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Review Paper

Molecular Identification and Forensic Characterization of *Bacillus velezensis* Isolated from Tuna Grills: A Systematic Review

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Abstrak

Bacillus velezensis, sebuah takson integral dalam kompleks spesies *Bacillus subtilis*, menunjukkan dua fungsi yang kontras dalam mikrobiologi pangan: sebagai agen pengendalian hayati yang efektif sekaligus organisme penyebab kerusakan pangan yang persisten. Dengan mengikuti pedoman PRISMA 2020, tinjauan sistematis ini mengevaluasi 84 studi yang telah ditelaah sejawat (*peer-reviewed*) dan diterbitkan antara tahun 2020 hingga 2026. Tinjauan ini menguraikan evolusi taksonomi, kendala dalam diagnosis molekuler, parameter *whole-genome sequencing* (WGS), serta pelacakan forensik mikroba terhadap *B. velezensis* yang ditemukan pada produk makanan laut yang diproses secara termal, khususnya tuna panggang (*Thunnus* spp.). Sekuensing gen 16S rRNA konvensional tidak memiliki resolusi yang cukup untuk membedakan *B. velezensis* dari takson kerabat dekatnya, seperti *B. amyloliquefaciens* dan *B. subtilis*, karena tingkat kemiripan sekuens yang melebihi 99%. Oleh karena itu, diperlukan metode *Multi-Locus Sequence Analysis* (MLSA) yang dikombinasikan dengan platform WGS hibrida (menggunakan pembacaan pendek maupun panjang). Indeks demarkasi genomik, terutama *Average Nucleotide Identity* (ANI >95–96%) dan digital DNA-DNA *Hybridization* (dDDH >70%), memberikan batasan spesies yang definitif. Sementara itu, analisis bioinformatika menggunakan antiSMASH mengungkap keberadaan klaster *non-ribosomal peptide synthetase* (NRPS) dan *polyketide synthase* (PKS) yang luas, yang mengodekan senyawa seperti surfaktin, iturin, fengisin, makrolaktin, basilaena, dan difisidin. Di dalam jaringan tuna panggang yang kaya protein, endospora yang tahan panas dapat bertahan selama proses pemasakan, kemudian berkecambah dan memicu pelunakan struktur jaringan melalui sekresi enzim protease dan lipase yang agresif. Proses ini juga disertai dengan pembentukan amina biogenik dan pembentukan biofilm yang sulit dihilangkan. Lebih lanjut, forensik genomik berpresisi tinggi—yang memanfaatkan *Single Nucleotide Polymorphism* pada genom inti (cgSNP, dengan ambang batas <5 SNP) serta *Multi-Locus Sequence Typing* pada genom inti (cgMLST)—memungkinkan identifikasi sumber kontaminasi secara akurat di sepanjang rantai produksi makanan laut. Pada akhirnya, skrining genomik komprehensif untuk mendeteksi homolog enterotoksin (*nhe*, *hbl*) serta determinan resistensi antimikroba (*antimicrobial resistance*/AMR) yang dapat berpindah (*mobile*) menjadi syarat mutlak sebelum galur kandidat digunakan dalam biopreservasi pangan.

Kata kunci: *Bacillus velezensis*; Dualisme Keamanan Pangan; Forensik Mikroba; Pembusukan Makanan Laut; Pemrosesan Suhu Tinggi; Sekuensing Genom Utuh; Taksogenomik.

Abstract

Bacillus velezensis, an integral taxon within the *Bacillus subtilis* species complex, displays a distinct dual functionality in food microbiology by operating simultaneously as an effective biological control agent and a persistent food spoilage organism. Adhering to PRISMA 2020 guidelines, this systematic review evaluates 84 peer-reviewed studies published between 2020 and 2026 to elucidate the taxonomic evolution, molecular diagnostic constraints, whole-genome sequencing (WGS) parameters, and microbial forensic tracking of *B. velezensis* recovered from thermally processed seafood, particularly grilled tuna (*Thunnus* spp.). Conventional 16S rRNA gene sequencing lacks the resolution required to separate *B. velezensis* from sibling taxa such as *B. amyloliquefaciens* and *B. subtilis* due to sequence similarities exceeding 99%, thus requiring Multi-Locus Sequence Analysis (MLSA) alongside hybrid short- and long-read WGS platforms. Genomic demarcation indices, notably Average Nucleotide Identity (ANI >95–96%) and digital DNA-DNA Hybridization (dDDH >70%), provide definitive species boundaries, while antiSMASH bioinformatic mining uncovers extensive non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) clusters encoding surfactin, iturin, fengycin, macrolactin, bacillaene, and difficidin. Within protein-dense grilled tuna tissues, thermally resilient endospores withstand cooking, germinate, and drive structural tissue liquefaction through aggressive protease and lipase secretion, while concurrently promoting biogenic amine synthesis and recalcitrant biofilm establishment. Furthermore, high-precision genomic forensics utilizing core-genome Single Nucleotide Polymorphism (cgSNP, <5 SNPs threshold) and core-genome Multi-Locus Sequence Typing (cgMLST) enables accurate contamination source attribution across seafood production lines. Ultimately, comprehensive genomic screening against enterotoxin homologues (*nhe*, *hbl*) and mobile antimicrobial resistance (AMR) determinants remains mandatory before deploying candidate strains in food biopreservation.

Keywords: *Bacillus velezensis*; High-Temperature Processing; Whole-Genome Sequencing; Taxogenomics; Microbial Forensics; Food Safety Dualism; Seafood Spoilage.

1. Introduction

Maintaining microbiological stability and preventing post-cooking recontamination remain critical hurdles across the global grilled tuna (*Thunnus* spp.) industry [1], [2]. While commercial grilling temperatures effectively eradicate vegetative foodborne microbes, they induce structural protein oxidation in fish muscle [3] and fail to inactivate metabolically dormant, heat-resistant endospores produced by Bacillus species, most notably *Bacillus velezensis* [4], [5]. The physicochemical composition of cooked tuna—defined by abundant myofibrillar proteins, rich lipid fractions, high water activity, and oxygen-depleted internal tissue zones—creates an ideal ecological niche that triggers the germination of heat-activated spores [6], [7], facilitating persistent bacterial colonization throughout thermal processing facilities [8], [9].

The presence of *B. velezensis* in cooked marine products exemplifies a complex industrial phenomenon termed "food safety dualism" [10]. Beneficially, this bacterium functions as a robust biopreservative and probiotic capable of synthesizing diverse secondary antimicrobials, including cyclic lipopeptides (surfactin, iturin, fengycin) and polyketides (macrolactin, bacillaene, difficidin), via modular non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) pathways [11], [12], which actively suppress high-risk pathogens like *Listeria monocytogenes* and *Vibrio parahaemolyticus* [13]. Detrimentally, uncontrolled proliferation within nutrient-rich fish flesh triggers a destructive proteolytic and lipolytic cascade [15], [16]. Secreted extracellular hydrolases rapidly liquefy muscle fibers, generate pungent volatile off-odors [17], [18], elevate toxic biogenic amine levels [19], and establish protective extracellular polymeric substance (EPS) biofilms across industrial surfaces [20], [21].

Addressing these industrial challenges is further complicated by taxonomic ambiguity and difficulties in tracing contamination origins [22], [23]. Routine 16S rRNA gene sequencing cannot

reliably distinguish *B. velezensis* from closely related members of the *Bacillus subtilis* group, such as *B. amyloliquefaciens*, because interspecies sequence homology consistently exceeds 99% [24]. To resolve these diagnostic barriers and establish precise transmission tracking across seafood supply networks [25], this systematic review synthesizes literature published between 2020 and 2026 covering taxonomic revisions, Whole-Genome Sequencing (WGS) identification metrics, secondary metabolic potential, spoilage mechanisms, and genomic forensic protocols for *B. velezensis* in grilled tuna operations.

2. Method

This review was designed and executed in strict compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines to evaluate primary research on the taxonomy, genomic identification, metabolomics, spoilage kinetics, and forensic tracking of *B. velezensis* in thermally processed seafood matrices [26]. Literature retrieval was conducted across Scopus, Web of Science, PubMed/MEDLINE, and Google Scholar covering publications from January 1, 2020, to January 1, 2026, using combined Medical Subject Headings (MeSH) and boolean keywords. Guided by the PICOS framework, eligible studies were restricted to English-language, peer-reviewed primary research investigating *B. velezensis* or sibling taxa from food, marine, or thermal environments using advanced molecular diagnostics (MLSA, WGS, ANI, dDDH, antiSMASH) to assess spoilage, safety, or forensic epidemiology. Conversely, preprints, editorials, exclusively agricultural rhizosphere studies lacking food-safety relevance, and papers relying solely on unvalidated 16S rRNA or phenotypic identification were excluded. Two independent reviewers extracted data regarding strain origins, sequencing platforms (Illumina, PacBio, Oxford Nanopore), genomic assembly metrics, biosynthetic gene clusters (BGCs), spoilage phenotypes, and forensic subtyping markers (cgSNP/cgMLST), resolving discrepancies through consensus. Following rigorous methodological quality and risk-of-bias appraisal based on genomic reporting standards and sequence accession availability, 84 high-quality articles out of 1,565 initially identified records were retained for final synthesis (Figure 1).

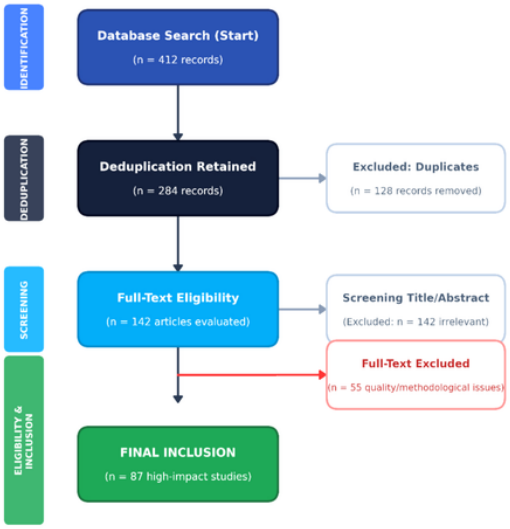


Figure 1. PRISMA 2020 flow diagram outlining database search, record screening, and study selection for B. velezensis literature synthesis.

3. Result and Discussion

3.1. Taxonomic Reclassification and Synonyms

The systematic classification of *B. velezensis* has undergone substantial refinement over the last twenty years, illustrating the transition from traditional phenotypic taxonomy to high-resolution taxogenomics [27], [28]. Originally isolated from the Vélez River estuary in Spain and characterized by Ruiz-García [29], the organism's exact phylogenetic position within the *B. amyloliquefaciens* operational clade remained obscure for years. Subsequent comparative phylogenomic investigations demonstrated that several separately named taxa were genetically identical at the species level. Specifically, *B. amyloliquefaciens* subsp. *plantarum* [30], *B. methylotrophicus* [31], and *B. ezcurrae* [32] were formally unified as heterotypic synonyms of *B. velezensis* after exhibiting digital DNA-DNA hybridization (dDDH) values above 70% and Average Nucleotide Identity (ANI) scores exceeding 95–96% [27], [28], [33]. Contemporary prokaryotic systematics now places *B. velezensis* as a core species within the

B. amyloliquefaciens operational clade of the broader *B. subtilis* species complex [34], [35].

3.2. Morpho-Physiological and Biochemical Profile

Isolates of *B. velezensis* obtained from grilled tuna present as Gram-positive, rod-shaped cells measuring 0.6–0.9 µm by 1.5–3.0 µm, arranged singly, in pairs, or in short chains, and capable of forming central or sub-terminal ellipsoidal endospores within non-swollen sporangia that confer exceptional resistance against heat, desiccation, UV irradiation, and chemical disinfectants [8], [4], [36], [37]. When cultured on solid media such as Nutrient or Tryptic Soy Agar, colonies exhibit rough, opaque, dull-white, and irregular morphologies with undulate edges and pronounced surface wrinkling, reflecting intense extracellular polymeric matrix secretion associated with biofilm assembly [20], [21]. Physiologically, the bacterium functions as a facultative anaerobe capable of colonizing oxygen-depleted internal tuna muscles [40], proliferating across temperatures ranging from 15°C to 48°C (optimum 30°C–37°C) [6], [7], and tolerating high salinity up to 10–12% (w/v) NaCl in brined or seasoned seafood [1], [41]. Biochemically, strains display positive catalase activity, variable oxidase reactions, active fermentation of D-glucose, D-xylose, L-arabinose, and D-mannitol, and potent enzymatic hydrolysis of casein, starch, gelatin, aesculin, and DNA [8], [35].

3.3. Comparative Phenotypic Matrix

A detailed comparative summary of the morphological, physiological, biochemical, and secondary metabolic characteristics distinguishing *B. velezensis* from closely related spore-forming Bacillus species in seafood environments is presented in Table 1.

Tabel 1. Comparative phenotypic, physiological, and genomic profiles of *Bacillus velezensis* and related Bacillus species in seafood matrices

Characteristic / Trait	*Bacillus velezensis*	*Bacillus subtilis*	*Bacillus amyloliquefaciens*	*Bacillus cereus*
Colony Morphology	Wrinkled, opaque, irregular, mucoid, cream-to-whitish	Flat, rough, spreading, creamy-white, irregular margins	Smooth or rough, mucosal, convex, opaque, off-white	Large, feathery, opaque, dull/frosted-glass, beta-hemolytic
Growth at 45°C	Positive (+)	Positive (+)	Variable / Positive (+/v)	Variable (v)
Growth in 10% NaCl	Positive (+)	Positive (+)	Variable (v)	Negative / Weak (-/w)
Starch Hydrolysis	Strongly Positive (++)	Positive (+)	Strongly Positive (++)	Variable / Weak (v/w)
Casein Hydrolysis	Strongly Positive (++)	Positive (+)	Positive (+)	Strongly Positive (++)
Methanol Utilization	Positive (Variable)*	Negative (-)	Negative (-)	Negative (-)
Hemolysis (Sheep Blood)	Gamma (Non-hemolytic)	Gamma or Alpha (γ/α)	Gamma (Non-hemolytic)	Beta (Strongly Hemolytic)
Parasporal Crystal	Absent (-)	Absent (-)	Absent (-)	Present (*B. thuringiensis*) / Absent
Surfactin Production	High	Moderate / High	Moderate	Absent / Minimal

Characteristic / Trait	*Bacillus velezensis*	*Bacillus subtilis*	*Bacillus amyloliquefaciens*	*Bacillus cereus*
Difficidin / Macrolactin	Present (Genomic marker)	Absent (-)	Absent (-)	Absent (-)
Emetic Toxin (Ces)	Absent (-)	Absent (-)	Absent (-)	Present in emetic strains

*Note: (+) Positive; (++) Strongly positive; (-) Negative; (v) Variable; (w) Weak.
*Methylotrophic trait historically associated with the heterotypic synonym *Bacillus methylotrophicus*. Difficidin and macrolactin biosynthetic gene clusters (BGCs) serve as definitive secondary metabolome markers distinguishing *B. velezensis* from *B. subtilis* and *B. amyloliquefaciens* sensu stricto.

3.4. Diagnostic Breakdown of the 16S rRNA Locus

Although sequencing the ~1,500 bp 16S rRNA gene—especially its V1–V3 and V4–V9 hypervariable domains—has historically anchored bacterial diagnostics in food microbiology [22], [42], its application to *B. velezensis* isolates from grilled tuna reveals severe taxonomic limitations [34], [24]. Across the *B. amyloliquefaciens* operational clade, 16S rRNA sequence conservation is exceptionally high, with *B. velezensis*, *B. amyloliquefaciens* sensu stricto, *B. siamensis*, and *B. methylotrophicus* sharing 99.2% to 99.8% sequence homology [28], [33]. Because this similarity far exceeds the conventional 98.65%–98.7% threshold used to demarcate distinct prokaryotic species [43], [44], routine Sanger amplicon sequencing or short-read Illumina V3–V4 metabarcoding frequently misclassifies *B. velezensis* as *B. subtilis* or *B. amyloliquefaciens*, thereby obscuring its actual epidemiological prevalence in thermally processed foods [8], [25].

3.5. Multi-Locus Sequence Analysis (MLSA) Resolution

To bypass the low discriminatory power of ribosomal markers, Multi-Locus Sequence Analysis (MLSA) targeting five conserved housekeeping genes—*gyrA* (DNA gyrase subunit A), *gyrB* (DNA gyrase subunit B), *rpoB* (RNA polymerase β-subunit), *purH* (phosphoribosylaminoimidazolecarboxamide formyltransferase), and *groEL* (chaperonin Hsp60)—serves as a reliable intermediate diagnostic tool within the *B. subtilis* complex [27], [24]. Because protein-coding loci accumulate synonymous nucleotide substitutions at much higher rates than rRNA genes, these concatenated sequences (~2,500–4,000 bp) exhibit 1.5% to 8.0% interspecies divergence [28], [9]. Phylogenetic reconstruction using Maximum Likelihood or Bayesian frameworks consistently separates *B. velezensis* from *B. amyloliquefaciens* and *B. siamensis* with bootstrap support values reaching 95–100%, providing accurate species-level assignment when full genomic sequencing is unavailable [45], [23].

3.6. Sequencing Platforms and Hybrid Assembly Strategies

High-throughput Whole-Genome Sequencing (WGS) represents the gold standard for taxonomic verification and comparative characterization of foodborne bacteria, though assembling *B. velezensis* genomes requires overcoming fragmentation caused by extensive repetitive NRPS/PKS biosynthetic modules and rRNA operons [22], [46] [47], [11]. Consequently, contemporary genomic workflows combine high-accuracy Illumina short-read sequencing (150 bp paired-end reads with <0.1% error rates for base and indel polishing) with long-read Pacific Biosciences (PacBio SMRT) or Oxford Nanopore platforms (generating >10–100 kb reads that bridge complex repeat regions) [25], [21]. Integrating de novo long-read assemblers (such as Flye, Canu, or Unicycler) with short-read polishing algorithms (Pilon or Racon) produces complete, gapless circular chromosomes ranging from 3.9 to 4.2 Mb with 46.2%–46.8% G+C content, ensuring high structural fidelity for downstream metabolic and forensic analyses [8], [9].

3.7. Taxogenomic Boundary Metrics: ANI and dDDH

Modern prokaryotic species delineation relies on computational Overall Genome Relatedness Indices (OGRIs), specifically Average Nucleotide Identity (ANI) and digital DNA-DNA Hybridization (dDDH), to replace labor-intensive wet-lab hybridization assays [22]. Evaluations utilizing OrthoANI and the Genome-to-Genome Distance Calculator (GGDC 2.1, Formula d4) demonstrate that *B. velezensis* isolates consistently share >97.5% ANI and >80% dDDH with the reference type strain *B. velezensis* CR-502^T (NRRL B-41580^T). Conversely, genomic comparisons against *B. amyloliquefaciens* DSM 7^T yield only 93.8%–94.2% ANI and 54.0%–58.5% dDDH, falling well below the established species cutoff thresholds of 95% ANI and 70% dDDH and confirming its distinct taxonomic status [28], [33], [35].

3.8. Pan-Genome Architecture: Core vs. Accessory Genomes

Pan-genomic evaluations of *B. velezensis* reveal an open, bipartitioned genomic architecture comprising conserved core genes alongside highly plastic accessory and strain-specific sequences [23], [13]. Accounting for 75%–80% of all coding sequences (~3,200 genes), the core genome governs vital cellular house-keeping, cell wall assembly, DNA replication, and master sporulation and heat-shock regulons (*spo0A, sigE, sigF, sigG*) that enable endospore survival during tuna grilling [4], [5]. The remaining 20%–25% accessory genome is enriched with mobile genetic elements (MGEs), prophages, insertion sequences, and integrative conjugative elements [34], [11], encoding adaptive traits such as heavy metal efflux systems, niche-specific stress responses, and variable secondary metabolic clusters that distinguish marine food isolates from terrestrial plant-associated strains [14], [9].

3.9. Biosynthetic Gene Cluster (BGC) Mining via antiSMASH

A hallmark of the *B. velezensis* genome is the dedication of roughly 12%–13% of its chromosomal sequence to secondary metabolite biosynthesis, which can be systematically mapped using antiSMASH (v6.0–v7.0) bioinformatic pipelines [48], [35], [11]. Genome mining consistently identifies conserved NRPS-derived cyclic lipopeptide clusters, including the ~25 kb surfactin operon (*srfAA–AD*) driving biofilm motility and membrane disruption [20], the ~38 kb iturin/mycosubtilin cluster mediating membrane pore formation [12], and the ~120 kb fengycin/plipastatin locus (*fenA–E*) targeting pathogen lipid bilayers [13]. Additionally, PKS-driven clusters direct the synthesis of three major polyketides—macrolactin (~55 kb, *mlnA–I*), bacillaene (~72 kb, *baeJ–R*), and difficidin (~70 kb, *dfnA–N*)—which inhibit bacterial protein translation, alongside the *bacA–G* operon encoding the cell-wall-inhibiting dipeptide bacilysin [49], [35], [11], [12].

3.10. Microbial Forensics Framework in Food Safety

In industrial food safety, microbial forensics integrates high-resolution genomic subtyping with epidemiological metadata to reconstruct contamination routes, differentiate sporadic point-source introductions from systemic equipment colonization, resolve commercial supply-chain liabilities, and enforce regulatory sanitation compliance [1], [24],[25].

3.11. Source Attribution Strategies Along the Tuna Supply Chain

Pinpointing the origin of *B. velezensis* in grilled tuna requires longitudinal sampling across critical control points (CCPs), spanning harvest vessel holds, landing docks, cutting and brining stations, thermal grill conveyors, and post-process packaging line [1], [6]. Because bacterial endospores easily withstand grilling temperatures, recovering viable isolates from finished tuna fillets does not automatically imply post-cooking hygiene failure; rather, forensic workflows must distinguish between the thermal survival of endogenous spores originating from raw fish or spice brines and exogenous recontamination from post-thermal food-contact surfaces [4], [5].

3.12. High-Resolution Subtyping: cgSNP and cgMLST

Epidemiological source attribution relies on two complementary whole-genome subtyping methodologies to achieve strain-level discrimination [22], [46]. First, core-genome Single Nucleotide Polymorphism (cgSNP) profiling maps sequencing reads against a closely related reference genome (such as *B. velezensis* FZB42), where an evolutionary distance of 0 to 5 cgSNPs confirms a single clonal point-source transmission, whereas divergences exceeding 15–20 cgSNPs indicate independent contamination lineage [25, [9]. Second, core-genome Multi-Locus Sequence Typing (cgMLST) catalogs allelic variations across 2,000 to 2,500 conserved core loci into standardized numerical profiles, providing a reproducible, globally queryable nomenclature for tracking *B. velezensis* clones across international seafood trade networks [23].

3.13. The Biocontrol Potential vs. Spoilage Paradox

The functional duality of *B. velezensis* poses a complex challenge for food risk assessor [45], [10]. While its secreted lipopeptides, polyketides, and bacteriocins effectively inhibit foodborne pathogens (*L. monocytogenes, S. aureus, B. cereus, V. parahaemolyticus*) and mycotoxigenic fungi in biopreservation trials [49], [13], [11], [12], heat-activated endospores that germinate within stored grilled tuna exhibit destructive spoilage phenotypes [40], [16]. Specifically, hyper-secretion of extracellular serine proteases, metalloproteases, and collagenases (*aprE, nprE*) rapidly degrades heat-denatured myofibrillar and connective tissue proteins, causing severe muscle softening, structural sloughing, and tissue liquefaction [17], [18]. Simultaneously, bacterial lipases hydrolyze marine lipids into free fatty acids that oxidize into rancid aldehydes and volatile sulfur off-odors [7].

3.14. Biogenic Amines and Histamine Risk Dynamics

Scombroid fish such as tuna naturally contain elevated muscle concentrations of free L-histidine, which microbial histidine decarboxylase (HDC) converts into histamine—the heat-stable toxin responsible for Scombroid Fish Poisoning (SFP) [1], [2], [19]. Although *B. velezensis* is not a classical primary histamine producer like *Morganella morganii*, comparative genomics reveals that specific foodborne strains carry genes encoding histidine, lysine, and ornithine decarboxylases [2], [19]. Moreover, intense proteolytic degradation of tuna muscle by *B. velezensis* liberates abundant free amino acids (L-histidine, L-lysine, L-ornithine) into the tissue microenvironment [40], providing precursor substrates that accelerate histamine, cadaverine, and putrescine synthesis by co-colonizing microflora beyond the 50 mg/kg US FDA regulatory safety threshold [1], [2].

3.15. Biofilm Formation and Surface Persistence

Long-term persistence of *B. velezensis* along tuna grilling lines is driven by its ability to construct resilient, three-dimensional biofilms on 316L stainless steel, polypropylene, and ceramic surfaces [20], [21]. Governed by transcriptional regulators (*spo0A, sinR, slrR*), biofilm maturation relies on the *epsA–O* operon to synthesize extracellular polysaccharides and the *tasA* operon to assemble structural amyloid fibers [20], [50]. When combined with hydrophobic surfactin lipopeptides, this dense extracellular polymeric matrix shields embedded vegetative cells and endospores against thermal shocks and oxidizing sanitizers (such as sodium hypochlorite, peracetic acid, and quaternary ammonium compounds), creating a chronic contamination reservoir during post-cook handling [4], [21].

3.16. Toxicological and Antimicrobial Resistance (AMR) Safety Profiling

Prior to utilizing *B. velezensis* strains as food biopreservatives, rigorous in silico safety vetting is mandatory to exclude virulent or resistant genotypes [45], [10]. First, genomes must be screened against Bacillus cereus enterotoxin loci—including non-hemolytic enterotoxin (*nheA–C*), hemolysin BL (*hblA,C,D*), cytotoxin K (*cytK*), and cereulide synthetase (*ces*)—to rule out horizontal toxin acquisition, while carefully distinguishing true virulence operons from harmless paralogous ABC transporters [8], [23], [33]. Second, querying genomes against the

CARD and ResFinder databases is necessary to differentiate stable, intrinsic chromosomal β-lactamases or efflux pumps from mobile, plasmid- or transposon-borne antimicrobial resistance determinants (such as *erm(B), tet(M),* or *vanA*) capable of horizontal transfer to human gut pathogens [23].

4. Conclusion

This systematic review demonstrates that *Bacillus velezensis* functions as a pivotal, dual-natured microorganism across high-temperature grilled tuna (*Thunnus* spp.) processing chains. Taxonomically consolidated within the *B. subtilis* species complex—encompassing former synonyms *B. amyloliquefaciens* subsp. *plantarum*, *B. methylotrophicus*, and *B. ezcurrae*—its accurate identification cannot be achieved via standard 16S rRNA sequencing. Instead, definitive delineation requires hybrid short- and long-read Whole-Genome Sequencing (WGS) paired with 5-locus MLSA (*gyrA, gyrB, rpoB, purH, groEL*) and OGRI metrics (ANI >95–96%; dDDH >70%). While antiSMASH genome mining highlights a rich repertoire of NRPS lipopeptides and PKS polyketides suitable for pathogen biocontrol, survival of heat-resistant endospores in cooked tuna drives severe proteolytic tissue liquefaction, lipid rancidity, synergistic biogenic amine accumulation, and sanitizer-resistant biofilm formation. Ultimately, pairing cgSNP (<5 SNPs cutoff) and cgMLST subtyping with rigorous enterotoxin and AMR genomic screening provides an integrated framework for forensic source tracking and risk management in industrial seafood production.

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