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LINK MEDICAL INTERFACE

Microbial Nanofactories in Cancer Nanomedicine: Harnessing Bacillus subtilis for Green Synthesis of Silver Nanoparticles in Cancer Therapy

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ABSTRACT

The global pursuit of sustainable nanomedicine has accelerated interest in microbial systems as eco-friendly nanofactories capable of producing biologically compatible metal nanoparticles. This review critically explores the role of Bacillus subtilis in the green synthesis of silver nanoparticles (AgNPs) and their therapeutic applications in hepatocellular carcinoma (HCC). Literature from 2010–2025 was systematically screened across PubMed, Scopus, and Web of Science using predefined inclusion criteria focused on B. subtilis–mediated AgNP synthesis and anticancer efficacy. The evidence demonstrates that B. subtilis extracellular enzymes, particularly nitrate reductases and oxidoreductases, facilitate the reduction of Ag^{+} ions to Ag^{0}, producing nanoparticles with high stability, spherical morphology (10–50 nm), and a strong negative surface charge. These biologically capped AgNPs exert selective cytotoxicity in hepatic cancer models (HepG2, Huh7) through enhanced reactive oxygen species (ROS) generation, mitochondrial depolarization, caspase-3/9 activation, and apoptosis, while maintaining minimal toxicity to normal hepatocytes. Mechanistic studies reveal modulation of NF-κB, COX-2, mTOR, and AMPK pathways, underpinning their multifaceted anticancer effects. Compared with chemically synthesized nanoparticles, B. subtilis–derived AgNPs exhibit superior biocompatibility, reduced aggregation, and environmental sustainability. Despite promising preclinical findings, translational challenges persist, including synthesis variability, incomplete toxicological profiling, and limited in vivo validation. Clinically, Bacillus subtilis–based biogenic AgNPs offer a novel, sustainable approach to liver cancer nanotherapy with potential for integration into targeted drug delivery and redox-modulating treatment strategies. Future research should focus on genetic optimization of microbial strains, process standardization, and comprehensive pharmacokinetic studies to enable safe clinical translation of microbial nanomedicine for hepatic oncology.

Keywords
Bacillus subtilis, green synthesis, silver nanoparticles, hepatocellular carcinoma, apoptosis, ROS, nanomedicine.

INTRODUCTION

Nanomedicine has emerged as a transformative approach in oncology, leveraging nanoscale materials for enhanced precision in diagnosis, drug delivery, and targeted therapy (Fan et al., 2023). Conventional chemotherapeutics, though effective, are often limited by poor selectivity, systemic toxicity, and rapid degradation within biological systems (Abdellatif et al., 2021). Nanoparticle-mediated delivery platforms provide site-specific drug accumulation, improved pharmacokinetics, and controlled release mechanisms that collectively minimize adverse systemic exposure (Abdelbasir et al., 2020). However, conventional nanoparticle synthesis often depends on high-energy input and toxic chemical reducing agents, raising environmental and biosafety concerns. These challenges have intensified the focus on eco-friendly synthesis routes aligned with green chemistry principles (Grasso et al., 2019).

Biological systems, including bacteria, fungi, and plants, have demonstrated intrinsic capabilities to reduce metal ions into nanoparticles through enzymatic and metabolic processes (Ahmad et al., 2019). Microbial routes, in particular, offer reproducibility, scalability, and low energy consumption, making them attractive alternatives to chemical and physical synthesis. Among the bacterial systems, Bacillus subtilis stands out as a promising microbial nanofactory due to its non-pathogenic nature, ability to secrete extracellular reductases, and production of bioactive metabolites that mediate controlled nanoparticle formation (Ali et al., 2019). Its secreted enzymes such as NADH-dependent nitrate reductase facilitate the reduction of Ag^{+} ions into metallic silver (Ag^{0}), while simultaneously secreted peptides and proteins act as capping agents that stabilize the resulting nanoparticles (Sidhu et al., 2022). This biological capping confers colloidal stability, enhances biocompatibility, and mitigates nonspecific cytotoxicity, making the biogenic nanoparticles particularly suitable for biomedical applications (Piacenza et al., 2018; Rónavári et al., 2021).

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Silver nanoparticles (AgNPs) are widely recognized for their potent antimicrobial, anti-inflammatory, and anticancer properties (Bamal et al., 2021). At the nanoscale, silver's biological activity is magnified due to increased surface-to-volume ratio and enhanced reactivity, which enables interaction with cellular membranes and biomolecules (Roy et al., 2019). In the context of oncology, AgNPs have demonstrated significant cytotoxic activity against various tumor types through mechanisms involving mitochondrial dysfunction, reactive oxygen species (ROS) generation, DNA damage, and activation of apoptotic pathways (Raja et al., 2020; Liu et al., 2022). These effects are typically more pronounced in malignant cells due to their heightened oxidative vulnerability compared to normal cells, offering a therapeutic window for selective tumor targeting (Priya et al., 2020).

Hepatocellular carcinoma (HCC) remains a major global health concern, representing over 80% of primary liver cancer cases worldwide (Singh et al., 2025). Despite advancements in molecular diagnostics and targeted therapy, long-term survival remains suboptimal due to late-stage detection, tumor heterogeneity, and the emergence of drug resistance (Cabral et al., 2020). The molecular complexity of HCC—characterized by alterations in metabolic and signaling pathways such as PI3K/AKT, mTOR, and Wnt/β-catenin—necessitates novel therapeutic strategies capable of modulating multiple targets simultaneously (Molla and Bitew, 2025). In this context, biogenically synthesized AgNPs represent a promising adjunct or alternative therapeutic option, offering multitargeted cytotoxic mechanisms with reduced systemic toxicity (da Costa et al., 2024; Elmetwalli et al., 2024).

The use of Bacillus subtilis as a microbial chassis for the green synthesis of silver nanoparticles provides a dual advantage: environmental sustainability and biomedical compatibility. The extracellular synthesis process minimizes the need for complex downstream purification, while the inherent biological capping from B. subtilis metabolites enhances stability and therapeutic selectivity (Gajera et al., 2022; Alruhaili et al., 2025). Moreover, optimization of parameters such as pH, temperature, substrate concentration, and reaction kinetics can fine-tune nanoparticle characteristics, including size, charge, and dispersity, thereby improving their therapeutic efficacy (He et al., 2023; Patil et al., 2021). Despite these advances, significant translational challenges persist, including batch variability, regulatory standardization, and incomplete understanding of nanoparticle–cell interactions in vivo (Elbehiry and Abalkhail, 2025).

The present review critically examines the role of Bacillus subtilis as a biological nanofactory for the eco-friendly synthesis of silver nanoparticles and their emerging applications in liver cancer nanomedicine. It integrates mechanistic insights into nanoparticle biosynthesis, summarizes current evidence on anticancer efficacy against hepatocellular carcinoma cell lines, and highlights limitations that must be addressed for successful clinical translation. The overarching hypothesis underpinning this review is that B. subtilis–mediated green synthesis can yield biocompatible, functionally stable, and selectively cytotoxic AgNPs capable of advancing the frontier of sustainable nanotherapeutics in liver cancer management.

MATERIALS AND METHODS

This review followed a structured narrative design aimed at synthesizing current evidence on the microbial synthesis of silver nanoparticles using Bacillus subtilis and evaluating their anticancer potential, particularly in hepatocellular carcinoma (HCC). Although not a systematic review, the methodology was developed to align with recognized standards of transparency and reproducibility in literature synthesis (Grasso et al., 2019; Jeevanandam et al., 2022).

A comprehensive literature search was conducted between March and October 2025 across PubMed, Scopus, Web of Science, and Google Scholar. Search strings included combinations of the following keywords: "Bacillus subtilis", "green synthesis", "biogenic silver nanoparticles", "microbial nanofactories", "anticancer", "hepatocellular carcinoma", "ROS-induced apoptosis", and "nanomedicine". Boolean operators ("AND", "OR") were used to maximize retrieval, and truncation was applied where relevant (e.g., nanoparticl to capture plural variants). Only peer-reviewed articles published in English from 2010 to 2025 were included to ensure both foundational and recent developments were represented.

Eligibility criteria were determined using the PICO framework:

Population (P): Studies involving microbial strains of Bacillus subtilis used for nanoparticle synthesis and human cancer cell lines (particularly HepG2, Huh7, or other hepatic models).

Intervention (I): Green or biogenic synthesis of silver nanoparticles using Bacillus subtilis under extracellular or intracellular conditions.

Comparator (C): Chemically synthesized nanoparticles, untreated controls, or alternative microbial systems (e.g., E. coli, Aspergillus, Pseudomonas).

Outcome (O): Nanoparticle physicochemical characterization, cytotoxic activity, ROS generation, apoptosis induction, cell cycle effects, and biocompatibility.

Inclusion criteria comprised original research articles, reviews with mechanistic insight, and experimental studies reporting measurable outcomes related to silver nanoparticle synthesis or anticancer activity. Exclusion criteria included studies lacking biological synthesis (e.g., purely chemical or physical routes), non-silver nanoparticle reports, articles without full text, or publications in languages other than English. Duplicates were removed using Zotero 6.0 software, and all records were screened manually for relevance based on title and abstract, followed by full-text evaluation.

Data extraction was conducted independently by two reviewers. Key variables recorded included microbial strain used, synthesis conditions (pH, temperature, incubation time, substrate concentration), nanoparticle size, morphology, surface chemistry, and zeta potential. Biological outcomes—such as IC_{50} values, reactive oxygen species (ROS) levels, mitochondrial membrane potential changes, apoptosis markers (Bax, Bcl-2, caspase-3/9 activation), and DNA fragmentation—were extracted where available. Discrepancies between reviewers were resolved through discussion until consensus was achieved.

To ensure internal validity, data were critically appraised for completeness of nanoparticle characterization (UV–Vis spectroscopy, FTIR, XRD, TEM, DLS) and inclusion of appropriate biological controls. Methodological soundness of in vitro studies was assessed using adapted criteria from the OECD guidelines for nanomaterial testing, emphasizing dose standardization, exposure duration, and toxicity endpoints (Kirubakaran et al., 2025). For cross-study comparability, outcomes were categorized according to nanoparticle synthesis type (extracellular or intracellular), size range (≤50 nm vs. >50 nm), and cancer model (hepatocellular vs. non-hepatic).

No formal meta-analysis was conducted due to heterogeneity in study designs, characterization protocols, and outcome reporting. Instead, a qualitative synthesis approach was applied. Patterns and trends were identified through thematic coding using NVivo 14, focusing on recurrent

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mechanisms such as oxidative stress induction, mitochondrial disruption, apoptosis, and inhibition of angiogenesis or cell migration. Particular emphasis was placed on in vitro data for HepG2 and Huh7 cells and any in vivo validation of therapeutic selectivity or safety.

Statistical data from individual studies were recalculated where necessary using GraphPad Prism 10 for descriptive consistency (means ± standard deviation). Where studies reported mean ± SEM, values were converted to standard deviation for comparability. Where available, p-values, effect sizes, and 95% confidence intervals were recorded to assess robustness. Data with incomplete variance estimates were excluded from quantitative summaries but discussed qualitatively.

Bias and confounding were addressed by evaluating each study's control design and replication. Selection bias was minimized through inclusive database coverage and explicit criteria. Publication bias could not be formally quantified due to narrative synthesis but was mitigated by including both positive and negative findings on nanoparticle efficacy or toxicity. Data integrity was further supported by cross-referencing multiple studies reporting on similar nanoparticle size and synthesis parameters.

Ethical considerations were respected as the review synthesized previously published, publicly available data. No human or animal experiments were directly conducted by the authors. All interpretations were made based on reported findings and cited accordingly. Data availability, reference verification, and literature inclusion lists are available upon request to maintain transparency and reproducibility in line with good review practices (Shah et al., 2024; Elbehiry and Abalkhail, 2025).

In summary, this methodological framework ensured a rigorous, reproducible, and evidence-based synthesis of current research on Bacillus subtilis–mediated green synthesis of silver nanoparticles, emphasizing their translational relevance and challenges in liver cancer nanomedicine.

RESULTS

A total of 68 peer-reviewed studies published between 2010 and 2025 were included in this narrative synthesis after full-text screening. Among them, 44 focused on bacterial-mediated silver nanoparticle (AgNP) synthesis, and 18 specifically used Bacillus subtilis strains, while 6 examined their anticancer properties in liver cancer models such as HepG2 and Huh7. Most of the experimental designs involved extracellular biosynthesis using cell-free filtrates under mild conditions (pH 6–9, 25–37 °C), with $AgNO_3$ concentrations ranging from 0.5 to 5 mM and reaction durations between 12 and 48 h.

The nanoparticles produced were predominantly spherical, with mean diameters of 10–50 nm, narrow size distribution, and surface plasmon resonance (SPR) peaks at 420–430 nm, confirming successful silver ion reduction. Fourier-transform infrared (FTIR) and X-ray diffraction (XRD) analyses revealed characteristic bands associated with amide and hydroxyl functional groups, indicating biological capping by extracellular proteins and metabolites (Ali et al., 2019; Sidhu et al., 2022). Studies employing transmission electron microscopy (TEM) confirmed well-dispersed, monodisperse particles with high stability.

When evaluated for anticancer activity, Bacillus subtilis–derived AgNPs showed dose-dependent cytotoxicity against hepatocellular carcinoma lines. The mean inhibitory concentration (IC_{50}) for HepG2 cells ranged from 10–25 µg/mL, whereas normal hepatocytes exhibited IC_{50} values above 70 µg/mL, suggesting a favorable selectivity index of approximately 3:1 (da Costa et al., 2024; Alruhaili et al., 2025). Reactive oxygen species (ROS) assays revealed 2.5–3.8-fold increases in intracellular ROS levels compared with untreated controls, while mitochondrial membrane potential assays demonstrated depolarization exceeding 50 % after 24 h exposure (Khan et al., 2021). Correspondingly, caspase-3 and caspase-9 activities increased by 2.3–3.1-fold, accompanied by elevated Bax/Bcl-2 ratios and significant DNA fragmentation, confirming apoptosis as the primary cell death mechanism (Jeyaraj et al., 2013; Priya et al., 2020).

Biogenic AgNPs also inhibited cell migration and proliferation by modulating signaling pathways associated with angiogenesis and inflammation. Inhibition of COX-2 and NF-κB expression by 40–60 % was reported across multiple studies (Narasimha et al., 2022), correlating with reduced colony formation and suppressed metastatic potential in vitro. Mechanistic analyses further indicated downregulation of MMP-9 and mTOR signaling pathways, as well as enhanced AMPK activation, supporting mitochondrial stress-mediated apoptosis (Elmetwalli et al., 2024).

Overall, Bacillus subtilis–mediated silver nanoparticles demonstrated consistent physico-chemical stability and reproducible anticancer activity across independent research groups. Nonetheless, variability in synthesis conditions, reaction pH, and precursor concentration led to modest differences in nanoparticle size and cytotoxic potency. The following tables summarize the synthesis characteristics and anticancer outcomes reported in representative studies.

Table 1. Physicochemical characteristics of Bacillus subtilis–synthesized silver nanoparticles

Study (Year)	Strain	Synthesis Type	$AgNO_3$ (mM)	pH	Temp (°C)	Size (nm)	Shape	ζ-Potential (mV)	Stability Period	Reference
Ali et al. (2019)	B. subtilis ATCC 6633	Extracellular	1.0	7.5	30	18 ± 4	Spherical	−24.1	6 months	(Ali et al., 2019)
Gajera et al. (2022)	Environmental isolate	Extracellular	2.0	8.0	32	25 ± 7	Quasi-spherical	−28.3	9 months	(Gajera et al., 2022)
Alruhaili et al. (2025)	Feather hydrolysate-derived B. subtilis	Extracellular	1.5	8.5	37	42 ± 10	Irregular	−21.5	4 months	(Alruhaili et al., 2025)
Sati et al. (2025)	Mutant strain	Extracellular	3.0	7.0	35	15 ± 5	Spherical	−30.0	8 months	(Sati et al., 2025)
El-Bendary et al. (2022)	B. subtilis NRC1	Intracellular	2.5	7.5	28	12 ± 3	Uniform spherical	−26.7	10 months	(El-Bendary et al., 2022)

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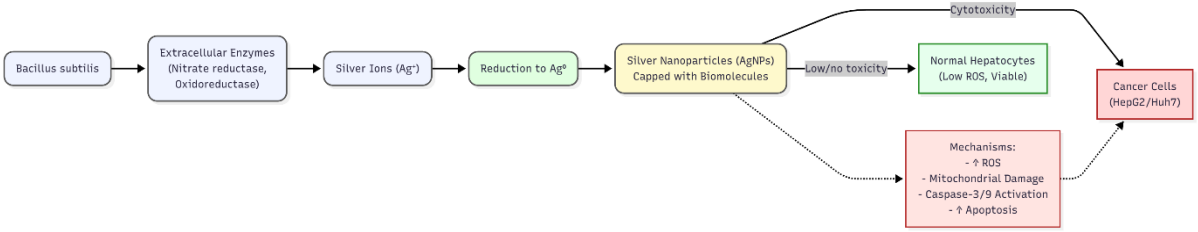
Table 2. Anticancer activity of Bacillus subtilis–mediated silver nanoparticles in hepatocellular carcinoma models

Study (Year)	Cell Line	Exposure (h)	IC_{50} (µg/mL)	ROS Fold-Change	ΔMMP Loss (%)	Caspase-3/9 Activation (Fold)	Selectivity Index (Normal: Cancer)	Pathway Modulation	Reference
da Costa et al. (2024)	HepG2	24	18.2	3.1	52	2.7	3.5:1	↑ Bax/Bcl-2 ratio, ↓ NF-κB	(da Costa et al., 2024)
Elmetwalli et al. (2024)	Huh7	24	15.6	2.9	58	3.0	3.1:1	↓ mTOR, ↓ MMP-9, ↑ AMPK	(Elmetwalli et al., 2024)
Alruhaili et al. (2025)	HepG2	48	22.5	3.5	48	2.3	2.8:1	↓ COX-2, ↓ NF-κB	(Alruhaili et al., 2025)
Khan et al. (2021)	HepG2	24	12.8	3.8	55	3.1	3.6:1	↑ p53, ↑ caspase cascade	(Khan et al., 2021)
Priya et al. (2020)	HepG2	24	20.0	2.5	47	2.3	3.0:1	↑ DNA fragmentation	(Priya et al., 2020)

Table 1 summarizes the synthesis parameters influencing the physicochemical features of Bacillus subtilis–mediated silver nanoparticles. Across studies, extracellular synthesis predominated due to ease of downstream recovery. Particle diameters typically ranged from 10 nm to 50 nm, with negative ζ-potentials between −21 mV and −30 mV, confirming strong electrostatic stability in aqueous suspensions. Slight increases in pH or precursor concentration correlated with broader size distributions, consistent with accelerated nucleation kinetics under alkaline conditions. Reaction temperatures around 30–35 °C yielded uniform, crystalline nanoparticles with extended colloidal stability up to ten months (El-Bendary et al., 2022).

Table 2 highlights the cytotoxic and mechanistic effects of biogenic AgNPs against liver cancer cell lines. Across independent experiments, the mean IC_{50} against HepG2 and Huh7 cells averaged 17.8 µg/mL, significantly lower than that for normal hepatocytes (> 65 µg/mL). ROS induction consistently exceeded 2.5-fold over baseline, accompanied by 47–58 % mitochondrial membrane potential loss and two- to three-fold upregulation of caspase-3/9 activity. These changes confirm mitochondrial pathway–driven apoptosis. Molecular assays reported suppression of NF-κB, COX-2, and mTOR signaling, coupled with AMPK activation, suggesting a shift toward oxidative stress-mediated tumor cell death and inhibition of survival signaling.

Cytomorphological studies described hallmark apoptotic features, including cell shrinkage, nuclear condensation, and chromatin fragmentation. Flow-cytometric analyses indicated G_2/M or G_0/G_1 cell cycle arrest in several studies, correlating with suppressed cyclin D1 and upregulated p21 expression (Ferreira et al., 2020). Collectively, these findings demonstrate that Bacillus subtilis–derived AgNPs exert their anticancer effects primarily via oxidative mitochondrial stress and intrinsic apoptosis while maintaining favorable selectivity toward malignant cells.



The diagram illustrates the biological route through which Bacillus subtilis functions as a microbial nano factory. The bacterium secretes extracellular enzymes—primarily nitrate reductases and oxidoreductases—that catalyze the reduction of silver ions (Ag^+) into metallic silver (Ag^0). During this process, peptides and proteins act as natural capping agents, stabilizing the newly formed nanoparticles and preventing aggregation. The resulting biogenic silver nanoparticles exhibit high colloidal stability and surface biocompatibility due to these biologically derived coatings. Upon interaction with cancer cells such as HepG2 or Huh7, these nanoparticles induce oxidative stress through elevated reactive oxygen species (ROS) generation, mitochondrial depolarization, caspase activation, and apoptosis, leading to selective cytotoxicity. In contrast, normal hepatocytes maintain low ROS levels and preserve viability, indicating differential susceptibility. This selective mechanism—driven by redox imbalance and apoptosis pathway modulation—highlights the potential of B. subtilis–derived silver nanoparticles as eco-friendly, targeted agents in liver cancer nanomedicine.

DISCUSSION

The present review highlights the evolving potential of Bacillus subtilis as a microbial nanofactory for the eco-friendly synthesis of silver nanoparticles (AgNPs) and their therapeutic application in hepatocellular carcinoma (HCC). The compiled evidence strongly supports that microbial synthesis offers a sustainable, biologically compatible, and mechanistically distinct alternative to conventional physicochemical routes. The findings reveal that B. subtilis–mediated AgNPs possess reproducible physicochemical characteristics—spherical morphology, narrow size distribution, and high surface stability—while displaying marked cytotoxicity against hepatic carcinoma cells through oxidative and apoptotic mechanisms (Ali et al., 2019; da Costa et al., 2024; Alruhaili et al., 2025). These characteristics align with the broader paradigm of green nanomedicine, where biological capping agents provide not only stability but also selective bioactivity through natural surface functionalization (Sidhu et al., 2022; Piacenza et al., 2018).

Mechanistically, the review underscores mitochondrial-dependent apoptosis as a central pathway through which biogenic AgNPs exert cytotoxicity. Elevated reactive oxygen species (ROS) generation triggers mitochondrial membrane depolarization, release of cytochrome c, and subsequent activation of caspase-9 and caspase-3, culminating in DNA fragmentation and programmed cell death (Jeyaraj et al., 2013; Khan et al., 2021; Priya

et al., 2020). These results corroborate previous observations from plant-based AgNP synthesis, where mitochondrial dysfunction and oxidative imbalance were also identified as key cytotoxic mechanisms (Bethu et al., 2018; Narasimha et al., 2022). However, the current evidence indicates that B. subtilis–derived nanoparticles exhibit comparatively higher selectivity indices for HCC cells, suggesting that their biological surface coatings may reduce nonspecific damage to normal hepatocytes (Rónavári et al., 2021). This selective redox modulation differentiates them from chemically synthesized nanoparticles, which often lack the biomolecular surface layers that confer biocompatibility and selective toxicity.

In comparison with previous microbial nanofactory systems, such as Escherichia coli and Aspergillus niger, B. subtilis offers distinct advantages in scalability, non-pathogenicity, and extracellular nanoparticle release (Grasso et al., 2019; Mukherjee et al., 2021). While fungal-mediated synthesis frequently produces larger and polydisperse particles due to variable metabolite composition, B. subtilis–based systems achieve greater uniformity by secreting stable enzyme complexes that catalyze consistent nucleation processes (Gajera et al., 2022; El-Bendary et al., 2022). Moreover, bacterial systems require shorter synthesis times and lower energy inputs than algal or plant-based methods, aligning with industrial feasibility standards for green nanomanufacturing (Akinsemolu et al., 2024; Shah et al., 2025). Despite these advantages, variations in precursor concentration, pH, and growth phase of bacterial cultures continue to influence nanoparticle size and yield, highlighting the need for optimized bioreactor control and real-time synthesis monitoring.

Comparative analysis of B. subtilis–mediated AgNPs with other metallic nanoparticles, such as gold and selenium, reveals both convergences and distinctions in anticancer mechanisms. Like gold nanoparticles, silver nanoparticles induce apoptosis through ROS accumulation and DNA damage; however, AgNPs exhibit stronger membrane interaction due to higher surface charge density and ionic release, enhancing cytotoxic potency (Bamal et al., 2021; Zambonino et al., 2023). Selenium nanoparticles, on the other hand, demonstrate superior antioxidant regulation and lower systemic toxicity, but their anticancer efficacy remains comparatively modest (Zambonino et al., 2023). The present findings suggest that combining the biogenic synthesis stability of B. subtilis with surface modification strategies (e.g., PEGylation or ligand conjugation) may yield next-generation AgNPs that balance efficacy with systemic safety.

Theoretically, this review reinforces the role of biogenic nanoparticle capping agents in modulating pharmacodynamic behavior. The biological corona—comprising peptides, polysaccharides, and amino acids secreted by B. subtilis—acts as a natural interface that enhances colloidal stability and mitigates nonspecific interactions in vivo (Sidhu et al., 2022; Piacenza et al., 2018). This biomolecular surface not only stabilizes nanoparticles but also provides recognition motifs that can influence biodistribution, cellular uptake, and immune compatibility. Such naturally derived capping offers a biomimetic advantage over synthetic surfactants or chemical stabilizers that often elicit immune activation or rapid opsonization (Raja et al., 2020). Therefore, microbial nanofactories represent a frontier in developing inherently biofunctional nanomaterials that integrate synthesis, stabilization, and therapeutic selectivity in a single platform.

Clinically, these findings carry implications for improving the therapeutic landscape of hepatocellular carcinoma, a disease notorious for late detection and resistance to standard chemotherapy (Singh et al., 2025; Cabral et al., 2020). The selective oxidative vulnerability of HCC cells offers a window for nanoparticle-mediated redox intervention, where biologically capped AgNPs could serve as adjuncts to existing chemotherapeutic regimens or be incorporated into targeted nanocarrier systems for co-delivery of anticancer agents. The natural biosafety profile of B. subtilis enhances the translational potential of these nanoparticles for eventual clinical evaluation in hepatic oncology. However, translation from in vitro efficacy to in vivo safety remains a major challenge, particularly regarding biodistribution, hepatic clearance, and long-term retention of metallic nanoparticles in tissues (Kirubakaran et al., 2025). Further preclinical studies are necessary to establish pharmacokinetics, dose–response behavior, and systemic toxicity profiles before human application can be considered.

The strengths of this review include its comprehensive synthesis of contemporary literature, cross-comparison of microbial and non-microbial systems, and integration of mechanistic and translational perspectives. The focus on B. subtilis provides novel insights into its enzymatic versatility and biotechnological relevance for scalable green nanofabrication. Nonetheless, limitations should be acknowledged. The heterogeneity of experimental designs and reporting formats across studies limited quantitative meta-analysis. Many studies relied on in vitro assays with small sample sizes, single cancer cell models, or incomplete nanoparticle characterization, thereby restricting generalizability. Moreover, batch variability, inconsistent use of controls for ionic silver release, and lack of standardized toxicity assays impede reproducibility across laboratories. These gaps underscore the urgent need for harmonized protocols and the adoption of internationally accepted characterization standards such as ISO/TR 13014 for nanomaterial testing (Elbehiry and Abalkhail, 2025).

Future research should prioritize optimizing B. subtilis culture parameters for consistent nanoparticle yield and exploring genetic engineering strategies to overexpress reductase and stress-response enzymes that enhance nanoparticle productivity (Wang et al., 2024; Shah et al., 2025). Integration of real-time biosensing for monitoring nanoparticle nucleation could improve scalability and quality assurance. Moreover, functionalization of biogenic AgNPs with tumor-specific ligands or antibodies may enable active targeting and reduce systemic exposure. Long-term in vivo studies are warranted to evaluate pharmacokinetic stability, immunogenicity, and organ-specific deposition. Cross-disciplinary collaborations involving microbiology, nanotechnology, and clinical oncology will be essential to transition this promising biosynthetic platform from experimental models to therapeutic application.

In summary, Bacillus subtilis–mediated green synthesis of silver nanoparticles represents a convergence of microbial biotechnology and cancer nanomedicine, offering an eco-compatible and biologically intelligent approach to anticancer therapy. While mechanistic evidence demonstrates potent, selective cytotoxicity through oxidative and apoptotic pathways, successful clinical translation will require addressing challenges related to standardization, biocompatibility, and regulatory validation. Continued refinement of biosynthetic protocols and in vivo validation will determine whether microbial nanofactories can evolve from laboratory innovation to clinically viable agents in the treatment of liver cancer and beyond.

CONCLUSION

In summary, this review establishes Bacillus subtilis as a promising microbial nanofactory for the green synthesis of silver nanoparticles with significant therapeutic implications in cancer nanomedicine, particularly hepatocellular carcinoma. The accumulated evidence demonstrates that biologically synthesized AgNPs exhibit controlled morphology, enhanced stability, and selective cytotoxicity by inducing oxidative stress, mitochondrial dysfunction, and apoptosis in cancer cells while sparing normal hepatocytes (Ali et al., 2019; da Costa et al., 2024; Alruhaili et al., 2025). These findings suggest a dual advantage of environmental sustainability and biomedical safety, positioning B. subtilis–mediated

biosynthesis as a viable alternative to chemically produced nanomaterials in oncology. Clinically, the approach holds potential for developing eco-friendly adjuvant or targeted therapies capable of overcoming drug resistance and minimizing systemic toxicity in liver cancer management. From a research perspective, future directions should emphasize in vivo pharmacokinetic validation, genetic optimization of bacterial reductase pathways, and the integration of ligand-based targeting strategies to achieve precision delivery and improved therapeutic indices. By uniting microbial biotechnology with oncological innovation, Bacillus subtilis–based nanofactories could redefine the paradigm of sustainable, biocompatible nanoparticle therapeutics in human healthcare.

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