



# *Bacillus velezensis*: phylogeny, useful applications, and avenues for exploitation

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## Abstract

Some members of the *Bacillus velezensis* (*Bv*) group (e.g., *Bv* FZB42T and AS3.43) were previously assigned grouping with *B. subtilis* and *B. amyloliquefaciens*, based on the fact that they shared a 99% DNA–DNA percentage phylogenetic similarity. However, hinging on current assessments of the pan-genomic reassignments, the differing phylogenomic characteristics of *Bv* from *B. subtilis* and *B. amyloliquefaciens* are now better understood. Within this re-grouping/reassignment, the various strains within the *Bv* share a close phylogenomic resemblance, and a number of these strains have received a lot of attention in recent years, due to their genomic robustness, and the growing evidence for their possible utilization in the agricultural industry for managing plant diseases. Only a few applications for their use medicinally/pharmaceutically, environmentally, and in the food industry have been reported, and this may be due to the fact that the majority of those strains investigated are those typically occurring in soil. Although the intracellular unique biomolecules of *Bv* strains have been revealed via in silico genome modeling and investigated using transcriptomics and proteomics, a further inquisition into the *Bv* metabolome using newer technologies such as metabolomics could elucidate additional applications of this economically relevant *Bacillus* species, beyond that of primarily the agricultural sector.

**Keywords** *Bacillus velezensis* · Beneficial compounds · Genome · Metabolome · Microbial · Omics · Biotechnological application

## Introduction

Over the past six decades, Gram-positive endospores have received a great deal of attention due to the various discoveries made pertaining to their use in various sectors of industry, especially agriculturally. Additional advantages when using these spore-forming bacteria are that they can be easily cultured, stored, and manipulated for biotechnological purposes (Cao et al. 2018). To date, *B. licheniformis*, *B. subtilis*, and *B. amyloliquefaciens* are among the most exploited species from the genus; however, over the last decade, a vast amount

of research has been done on *B. velezensis* (*Bv*). Although *Bv* is categorized as a heterotypic synonym of *B. amyloliquefaciens* subsp. *plantarum* FZB42T, *B. methylotrophicus* KACC 13015T, and *B. oryzicola* KACC 18228, based on DNA–DNA hybridization values greater than 84% (Dunlap et al. 2016; Fan et al. 2017), strains within the species still show distinct genomic characteristics (Adeniji and Babalola 2018). *Bv* was originally cultured from the river bank of Vélez in Málaga in Southern Spain, was reported to grow at a pH of between 5.0 and 10.0 and at temperatures of between 15 and 45 °C (Ruiz-García et al. 2005), and produce diverse metabolic intermediates, which include antibiotics, enzymes, phytohormones, iron chelators, antioxidants, growth promoters, and antitumor agents (Gao et al. 2017b; Liu et al. 2010b; Meena et al. 2018).

Genome sequence reports for many of the *Bv* subspecies are now readily available (Baptista et al. 2018; Chen 2017; Kim et al. 2017a; Kim et al. 2017b; Lee et al. 2015, 2017; Li et al. 2018; Liu et al. 2017) which is also considered extremely advantageous from a biotechnological exploitation perspective. To date, *Bv* has been extensively utilized in the area of

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biocontrol and plant growth promotion; however, studies involving their use in other industries or various other environmental applications are scarce and hence a topic of further research. Better described *Bacillus* sp. such as *B. licheniformis*, for instance, has been comparatively well exploited for various commercial applications ranging from the manufacture of enzymes (proteases, aminopeptidases, pectinolytic enzymes) to the production of antibiotics, biochemicals, and also applied in the textile industry. *Bv*, on the other hand, although considered extremely robust to work with, and despite the numerous studies on its possible biotech applications, has as yet not been extensively applied commercially, nor if these applications have reduced reliance on the use of synthetic products for industrial, agricultural, environmental, and medical purposes.

Comparative genomic, mutagenic, transcriptomic, histology, and proteomic analyses have all contributed to characterizing these beneficial *Bacillus* strains and in the production of improved bioformulations for various biotech applications (Wu et al. 2015b). A limitation to using these technologies, however, is that it appears that only those compounds previously known to be present in other *Bacillus* spp., such as difficidin, surfactin, bacilysin, macrolactin, bacillaene, bacillibactin, and fengycin, are being re-identified in many of the *Bacillus* strains and species reported lately in literature. Considering this, there is a desperate need for using other research approaches for identifying and characterization new *Bv* secondary biomolecules in vitro. Metabolomics, one of the newest additions to “omics” research, is likely to identify new microbial biomarkers and, in so doing, expand our knowledge and subsequently the applications and commercial value of *Bv* and other species of *Bacillus*. Here, we expound on the genomic relatedness existing among the *Bv* species, their rapid deployment over the years, and we suggest future approaches for exploiting underutilized beneficial microbes such as *Bv*.

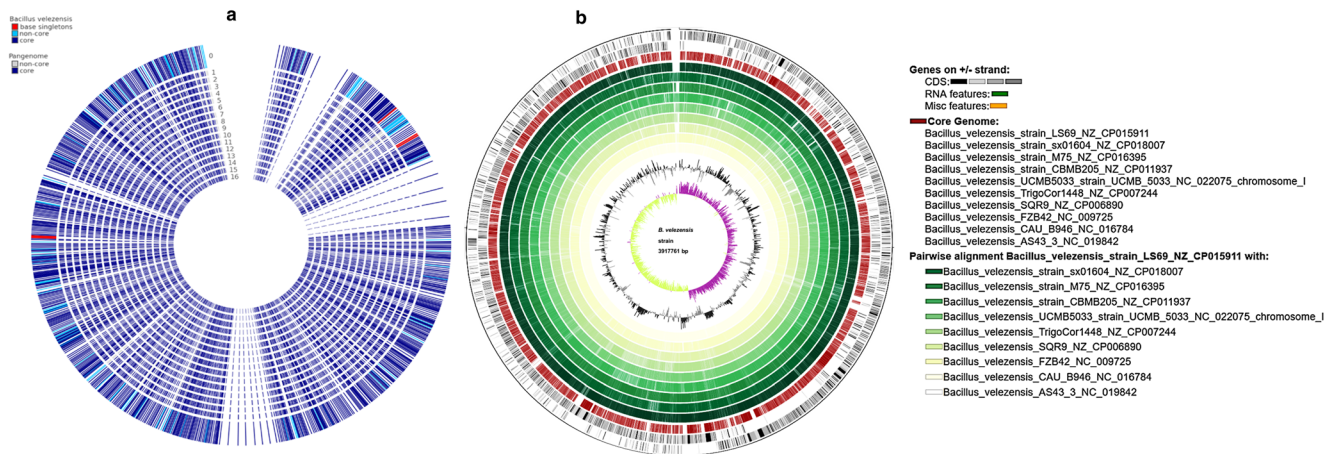
## Phylogenomic interrelation between the *B. velezensis* strains

Dunlap et al. (2016) report that members of the *Bv* sp. share phenotypic and genotypic coherence based on their morphology, physiology, chemotaxonomy, and phylogeny and since then confirmed by others (Ye et al. 2018). In order to fully understand the genetic relationship and biochemical diversity which exists within the species, we collected 17 *Bv* genome sequences from the National Center for Biotechnological Information (NCBI), with identity similarity  $\geq 98\%$  and phylogenomically surveyed them using the Kbase bioinformatics platform (Arkin et al. 2016). Subsets (10) of the genomes were also compared using the online web server of EDGAR 2.3 <https://edgar.computational.bio.uni-giessen.de/>. From the in silico analysis, the conserved genes located in

all of the collected strains (representing the core genome), and the supersets of all the genes (the pan-genome) (Dunlap et al. 2013) were identified and described (Fig. 1a, b).

In Fig. 2a, b, the values overlapping are the gene coding proteins (GCPs) common within the genomes and values outside the overlaps signify the GCPs in each genome without orthologs in the other genomes. The Venn diagram was computed as described by Bardou et al. (2014) using the BIOiPLUG Comparative Genomics Database (<https://www.bioiplug.com/>). Also, using the default parameters of the web server BIOiPLUG Apps (<https://www.bioiplug.com/apps>), the Pan-genome Orthologous Groups (POGs) and Gene Content (GC) of a subset of the 17 strains (5 highly referenced and 5 somewhat less popular *B. velezensis* strains) were determined. The Pan-genome Orthologous Groups (POGs) which represent the basic unit of core genes/pan-genome and gene presence/absence representing total genetic content were analyzed using BIOiPLUG Comparative Genomics Database (<https://www.bioiplug.com/>) (Fig. 3a, b).

Both the core genes and dispensable genes are crucial for determining bacterial species diversity. The core genes are responsible for indicating fundamental functions of the organism’s biology which includes replication, translation, and maintenance of cellular homeostasis, and in Fig. 3a, b, these core genes indicate the genetic diversity among the strains compared. The dispensable genes are associated with survivability, antimicrobial resistance, virulence traits, and development of novel gene functions. Some of these genes, when present within the strains, also confer an adaptive superiority over others lacking them (Carlos Guimaraes et al. 2015). Average nucleotide identity (ANI) was also computed on the online web server of EDGAR 2.3 <https://edgar.computational.bio.uni-giessen.de/>. Majority of the core genes irrespective of their functions and location are necessary for better phylogenomic reassignments among the *Bv* strains. The distance matrices heatmap (Fig. 4) indicates the genomic similarities and differences among 10 of the 17 *Bv* strains. Based on this ANI analyses, we reported that the phylogeny of these closely related bacteria corresponds to previous reports. A total of 154 core functions and 784 core protein families were peculiar to all 17 the strains probed. Additionally, the 17 strains have in combination 60,538 protein-coding genes of which 59,263 are present in homolog families and 1275 in singleton families. The total numbers of families identified in the 17 strains are 5538, of which 4207 are in the homolog families and 1331 in the singleton families (Table 1). Figure 5 indicates the collinearity of genes among 10 of the *Bv* subset. The conserved genomic regions of the *Bv* strains were probably due to their common ancestry, either by duplication or by speciation of the genes. Several industries have benefited from the versatile genomic resource of *Bv*, and this will subsequently be discussed in greater detail in the latter sections of this review.



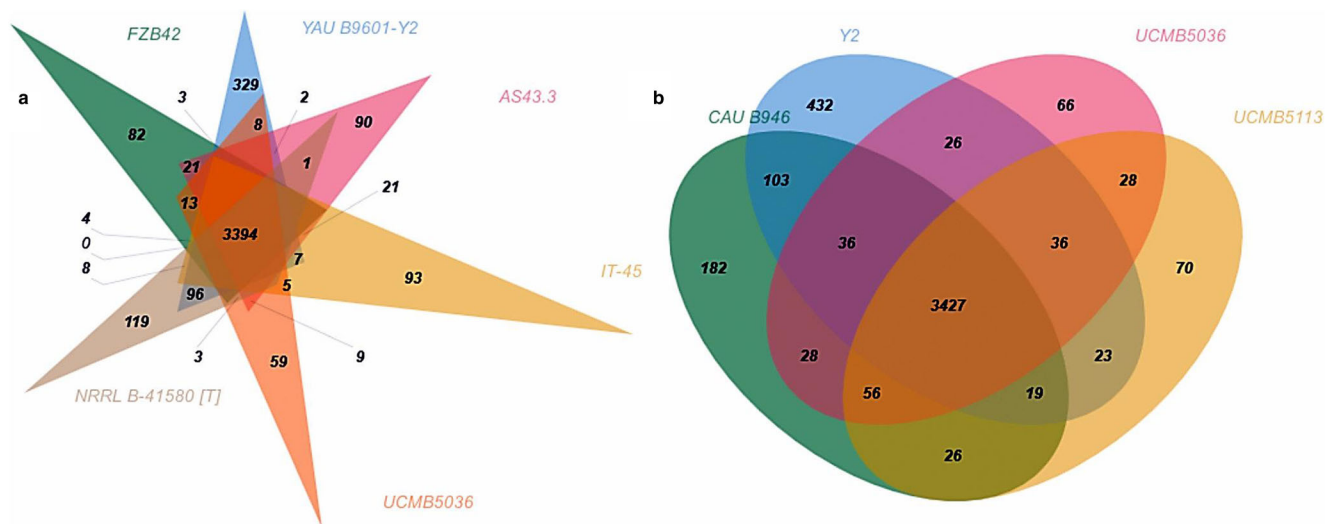
**Fig. 1** **a** Pan-genomic atlas of 17 *Bv* strains computed on the Kbase platform (Arkin et al. 2016). Core and non-core genome similarities and dissimilarities are indicated on the left key of the figure. Peculiar genes in the genome 0 (*Bv* NWUMFkBS10.5) are indicated in its ring as light blue arcs; genome 1: VCC genbank genome (gbg) genome; genome 2: ATCC19217.gbg; genome 3: BS10.5.gbg; genome 4: SX.gbg; genome

5: AS43.3.gbg; genome 6: LABIM40.gbg; genome 7: J-5.gbg; genome 8: DR08.gbg; genome 9: GYL4.gbg; genome 10: UCMB5033.gbg; genome 11: M7.gbg; genome 12: SQR9.gbg; genome 13: TrigoCor1448.gbg; genome 14: CBMB205.gbg; genome 15: WS8.gbg; Genome 16: LS69.gbg). **b** Circular map showing pairwise alignment of *Bv* strain LS69 (NZ\_CP015911) with other 9 *Bv* genome subsets

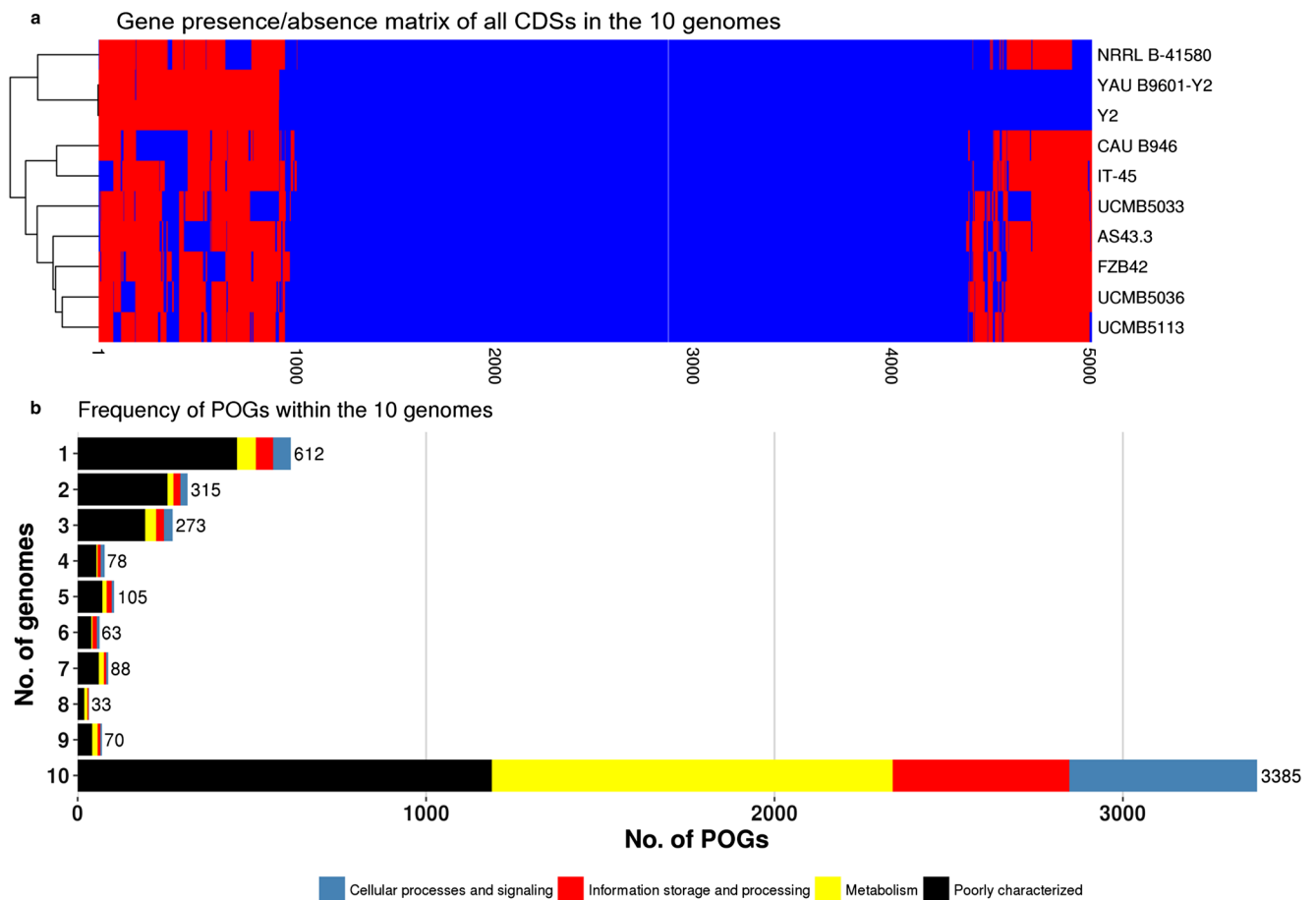
### Agricultural applications of *B. velezensis*

As previously mentioned, the majority of research supports the use of *B. velezensis* for its possible utilization in the agricultural industry, as alternatives to the current synthetic fertilizers and chemical pesticides (as summarized in Table 2). In an attempt to reduce the incidence of wheat powdery mildew (Cai et al. 2017), Cai et al. (2016) performed a field trial showing the capacity of a bioactive metabolite extract of *Bv* CC09 to reduce the severity of mildew disease by 86.12% when compared to a commercial fungicide triazolone which only showed a 50.39% reduction when applied as pretreatment samples. Similarly, Chen et al. (2018c) indicated that *Bv* extract, LM2303, significantly reduced the incidence and severity of wheat FHB (caused by *F. graminearum*) under *in planta* conditions and

similarly to CC09, it also had a higher biocontrol efficacy when compared with that of a chemical fungicide. The authors further proposed that the biocontrol potential of *Bv* LM2303 was most likely via four major mechanisms: (i) antibiosis mediated by *Bacillus* antibacterial metabolites and lipopeptides, (ii) activation of induced systemic resistance (ISR) in wheat by the volatiles and the lipopeptide surfactin, (iii) enhanced growth of wheat due to an elevated production of growth hormone and nutrient uptake, and (iv) competitive exclusion of other microflora. Gilardi et al. (2015) investigated a commercially obtained *Bv* (Cilus Plus IT45) towards the biocontrol of *Phytophthora capsici*, which results in root and crown rot of zucchini in parts in Italy, however, in their investigation the biocontrol effect of the strain was determined to be non-significant. Using an HPLC-MS, in combination with genomic analyses, Jin et al.



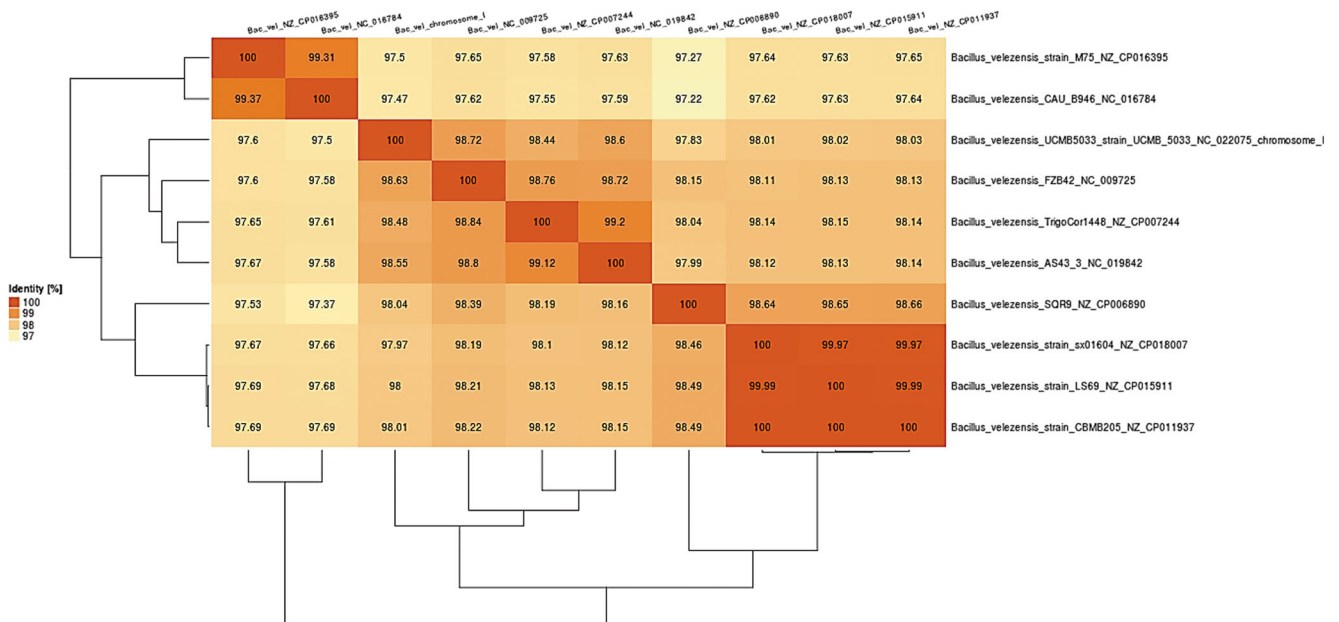
**Fig. 2** Venn diagrams showing the gene coding proteins (GCP) shared between highly referenced *Bv* strains (a) and somewhat less popular *Bv* strains (b)



**Fig. 3** Clustering of the gene content and the conserved pan-genome orthologous groups (POGs) of 10 *Bv* genomes subsets (a, b)

(2017) identified the metabolic intermediates that were responsible for the plant protection and growth promoting attributes of *Bv* S3-1 during an anti-*Botrytis cinerea* experiment. The

bioactivity of the *Bv* was attributed to the production of various lipopeptide antibiotics (iturin, fengycin, and surfactin). It is important to note, however, that many of the aforementioned



**Fig. 4** Heatmap showing intrastrain similarities of subsets from the 17 *Bv* based on the ANI of their genome sequences



**Table 1** Summative statistics of the pan-genome datasets and shared genes of 17 *B. velezensis* strains retrieved from the NCBI data base

Genome accession nos.	Genes	Homologs	Homolog families	Singletons
<i>Bv</i> SQR9 NZ_CP006890.1	3985	3795	3701	190
<i>Bv</i> TrigoCor1448 NZ_CP0070244.1	3683	3623	3542	60
<i>Bv</i> SX01604 CP018007.1	3773	3626	3560	147
<i>Bv</i> NWUMFkBS10.5 CP007244.1	968	910	893	58
<i>Bv</i> UCMB5033 HG328253.1	3952	3770	3691	182
<i>Bv</i> M7 CP016395.1	3775	3632	3566	143
<i>Bv</i> LABIM40 CP023748.1	3999	3763	3687	236
<i>Bv</i> FZB42 CP000560.1	3797	3560	3490	237
<i>Bv</i> VCC CP027429.1	3792	3559	3494	233
<i>Bv</i> J-5 CP018295.1	3768	3640	3561	128
<i>Bv</i> DR-08 CP028437.1	3772	3634	3568	138
<i>Bv</i> GYL4 CP020874.1	3803	3600	3530	203
<i>Bv</i> AS43.3 CP003838.1	3856	3613	3547	243
<i>Bv</i> LS69 CP015911.1	3805	3633	3567	172
<i>Bv</i> ATCC19217 CP009749.1	3882	3634	3565	248
<i>Bv</i> WS-8 CP018200.1	3771	3633	3567	138
<i>Bv</i> CBMB205 CP011937.1	3770	3770	3564	132

compounds ascribed to *Bv* functionality are not necessarily novel compounds.

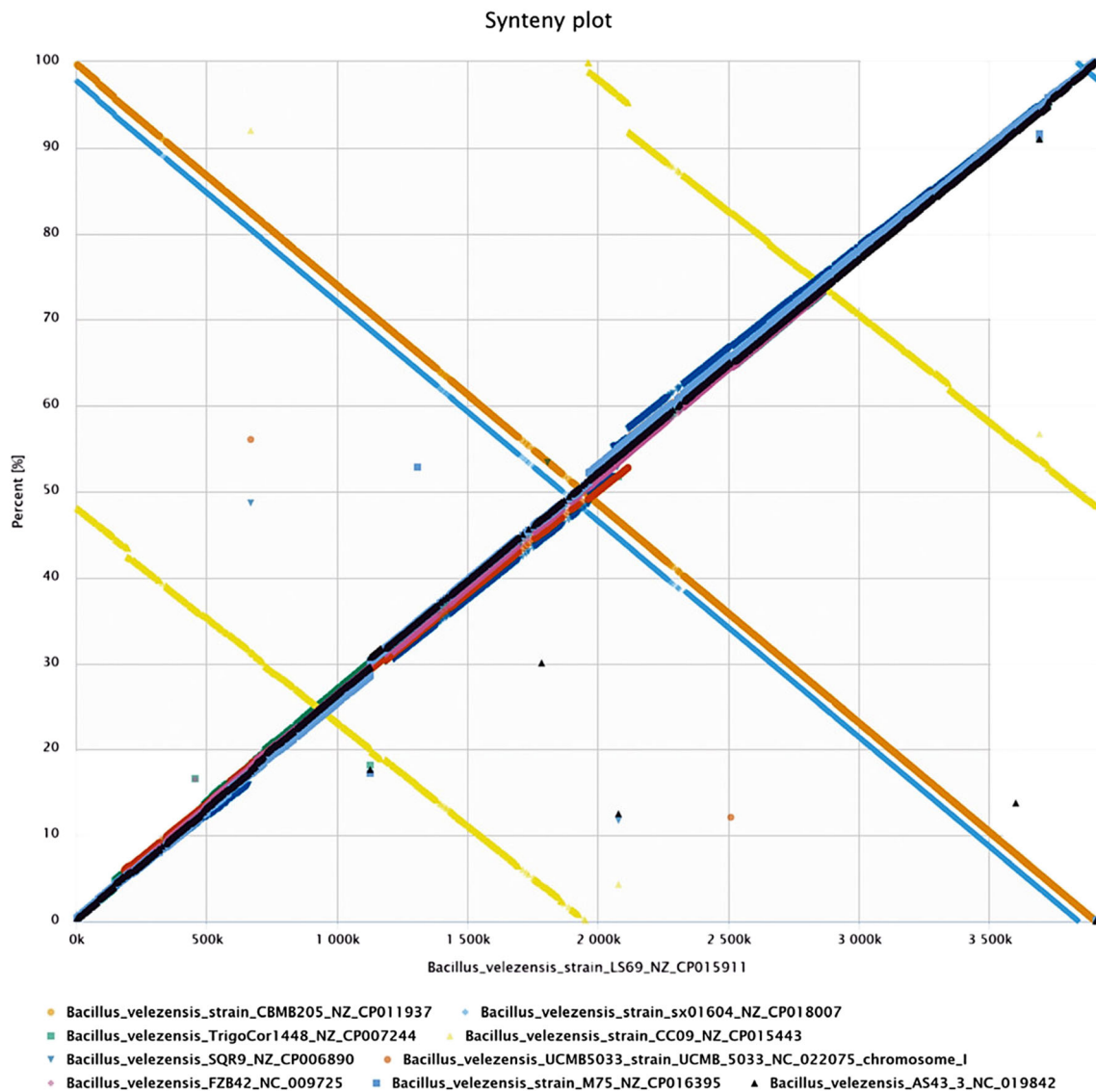
*Streptomyces scabies* is a pathogen that affects diverse crop types, ranging from, but not limited to the following: beet, carrot, cucumber, pepper, potato, radish, squash, tomato, and turnip. *Bv* BAC03 was identified as an effective antagonist of *S. scabies*; however, antagonism and plant growth promotion of the strain was dependent on its prior application before disease onset. Additionally, strain BAC03 increased the biomass of the radish roots and leave harvest, in spite of *S. scabies* infection (Chen et al. 2015; Meng and Hao 2017; Meng et al. 2016). Likewise, *Bv* YC7010, a multi-functional endophyte with the capacity to induce systemic resistance against several rice pathogens, was also found to induce systemic resistance in Arabidopsis against *Myzus persicae* (Rashid et al. 2017). Green peach aphid (GPA), *Myzus persicae*, is a destructive phloem sap insect–pest that causes significant agricultural losses. Root drenching of Arabidopsis with the aforementioned *Bv* strain resulted in a significant reduction in the settling, feeding, and reproduction of the pest on the Arabidopsis leaves. The authors attributed the ISR to the expression of the senescence-promoting gene, phytoalexin deficient 4 (PAD4), and the subsequent suppression of botrytis-induced kinase 1 (BIK1) of *M. persicae*, which warrants further experiments on the use of *Bv* YC7010 as a pesticide.

Lastly, Nam et al. (2009) proved the efficacy of various formulations made up of *Bv* BS87 and RK1 to be highly effective for the bioprotection of strawberries against *Fusarium oxysporum* f. sp. *fragariae*, by performing in vitro and in planta studies. Although the bioprotective efficacy of the RK1 formulation was higher than its counterpart BS87, it

exhibited similar antagonism in planta in comparison to a conventional fungicide (copper hydroxide). Application of *Bv* along with a *Glomus intraradices* (mycorrhizal fungi) as a potential biofertilizer for strawberry crops was later reported by Palencia et al. (2015). These studies show the capacity of *Bv* for use as a very effective alternative for conventional biofertilizers and chemical pesticides, with subsequently comparatively even better crop yields with less harm to the environment.

### Industrial and environmental applications of *B. velezensis*

Various *Bv* strains have been shown to have applications for degrading various toxic and harmful industrial byproducts. The azo dyes, for instance, found in textile industry effluents, are toxic to aquatic environments, due to their mutagenic and recalcitrant properties. A preliminary evaluation by Bafana et al. (2008) indicated the capacity of *Bv* strain AB to decolorize and detoxify these azo dyes and, subsequently, its applications for treating and preventing azo dye effluent pollution. Similarly, Lan (2016) indicated a total reduction in nitrogen and COD of 83.5% and 93.2% respectively, in a study testing the capacity and possible applications of *Bv* strain M2 for application for slaughterhouse wastewater bioremediation. An in silico study by Chen et al. (2018a) indicated that a number of *Bv* strains, employed for in vivo pretreatments of lignocellulosic material, had the capacity to efficiently degrade cellulose and hemicelluloses due to the presence of genes coding for various lignocellulolytic



**Fig. 5** The syntenic genes shared among the *Bv* strains. In each, the loci set are found on the same chromosome and are not necessarily conserved sequentially

enzymes including endo-glucanases, endo-glucosidases, phospho-glucosidases, phospho-galactosidase, sucrose-phosphate hydrolase, and levanases.

In a follow-up study, Chen et al. (2018b) isolated a *Bv* strain (157) with the capacity for degrading various agro-industrial byproducts including soybean meal, wheat bran, sugarcane bagasse, wheat straw, rice husk, maize flour and maize straw, and the subsequent utilization of these degraded agro-materials in biofuel production. The authors additionally indicated that during solid-state fermentation, several important industrial enzymes are produced by this *Bv* strain 157. In an explorative study, searching for *Bv* strains with the capacity to efficiently depolymerize various types of lignocelluloses (cellulose, hemicellulose, and lignin) into fermentable sugars (Nair et al. 2018) isolated *Bv* ASN1 which had the capacity to synthesize cellulase, which has a broad range of applications

in food, textile, animal feed, petroleum, waste management, biosurfactant, and pulp/paper industries. Cellulase from *Bv* ASN1 is a candidate for depolymerizing recalcitrant lignocellulosic biomass from waste office paper.

There is currently a high demand for biosurfactants, for application as commercial bioemulsifiers due to their improved biodegradability as compared to their synthetic surfactant counterparts. A comprehensive analysis by Liu et al. (2010b), using rapid molecular PCR and chemical elucidation of various *Bv* H3 metabolomic intermediates, indicated that the investigated strain has excellent bioemulsifying properties. Due to the fact that primer detection efficiency for *Bv* PCR analysis is suboptimal at present, a species–subspecies in silico exploration of *Bv* and *B. subtilis* genomes was carried out by Cho et al. (2018) in order to identify and produce *Bacillus*-specific primers. The study selected various

**Table 2** Areas of potential use of *B. velezensis* in agriculture research

<i>B. velezensis</i> strain	Source	Potential application	Omics analytical resource	References
<i>Bv</i> LM2303	Dung of wild yak	Biocontrol agent (antiphytopathogenic fungi)	Molecular and metabolomics (UHPLC-ESI-MS)	(Chen 2017; Chen et al. 2018c)
<i>Bv</i> CC09	Leaf tissue of <i>Cinnamomum camphora</i>	Biocontrol agent and PGPB ( <i>Glomerella glycines</i> , <i>Rhizoctonia solani</i> , <i>B. graminis</i> f. sp. <i>tritici</i> and <i>Alternaria alternata</i> ); potential Biocontrol vaccine	Not employed	(Cai et al. 2017, 2016; Kang et al. 2018)
<i>Bv</i> Y6 and F7	Tomato soil Rhizosphere	Biocontrol agents ( <i>Ralstonia solanacearum</i> and <i>Fusarium oxysporum</i> )	Molecular, mutagenic and metabolomics (UPLC-MS)	(Cao et al. 2018)
<i>Bv</i> strain AB	Effluent treatment plant	Production of azoreductase and DR28 dye detoxification	Molecular and metabolomics (HPLC-MS)	(Bafana et al. 2008)
<i>Bv</i> 157	Bark of <i>Eucommia ulmoides</i>	Lignocellulosic biomass conversion	Genomics	(Chen et al. 2018b)
<i>Bv</i> Blends	Commercial	Biopesticide (anti- <i>Diabrotica virgifera virgifera</i> and anti- <i>Ostrinia nubilalis</i> ); PGPB (corn seed treatment)	Metabolomics (GC-MS)	(Disi et al., 2018a, b)
<i>Bv</i> V4	Marine (recirculation aquaculture systems)	Probiotic supplement (anti- <i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> ) and PGPB	Molecular/genomic and metabolomic (LC-MS/MS and NMR)	(Gao et al. 2017a)
<i>Bv</i> ZSY-1	Garden soil	Biocontrol agent (anti- <i>Alternaria solani</i> and anti- <i>Botrytis cinerea</i> )	Molecular and metabolomics (SPME-GC-MS)	(Gao et al. 2017b)
<i>Bv</i> GF610	Sea sediment sample	Biocontrol (anti- <i>Listeria innocua</i> and anti- <i>Listeria monocytogenes</i> )	Molecular/genomic and metabolomic (MALDI-TOF-MS and FTICR-MS)	(Gerst et al. 2017)
<i>Bv</i> 9912D	Rhizospheric of <i>Sporobolus airoides</i> (Torr.) Torr.;	Biocontrol agent (antiphytopathogenic fungi)	Genomic	(Pan et al. 2017)
<i>Bv</i> 2A-2B strain and 3A-25B	Branches of <i>Euconymus japonicus</i> and soybean soil rhizosphere. Laboratory strains	Biocontrol agents (antiphytopathogenic fungi) and PGPB (IAA and ISR inducers)	Genomic	(Martínez-Raudales et al. 2017)
<i>Bv</i> HYEB5-6 and other <i>B. velezensis</i>	Roots of Korean ginseng	Biocontrol agents for anthracnose (anti- <i>Colletotrichum gloeosporioides</i> ) and soybean root rot ( <i>Fusarium oxysporum</i> )	Molecular	(Huang et al. 2017a, b)
<i>Bv</i> 5YN8 and DSN012	Chicken feces	Biocontrol agent for <i>Botrytis cinerea</i>	Molecular	(Jiang et al. 2018)
<i>Bv</i> G341	Rhizoplane of Rice	Biocontrol agent (antiphytopathogenic fungi)	Molecular and metabolomics (GC-MS)	(Lim et al. 2017)
<i>Bv</i> CN026	Wheat anthers	Biocontrol agent (food borne pathogens; <i>Escherichia coli</i> , <i>Salmonella enterica</i> , <i>Campylobacter jejuni</i> , <i>Listeria</i> spp., and <i>Bacillus</i> spp.)	Genomic	(Nannan et al. 2018)
<i>Bv</i> CBMB205	Commercial compost	PGPB (phosphate-solubilizer)	Genomic	(Hwangbo et al. 2016)
<i>Bv</i> RC 218	Brackish water	Biocontrol agent (antiphytopathogenic fungi)	Molecular/genomic and metabolomic (LC-MS)	(Palazzini et al. 2016)
<i>Bv</i> QST713		Biocontrol agent ( <i>T. aggressivum f. europaeum</i> . Button mushroom)	Genomic	(Pandin et al. 2018)
<i>Bv</i> 40B		Biofloculant	Molecular and metabolomic (FT-IR)	(Zaki et al. 2013)

IAA indole acetic acid, *ISR* induce systemic resistance, *PGPB* plant growth promoting bacteria, *SPME-GC-MS* gas chromatography–mass spectrometry of solid phase microextraction, *LC-MS/MS* liquid-chromatography/mass spectrometry, *UHPLC-ESI* ultrahigh performance liquid chromatography–electrospray ionization, *MALDI-TOF-MS* matrix-assisted laser desorption ionization–time of flight mass spectrometry, *FTICR-MS* Fourier transform ion cyclotron resonance mass spectrometry, *NMR* nuclear magnetic resonance

*Bacillus*-specific genes, and the designed primers were for the purpose of improved detection efficiency during quantitative and real-time PCR procedures. The improved PCR assay was subsequently further applied towards a targeted detection of the *Bacillus* strains in a fermented food sample.

Microbial inoculants containing a number of different *Bv* strains have also been previously investigated for their capacity for reducing N<sub>2</sub>O and CO<sub>2</sub> emissions occurring due to various anthropogenic activities when manufacturing and using fertilizers, which is considered an environmental hazard (Calvo et al. 2013; Calvo et al. 2016). The data provided preliminary proof for the ex-situ application of the *Bv* bio-inoculants for these applications since the *Bv* strains tested show excellent nitrogen use capacities. Lastly, an inoculant containing *Bv* strain NRRL B-23189 was indicated to be an antagonist of *Penicillium roqueforti* sensu stricto (*s.s.*) (toxigenic mold) which can have a profound effect on improving processes related to corn silage (Wambacq et al. 2018). Although the initial inhibitory tests showed promise, the latter in vitro and in vivo results from the study were less encouraging.

## Applications in biomedicine

The increased prevalence of drug-resistant pathogens and subsequent shortage of possible drugs for eliminating these organisms and curing the associated diseases have led to an upsurge in research on possible new drug compounds, in particular, those from natural origins. In recent years, microbial produced exopolysaccharide (EPS) biopolymers have been shown to be of particular value for various applications relating to medical health, bio-nanotechnology, food and cosmetic industry gelling agents, and bio-flocculation (Moghannem et al. 2018). Moghannem et al. (2018) applied a Plackett–Burman statistical experimental design, in order to screen for microbes producing EPS and identify the most optimal culture and environmental conditions conducive for EPS production by these species, using a response surface methodology (RSM). The *Bv* KY498625 strain tested and produced comparatively the highest EPS, which was easily extracted and purified using diethylaminoethyl (DEAE) cellulose. Further identification of the EPS was carried out using gel permeation chromatography (GPC), Fourier transform infrared (FTIR), and gas chromatography-mass spectrometry (GC–MS). Since this microbial produced EPS is biodegradable, it is considered a considerably substantial breakthrough in terms of environmental sustainability.

Microbial produced compounds with possible anticancer/tumor properties (e.g., *B. subtilis* surfactins) are also now being investigated due to their high pharmacological potency and medicinal value for treating these conditions (Wu et al. 2017). An exopolysaccharide (EPS) compound, isolated from a marine *Bv* MHM3 strain designated as MHM3EPS, showed

an extremely high anticancer (MCF-7 cells) capacity at very low concentrations, with no apparent cytotoxic effects against health host cells (Mahgoub et al. 2018). In a similar study, Meena et al. (2018) indicated a lipopeptide antibiotic extract from a novel *Bv* strain (KLP2016), inhibited both *Aspergillus niger* and a *Mucor* sp., and additionally exhibited high (~90%) cytotoxicity against human cervical carcinoma cells Hep2-C. Furthermore, Rehman et al. (2018) recently reported the antiproliferative activity of five compounds extracted from *Bv* RA5401 against breast cancer cell lines, two of which function via inhibition of the intracellular cancer proteases and the remaining three via inhibition of the G protein-coupled receptors of the cancer cells (Meena et al. 2018).

A *Bv* V4 strain, isolated from an aquaculture system, with biocontrol activity against *Aeromonas salmonicida* subsp. *salmonicida*, the causative agent of furunculosis in fish (Gao et al. 2017a), was also investigated for possible probiotic activity on *Oncorhynchus mykiss* (rainbow trout). In the study, *Bv* V4 caused a significant reduction in the fish's mortality while also improving its growth. The authors used metabolic profiling (genomics and metabolomics (LC-MS/MS and NMR)) to identify the active metabolites produced by *Bv* V4, which were lipopeptides from the iturin, difficidin, and macrolactin group, with known mechanisms of action. *Bv* V4 has the potential for use as an aquaculture probiotic and as an antifurunculosis agent. With similar objectives, Van Doan et al. (2018) reported an improved immunity (via significantly elevated serum and skin mucus lysozyme and peroxidase activities, alternative complement activity, phagocytic activity, and respiratory burst activities) of Nile Tilapia when treated with a probiotic mixture of *Lactobacillus plantarum* N11 and *Bv* H3.1., as a feed additive. However, adequate information on the specific identity of the active metabolites (from the *Bv* strain) responsible for the immune boosts and mechanism by which the metabolite worked synergistically with other components of the probiotic is currently lacking.

And lastly, Yoo et al. (2018) isolated *Bv* K68 from traditional Korean-fermented foods and indicated its functionality and possible use in the prevention of dental caries, caused by *Streptococcus mutans* by inhibiting *S. mutans* biofilm formation, adhesion, and GTF gene expression, through deoxynojirimycin (DNJ) production. This study and all of the aforementioned literature substantiate the use of *Bv*, various *Bv* extracts and purified compounds from *Bv* for various medicinal/clinical applications in both humans and animals.

## Possible future use of metabolic information in combination with other omics derived data

Microbial genome-scale metabolic models (GEMs) have been previously developed but to date rarely used as systems' metabolic engineering strategies for strain design and



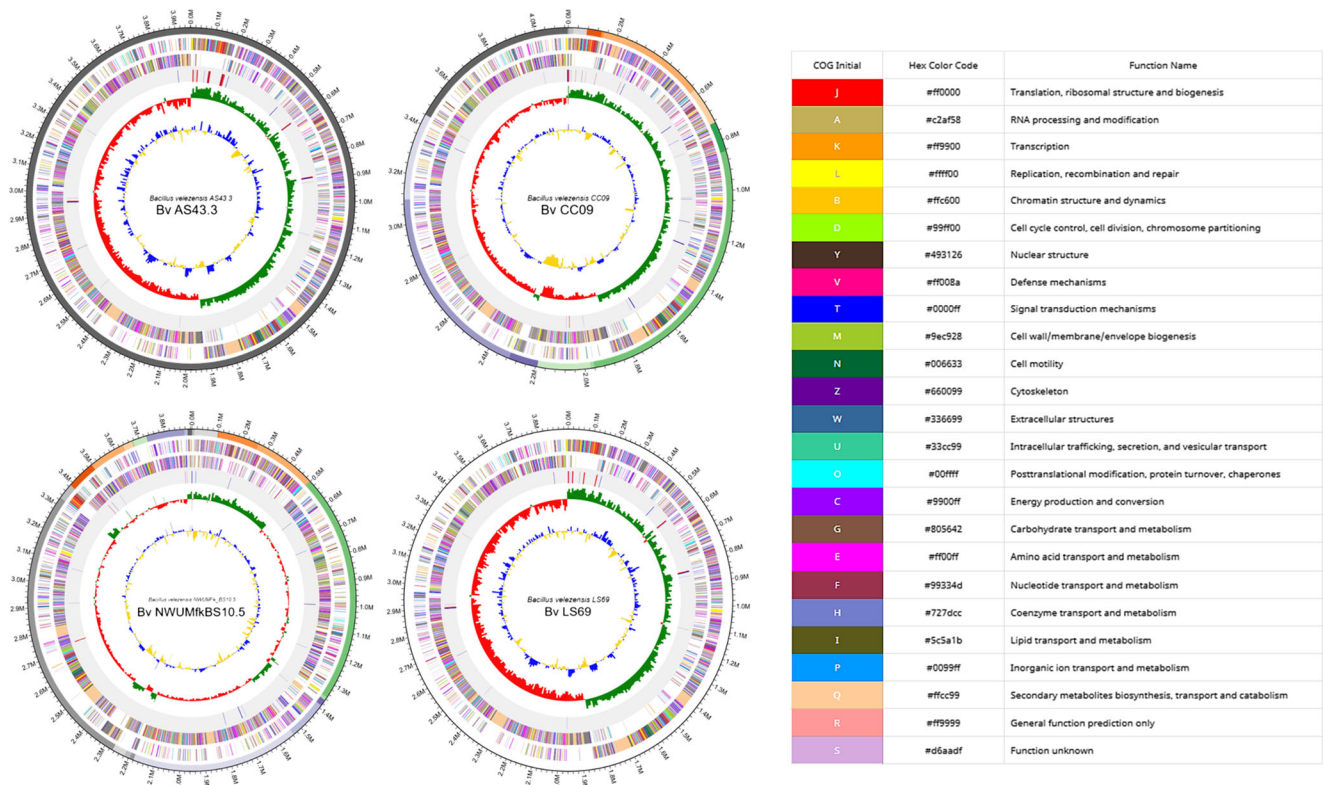
development (Covert et al. 2001; Lee et al. 2010; Oh et al. 2007). Applying microbial metabolic models or predictive pathway analyses could greatly assist researchers in predicting structural and functional properties of various microorganisms, including that of the *Bv* strains discussed in this review. Microbial metabolic models can additionally be used as a tool for generating hypotheses and engineering the metabolism of several organisms (Liu et al. 2010a; Patil et al. 2004). For example, a recombinant *B. subtilis* strain (BBG100), obtained from a wild strain ATCC 6633, was engineered to over secrete mycosubtilin (15-fold increase), via manipulation of its internal mycosubtilin operon (Leclère et al. 2005). A similar approach was used to generate various microbial strains for the production of novel bio-based compounds, medical antibiotics, plant/crop inoculants, and industrial chemicals via fermentation (Mienda 2017; Zachow et al. 2015).

The discovery of the non-ribosomal peptide synthetase (NRPS) siderophores (cupriachelin and taiwachelin) via in silico genome mining of the nitrogen-fixing bacteria *Cupriavidus necator* H16 (syn. *Ralstonia eutropha* H16) and *C. taiwanensis* LMG19424 by Kreutzer et al. (2012); Kreutzer and Nett (2012) also follow a genome-scale metabolic modeling. The predicted metabolic map (from the genome sequence data) by the same researchers subsequently assisted in the reconstruction of the putative pathways for the biosynthesis of the aforementioned lipopeptides. The reconstructed pathway was then used in the formulation of the

fermentation growth media for the synthesis of these aforementioned compounds, which were subsequently purified, extracted, and identified.

## Biosynthetic modeling of *B. velezensis* NWUMFkBS10.5 genome

Using the KEGG platform and the RAST server genome annotation of the *Fusarium*-biosuppressor *Bv* NWUMFkBS10.5 Adeniji et al. (2018), a genome-scale metabolic model of the *Bv* NWUMFkBS10.5 was mapped and or constructed. The final model includes 1558 chemical reactions, employing 1559 compounds, associated with 1000 genes. Twenty-eight possible significant compounds were predicted from the modeled metabolic pathway. In Fig. 6, the biosynthetic uniqueness of this genome is illustrated and compared to the genomic map of three other referenced *Bv* strains (using the default parameters of the web server BIOiPLUG Apps (<https://www.bioiplug.com/apps>)). One of the major novelties of the NWUMFkBS10.5 genome is the presence of the betalain and novobiocin metabolic pathways, responsible for the synthesis of interesting compounds including gomphrenin-1, lampranthin-2, celosianin-2, 2-decarboxybetanidin, betalamic acid, miraxanthin V, albamycin, and maltol. These compounds have been previously documented to be useful antioxidants, food



**Fig. 6** Uniqueness of the NWUMFkBS10.5 genome compared with three other highly referenced *Bv* genomes

**Table 3** Biosynthetic pathways and possible beneficial compounds identified from the metabolic modeling of the *B. velezensis* NWUMFkBS10.5 genome

Biosynthetic pathway	Biomolecule	Beneficial significance
Tetracycline biosynthesis	Oxytetracycline and tetracycline	Biomedical antibiotic
Brassinosteroid biosynthesis	Brassinolide	Phytohormone
Puromycin biosynthesis	O-beta-D puromycin	Antibiotic
Zeatin biosynthesis	Xylosylzeatin, zeaxanthin, and lupinate	Phytohormones
Anthocyanin biosynthesis	Cyanidin, malvidin, delphinidin, malonylshisonin, and pelargonidin	Plant pigments
Benzoxazinoid biosynthesis	DIMBOA	Plant antibiotic
Vancomycin biosynthesis	Chloroeremomycin and vancomycin	Antibiotics
Ansamycins biosynthesis	Rifamycin and protorifamycin	Antibiotics
Chondroitin biosynthesis	Chondroitin	Medical supplement
Monoterpenoid biosynthesis	Asperuloside and myrcene	Plant defense compounds and anti- <i>Helicobacter pylori</i>
Betalain biosynthesis	Gomphrenin-1, lampranthin-2, celosianin-2,2-decarboxybetanidin, betalamic acid, and miraxanthin V	Novel antioxidants and food additives
Novobiocin biosynthesis	Albamycin and maltol	Antibiotic, antioxidant, and flavor enhancer

additives, and flavor enhancers. A summary of the possible metabolic pathways for the synthesis of some of these beneficial compounds is given in Table 3.

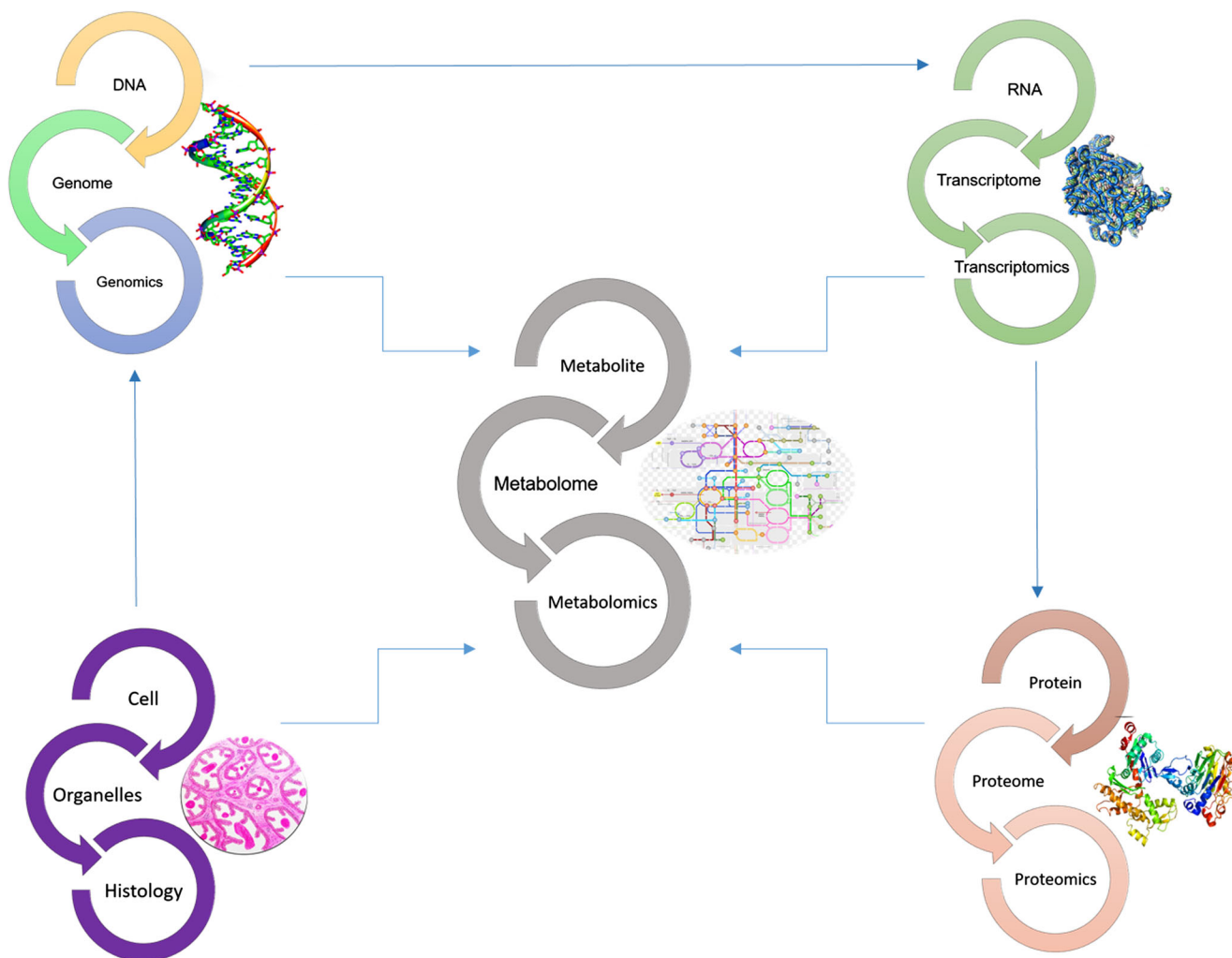
### Exploiting the *Bacillus velezensis* metabolome using practicable metabolomic insights

Interpretation of microbial intracellular information derived from all the other omics data, in conjunction with metabolomics data, would potentially revolutionize the quest for identifying and synthesizing various novel and useful natural microbial compounds (Adeniji and Babalola 2018), thereby further enhancing natural products discovery (Fig. 7). The benefits of using the multi-omics platform approach with deliberate convergence to metabolomics, for microbial bioengineering applications, are clear. Various studies (as discussed in this review) have attempted to use a multi-dimensional omics approach to determine the role of several candidate biocontrol compounds synthesized by various *Bv* strains (Chen et al. 2018c). Other practicable insights are further described below. For instance, during a host–microbe interaction (within a clinical, industrial, environmental, and agricultural setting), various metabolic processes may be disrupted within a treated pathogen or living host, after the application of a beneficial microbe, or its purified beneficial compounds. Metabolomics can provide valuable information on the bio-transformation that typically occurs in the metabolome of these beneficial microbes when they are utilized directly as a treatment alternative (Xu et al. 2014).

Furthermore, metabolomics can be used to monitor systemic acquired resistance/induced systemic resistance in plants

induced by exogenous microbial treatment. Its application during microbial industrial fermentation studies may additionally elucidate changes to the metabolic flux, enzyme functions, and gene function, which in turn can be applied to optimizing fermentation protocols (Gao and Xu 2015). Zhao et al. (2016) investigated the inhibitory effect of a phage protein (gp70.1) on *Pseudomonas aeruginosa* using transcriptomics and NMR-based metabolomics. The phage protein caused a significant reduction in the rate of amino acid consumption by *P. aeruginosa*. The up-production of alanine and pyroglutamate and downregulation of ornithine which was revealed by the NMR spectroscopy confirmed the phenotypic disruptions observed in the extracellular components of the organism.

Lastly, Wu et al. (2015a) elucidated the mechanism by which the biocontrol bacteria *Penicillium citrinum* W1 could improve its production of the antifungal protein, PcPAF. Using a GC-MS metabolomics approach, they determined an upregulation in the fatty acid synthesis and TCA cycle intermediates when cultured in media supplemented with glycine, serine, and threonine, correlating to the phenotypic expression of increased bacterial growth and improved antagonism (Wu et al. 2015a). Similarly, Koen et al. (2018a) and Koen et al. (2018b) used a GCXGC-TOFMS metabolomics approach to determine the intracellular response of the organism *Mycobacterium tuberculosis* (*Mtb*) to the antimicrobials colistin sulfate and colistin methanesulfonate respectively, in order to determine possible mechanisms of action of the antimicrobial. Increased uptake of various metabolic precursors (e.g., glucose) and a subsequent elevation of other underutilized precursors within the cell, all of which were related to fatty acid synthesis and cell wall repair, were



**Fig. 7** A multi-dimensional omics approach for enhancing natural compound discovery. The sequential events will focus on the metabolomic interpretation of molecular and or cellular data stemming from microscopic, genomic, transcriptomic, and proteomic evaluations

reported by the authors. The metabolomics study corroborated previously proposed genomics and histological generated hypotheses, regarding colistin's mechanism of action against *Mtb*. Negative alterations in the metabolome of beneficial microbes (like *Bv*) could be monitored and controlled borrowing from the perspectives above.

## Final remarks and conclusion

It is becoming paramount that the biosynthetic capacity of microbes identified through other omics strategies be validated via metabolomics, and the presupposed or predicted significant microbial biomolecule (or the products of microbial fermentation) is subsequently detected or synthesized in vitro (Palazzotto and Weber 2018). Apparently at inception, research into microbial metabolomics was limited by the capacity of analytical techniques available at the time; however, the recent advancement of the platform (metabolomics) has

resulted in an exponential increase in new and exciting discoveries (Xu et al. 2014). We anticipate an exponential increase in the use of systems biology research approaches towards the discovery of new compounds from microbial sources over the course of the next 10 years. A number of challenges however still exist, and these include the difficulty in extracting or purifying novel compounds from fermentation broths produced by these beneficial microbes (e.g., *Bv*). The latter limitation is largely attributed to suboptimized growth conditions, improper selection of extraction solvents, and a lack of availability for novel compounds commercially for absolute identification of novel compounds, especially those synthesized in lower concentrations (Machado et al. 2017). The reported non-selectiveness of many microbial-derived beneficial agents and their apparent inability to competently function in vivo as detected in vitro, in comparison to synthetic antimicrobials, is a challenge. A further question is whether the location or sources of isolation of these beneficial bacteria (e.g., *Bv*) influence the diversity of their applications.



This study expounds on the phylogeny of *Bv* (using current omics tools) to actually corroborate previous claims on the close knitting of the species, while comprehensively revealing the omics techniques employed in the past investigations relating to *Bv*. Using metabolic modeling, we identified possible novel biomolecules from our *Bv* strain (NWUMfkBS10.5). We have also shared interesting perspectives on how the *Bv* metabolome could be further exploited, using current metabolomic trends capable of detecting biomarkers pertinent to novel biosynthetic pathways. Despite the inexhaustible diversity of microbial metabolites and the beneficial compounds produced by beneficial bacteria, there are only a few databases which currently exist, documenting these compounds and their biotechnological applications. Metabolomics would serve a useful approach to generating such info quickly, which deserves attention considering the need for sustainable production of novel biotechnological products.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies involving human participants or animals.

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