*		x	x	x	x	x
gi|946904936	two-component system response regulator	*Xanthomonas*		x	x	x	x	x

Proteins involved in specific RH biological processes that are expressed by genera **which** are present both in BS and in RH soils.

Proteins involved in specific RH biological processes that are expressed by genera **which** are present only in RH soil.
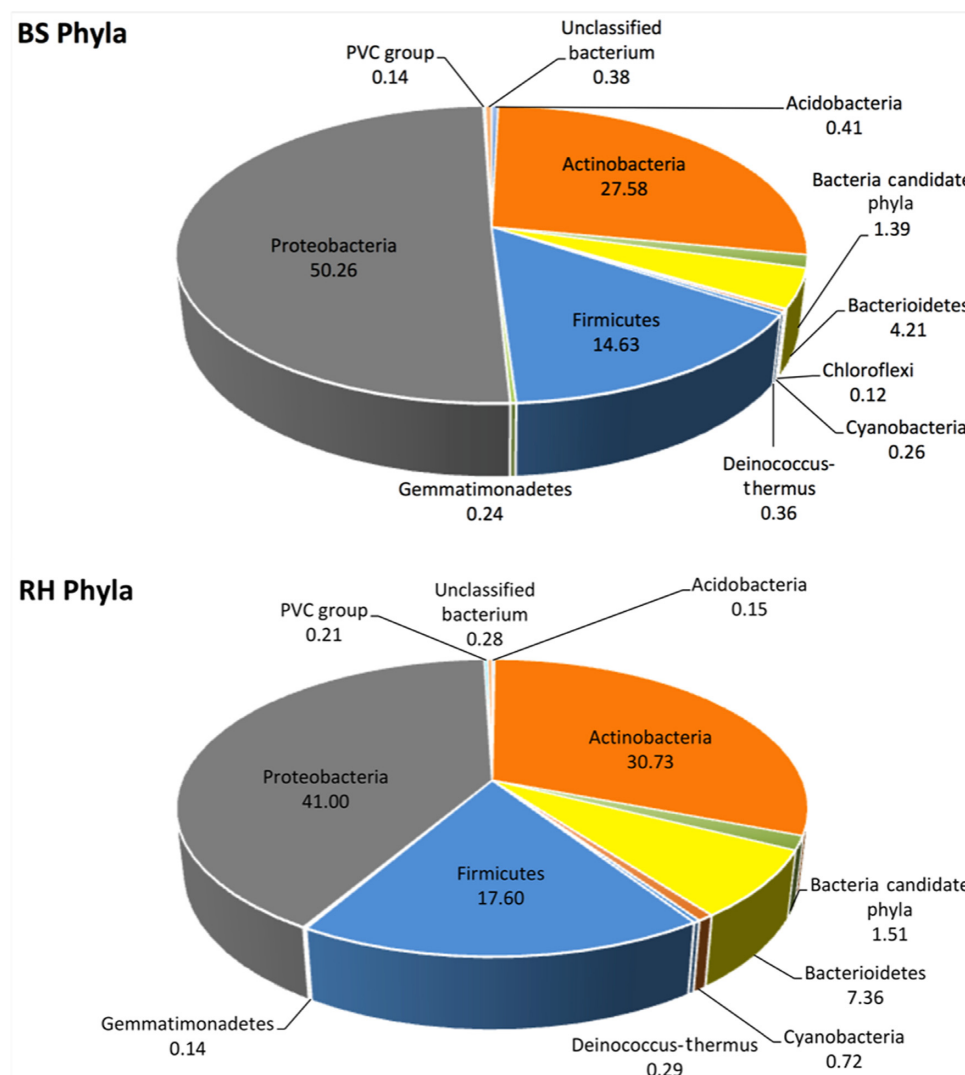
Figure 3. Pie charts of the phyla frequencies (%) distribution in the bulk soil (BS) and in the soil associated with the roots of *Vitis vinifera* cv. Pinot Noir (RH). Mean phyla frequency was calculated as the mean of the percentage ratio between the number of identified proteins (in each soil replica) expressed by the considered phyla and the total number of identified proteins (in each soil replica).

the expression of transcriptional regulators. The active genera were: *Streptomyces* (expressing a MerR family transcriptional regulator, another transcriptional regulator, three helix-turn-helix transcriptional regulators, a histidine kinase and a two-component system response regulator), *Bradyrhizobium* (expressing two different transcriptional regulators), *Amycolatopsis*, *Micromonospora* and *Nocardia* (expressing different MerR family transcriptional regulators), *Actinoplanes*, *Bacillus*, *Caulobacter*, *Pseudomonas*, *Sorangium* and *Xanthomonas* (expressing other types of transcriptional regulators) and *Mesorhizobium* (expressing a valine–tRNA ligase).

DISCUSSION

The rhizosphere has been studied with different approaches and many papers characterizing bacterial selection near plant roots are present in the literature. The effects of these interactions on plant growth, yield and production quality have been published (Nannipieri *et al.* 2003; Lingua *et al.* 2013; Bevivino *et al.* 2014; Bona *et al.* 2015; Bona, Lingua and Todeschini 2016; Bona *et al.* 2017). The present study, relying on a proteomic approach, shows, for

the first time, that *Streptomyces* was the genus with the highest number of expressed proteins in the vineyard rhizosphere, followed by *Bacillus*, *Bradyrhyzobium*, *Burkholderia* and *Pseudomonas*. These data are in agreement with the literature concerning culturable soil bacteria (Bevivino *et al.* 2014). Moreover, we identified different genera specifically involved in vineyard rhizosphere interactions, such as *Comamonas*. The most active phyla were *Proteobacteria*, *Actinobacteria* and *Firmicutes* in both soils. In RH soil, *Actinobacteria* expressed a larger number of proteins compared with BS soil, while the protein expression of *Proteobacteria* suggests reduced activity of this phylum in the rhizosphere. In addition, proteins from *Chloroflexi* were only found in BS soil. The *Deinococcus-thermus* phylum was also reported to be active in two proteomic works, by Knief and co-workers (2012), and Lin *et al.* (2013), in the rhizospheres of rice and sugarcane, respectively. Proteomic analysis showed that *Bacteroidetes* were more active in RH than in BS soil. Our findings indicate that *Proteobacteria* were the most active phylum in the rhizosphere, followed by *Actinobacteria* and *Firmicutes*; these data are partially in agreement with those obtained using a metagenomic approach, by Opsi
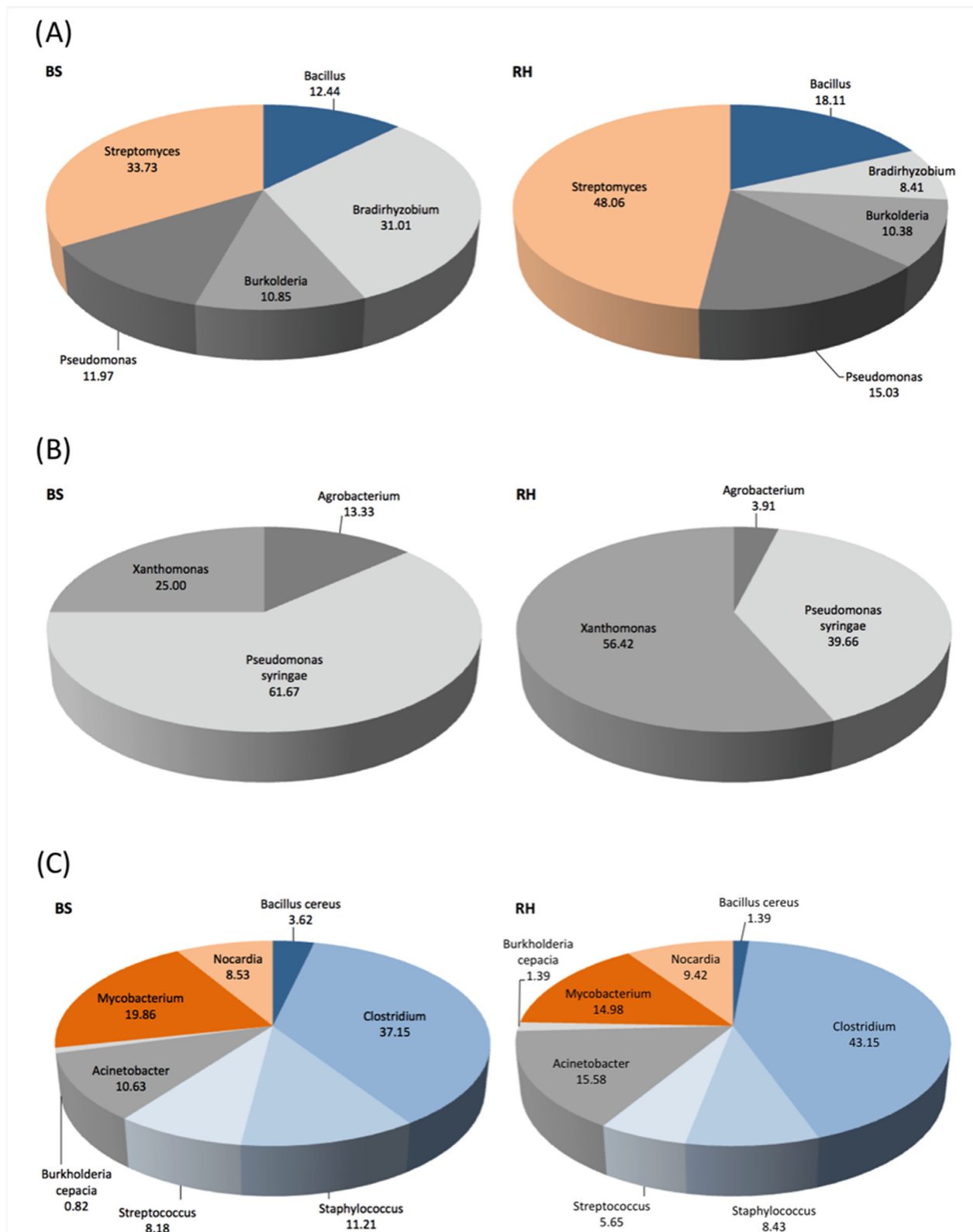
Figure 4. Pie charts showing the distribution (%) of the proteins produced by (**A**) beneficial, (**B**) plant and (**C**) human pathogen genera in the bulk soil (BS) and in the soil associated with the roots of *Vitis vinifera* cv. Pinot Noir (RH). Mean distribution was calculated as the mean of the percentage ratio between the number of identified proteins (in each soil replica) expressed by the considered genus and the total number of identified proteins in the considered category (in each soil replica). In the case of genera including both beneficial and pathogen microorganisms, only species which were definitely attributable were ascribed to the pathogens, while those recognized at genus level were included in the 'good' group.
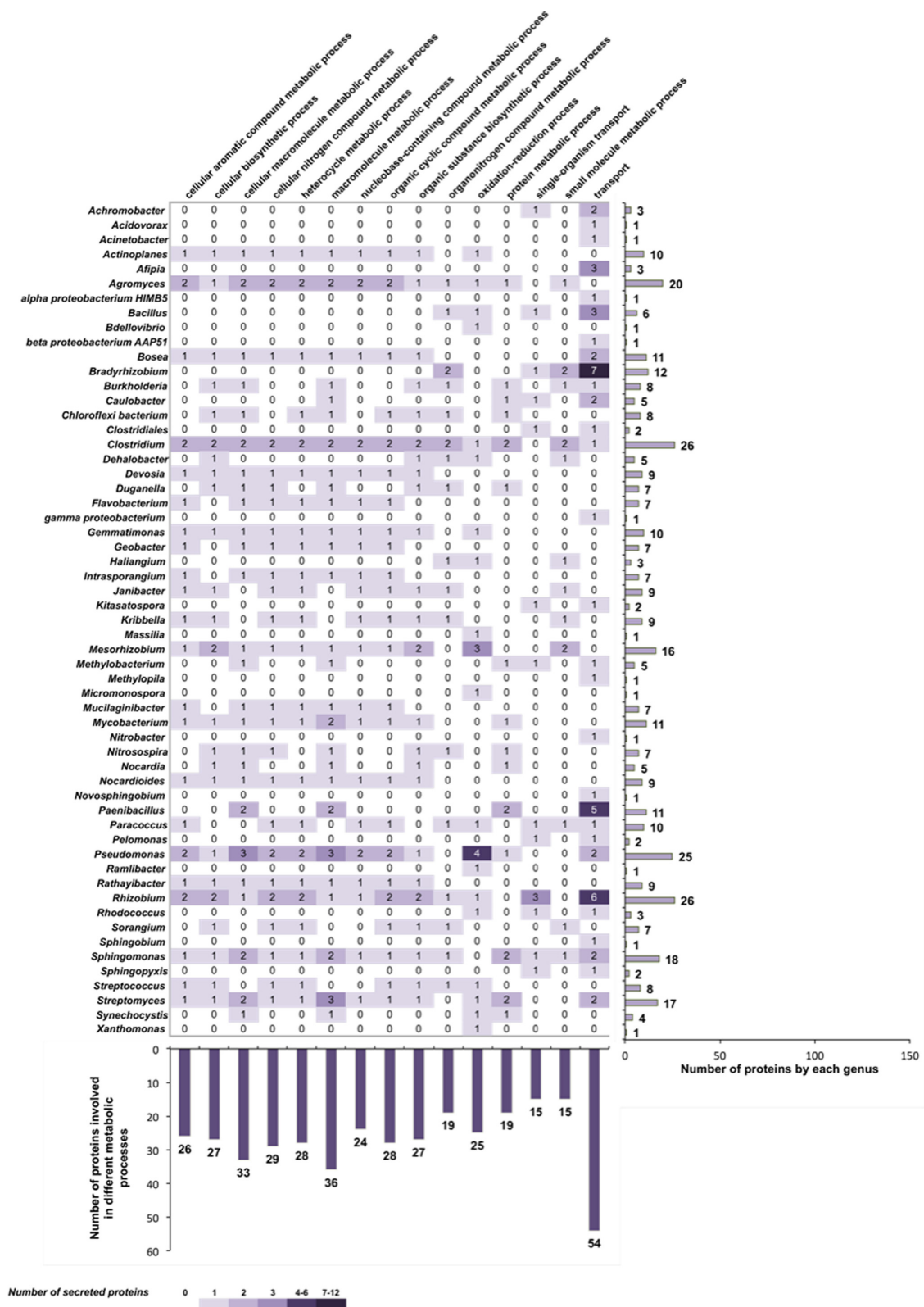
Figure 5. Number of proteins with a Blast2GO assignment expressed by each genus and involved in different biological processes in bulk soil (BS). The horizontal histogram on the right shows the number of proteins expressed by each genus. At the bottom, the number of proteins involved in each biological process is reported.
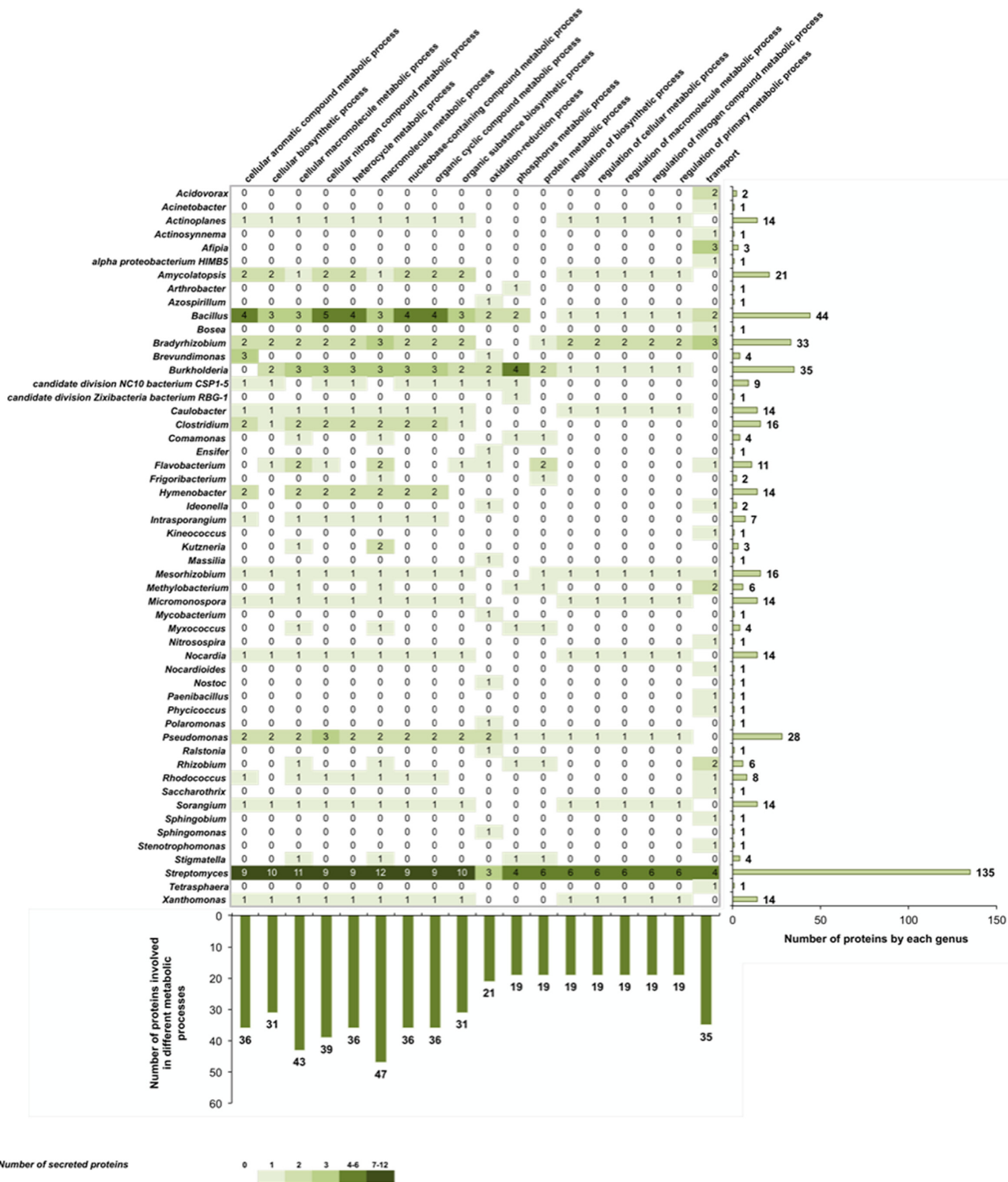
Figure 6. Number of proteins with a Blast2GO assignment expressed by each genus and involved in different biological processes in the soil associated with the roots of *Vitis vinifera* cv. Pinot Noir (RH). The horizontal histogram on the right shows the number of proteins expressed by each genus. At the bottom, the number of proteins involved in each biological process is reported.

et al. (2014), who reported *Proteobacteria* (36%), followed by *Actinobacteria* (26%) and *Acidobacteria* (15%), as the prevalent phyla in the vineyard. These phyla have also been described as the most common in other kinds of soil and rhizospheres such as human-, penguin- and seal-colony impacted soils and pristine soil in the Fildes Region (King George Island, Antarctica) (Wang *et al.*

2015). Wang *et al.* (2011b) report *Proteobacteria* (44%), *Actinobacteria* (14%) and *Firmicutes* (9%) as the most relevant phyla in the rice rhizosphere, using a metaproteomic approach. The Shannon-Wiener index—an entropy measurement that increases according to the number of species in the sample—was higher in RH

than in BS soil, even if the difference was not statistically significant. The Simpson index, which is based on the probability of assigning two independent individuals taken randomly from the community into the same species, did not change in the two soils. These results are partially in agreement with what was observed by Novello *et al.* (2017), in which, using a metagenomic approach, the values of the Shannon-Wiener index were higher than those obtained in this work, where a metaproteomic approach was applied. These results underline the difference between the metagenomic and metaproteomic methods in describing the environmental bacterial community. In fact, while the metagenome provides a description of the whole bacterial community (using DNA), the metaproteome clearly indicates the active species, which are necessarily included in those described by the DNA presence.

Considering the model proposed by Mendes and colleagues (2013), our work confirms that the most active microorganisms are good while bad and ugly are present, but have a marginal role in terms of protein expression.

Our results regarding the detailed protein expression in BS and in RH soils enabled us to highlight two main points: (i) a set of proteins expressed by the same genera was identified in both soils and must therefore represent some constitutive mechanisms occurring, possibly in a general fashion, in soils; and (ii) a second set of proteins was specific to the rhizosphere compartment. Specifically, where the first set is concerned, 20 proteins involved in mechanisms of bacterial metabolism and responses to environmental stimuli not linked with plant root presence were commonly expressed both in BS and RH soils by the same bacterial genera. Major facilitator superfamily transporter (MFSt) (identified in our work in *Acidovorax*), together with beta-lactamase, are included in the ancient and diverse group of proteins encoded by antibiotic resistance genes (ARGs). These genes have previously been identified by Forsberg and co-workers (2014) in agricultural soil and they include genes identical to those found in human pathogens. Despite the apparent overlap between soil and clinical resistomes, the factors influencing ARG composition in soil and their movement between genomes and habitats remain largely unknown. MFSts are enriched by *Actinobacteria* and *Proteobacteria* (Forsberg *et al.* 2014), and this result was confirmed by our study. Although MFSt is linked with antibiotic resistance, it is important to highlight that it is also involved in nutrient transport: in fact, it was identified in *Nitrosospira multiformis*, an ammonia-oxidizing bacteria from the soil environment (Norton, Klotz and Stein 2008) related to nitrogen transport; sulfate transport involves both MFS and ABC transporters, while phosphate transport is mediated by a complete ABC transporter (ABCt) and by three phosphate-selective porins (Norton *et al.* 2008). Porins from *Bradyrhizobium* were detected in our samples, confirming their role in soil.

Regarding phosphate metabolism in soil bacteria, two phosphate ABCt substrate-binding protein PstS were detected from *Afipia*. This expression could be related to phosphate starvation, as would be expected in a soil low in phosphate, as demonstrated by Aguena, Ferreira and Spira (2009) in *Escherichia coli*: PstS is the substrate-binding component of the ABC-type transporter complex pstSACB, involved in phosphate import, and its accumulation is enhanced under phosphate starvation. The major function of ABC import systems is to provide essential nutrients to bacteria (Lin *et al.* 2013). A second identified membrane protein was the outer membrane protein A38 (OmpA38), which is a porin and the most abundant protein in the outer membranes of *Acinetobacter baumannii* (Choi *et al.* 2005). In this

work, it was detected in both BS and RH soils. Omps of Gram-negative bacteria are known to be key players in bacterial adaptation and pathogenesis (Lin, Huang and Zhang 2002). We also detected a protein-export membrane protein called SecF, which is involved in the secretion across the inner membrane mediated by the preprotein translocase pathway, typical of some Gram-negative bacteria (Tseng *et al.* 1999).

Expression of MERK protein in our samples could be linked to constitutive mechanisms of stress resistance. In fact, Petrus and c-oworkers (2015) identified the presence of a new Mer gene (MerK) in *Xanthobacter autotrophycus*, a mercury-resistant soil bacteria. These genes encode proteins with homology to members of the pyridine nucleotide disulfide oxidoreductase family, and are most similar to a glutathione reductase (Petrus *et al.* 2015).

Glycosyl transferase family proteins from *Pseudomonas* (detected in both soils) could be involved in response to osmotic stress in soils. In a transcriptomic study by Johnson *et al.* (2011), this protein is upregulated in *Sphingomonas wittichii* under salinity stress (Csonka 1989).

TonB-dependent receptors (TBDRs) that were detected from *Sphingobium* could be linked with iron starvation. TBDRs are outer membrane proteins mainly known for the active transport of iron siderophore complexes in Gram-negative bacteria (Blanvillain *et al.* 2007). Both in BS and in RH soil proteome, a peptidase from *Variovorax* was detected. This protein is commonly found in extracellular proteomes of the various *Bacillus* species, which contribute to the virulence and supply of nutrients (Antelmann *et al.* 2005).

Regarding proteins differentially expressed in the two soils (BS and RH), in the rhizosphere a higher number of proteins involved in macromolecule, cellular macromolecule, cellular nitrogen compound, cellular aromatic compound, heterocycle, nucleobase-containing compound and organic cyclic compound metabolic processes was detected. This higher metabolic rate is part of the stimulating effect of the root presence on bacterial community metabolism, as well as the appearance of the regulation of the primary metabolism, involving the main genera present in RH soil. In our opinion, the appearance of the phosphorus metabolic process and the regulation of the nitrogen compound metabolic process are useful to help explain and clarify the role of microorganisms and the specific enzyme involved in rhizosphere metabolism. Specifically, proteins involved in the phosphorus metabolic process are enzymes with phosphate transfer and kinase activity: *Bacillus* expresses a carbamate kinase, whose expression is regulated in a manner that allows the enzyme to function as a provider of ammonia under aerobic conditions or of ATP under anaerobic conditions (Abdel, Bibb and Nainan 1982), and a thymidylate synthase that produces *de novo* thymidylate, an essential DNA precursor; *Burkholderia* expresses a sensor histidine kinase, a polyphosphate kinase 2 and a pantetheine-phosphate adenyltransferase that catalyzes, as reported by Edwards *et al.* (2011), the fourth of five steps in the coenzyme A biosynthetic pathway in *Burkholderia pseudomallei*; *Comamonas*, *Myxococcus*, *Rhizobium* and *Stigmatella,* that express different histidine kinases; *Methylobacterium*, an ATPase; and *Streptomyces*, a two-component sensor histidine kinase, phosphoenolpyruvate synthase and putative molybdopterin biosynthesis protein. Finally, this part of the metabolic process involved a glycosyl hydrolase family 15 from *Arthrobacter* linked to lignin degradation as reported by Jiménez *et al.* (2016) in a metatrascriptomic study of soil-derived microbial consortia that were trained to degrade once-used wheat straw, switchgrass and corn stover. The set of proteins involved

in the regulation of the nitrogen compound metabolic process comprised different transcriptional regulatory protein polypeptides such as: yhcZ-like uncharacterized transcriptional regulatory proteins from *Actinoplanes*, a transcriptional regulator from *Bacillus*, two transcriptional regulators from *Bradyrhizobium*, an IclR family transcriptional regulator from *Caulobacter*, valine–tRNA ligase from *Mesorhizobium*, a LysR family transcriptional regulator from *Pseudomonas*, an AraC family transcriptional regulator from *Sorangium*, a transcriptional regulator, two helix-turn-helix transcriptional regulators and a transcriptional regulator from *Streptomyces*, and finally two-component system response regulators from *Xanthomonas*. The expression of different kinds of transcriptional regulator could be linked to environmental responses; in fact, very often, adaptive responses in bacteria are mediated by transcriptional regulators which, upon receiving the appropriate signal, trigger the specific transcriptional response. For example, a number of regulators belonging to the IclR family are involved in the control of catabolic pathways for the degradation of aromatic compounds (Molina-Henares *et al*. 2006). MerR family transcriptional regulators were found in *Amycolatopsis*, *Micromonospora*, *Nocardia* and *Streptomyces*. The MerR family is a group of transcriptional activators with similar N-terminal helix-turn-helix DNA binding regions and C-terminal effector binding regions that are specific to the effector recognized (Brown *et al*. 2003). Mer genes are linked with mercury resistance in bacteria (Brown *et al*. 2003).

Concluding, our results demonstrate that a metaproteome approach allows an in-depth investigation of the mechanisms occurring in the rhizosphere; in the case of *V. vinifera* subjected to IPM, we showed that bacteria belonging to *Streptomyces, Bacillus, Bradyrhizobium, Burkholderia* and *Pseudomonas* genera were the most active in protein expression, and were mainly involved in phosphorus and nitrogen rhizosphere metabolism. Variation in rhizosphere microbial communities among genotypes has been demonstrated experimentally for *Arabidopsis thaliana* (Lundberg *et al*. 2012; Micallef *et al*. 2009) and has been attributed to differences among genotypes in root exudates (Micallef *et al*.2009). Moreover, evidence for an association between the genetic structure of the plant population and the structure of the microbial community in a natural salt marsh has been demonstrated by Zogg, Travis and Brazeau (2018). Also in this work, the plant roots (grapevine of the cultivar Pinot Noir in this case) exert a selection of the active genera stimulating an effect on the bacterial community metabolism. Finally, comparing metagenome (Novello *et al*. 2017) and metaproteome approaches, it is clear that the former gives a wider view of the bacterial composition of an ecosystem, but the latter is more focused on what is really vital and active; so, in order to have a complete description of 'actors' and 'roles', it is fundamental, in our opinion, to adopt both these methods in an integrated manner.

Future perspectives of this work could be: (i) to apply this metaproteome approach to other important grapevine cultivars in order to better understand the impact of the genetic structure of the plant in the modulation of the composition and the activity of the associated microbial communities; and (ii) to isolate and to screen beneficial bacteria based on probes designed on the basis of the identified proteins in order to use them as biostimulants in degraded vineyards.

DATA AVAILABILITY

The genomic datasets generated and/or analyzed during the current study are available in NCBI using BioProject ID PRJNA394211.

The mass spectrometry proteomics data have been deposited with the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD007670.

Submission details: Project Name: *Vitis vinifera* rhizosphere characterization: a metaproteome approach. Project accession: PXD007670. Project DOI: Not applicable; Reviewer account details: Username: reviewer66231@ebi.ac.uk Password: MmGK1gWH.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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