Medical Research, Nursing, Health and Midwife Participation
https://medalionjournal.com | ISSN: 2986-7754

"UNLOCKING THE HIDDEN POTENTIAL OF BACILLUS TOYONENSIS: A JOURNEY INTO VERSATILITY"

Arif Hussain Pandith[1], Vicky Kumar[2]
[1]Annamalai University, Chidambaram, Tamil Nadu, 608002 India
[2]Researcher
Email : arifpandith141@gmail.com, rkthakur075@gmail.com

Abstract

Bacillus toyonensis, a gram-positive, aerobicspore-producing bacterium included in the Bacillus cereus group, exhibits versatile applications across microbiology, medicine, pharmaceuticals, agriculture, and industry. The multifaceted roles and applications of this bacterium across different scientific domains are explored in detail. Furthermore, we underscore the necessity for ongoing research and thorough evaluation to comprehensively grasp and leverage the capabilities of Bacillus toyonensis in its diverse applications. The review offers an inclusive and enlightening portrayal of B. toyonensis, elucidating its positive applications and potential drawbacks, and promoting a well-rounded perspective for future exploration and utilization in various scientific and industrial settings.

Keywords: ***Bacillus toyonensis, Probiotic, Bioremediation, Pathogenesis, Pharmaceutical properties, Agricultural benefits***

1. INTRODUCTION

Bacillus species are gram-positive, aerobic rods that form spores. Bacillus toyonensis belongs to the Bacillus cereus group and is found in diverse habitats, including tree hollows, deep-sea environments, agricultural soil, and the digestive tracts of different organisms. Most of them are non-pathogenic, with significant microbiological applications and few associated diseases in humans or animals [1]. They are diverse bacteria found everywhere. Some, like B. cereus, can be occasional human and livestock pathogens, while others are harmless saprophytes. Specific Bacillus species target certain insect groups as pathogens. They play important roles in healthcare, medicinal products, farming, and manufacturing operations due to their various physiological features and the synthesis of enzymes, antimicrobial agents, and other compounds. Antibiotics like bacitracin and polymyxin, and some others, serve as standards in medical and pharmaceutical assays [2]. Enhanced surveillance and genome sequencing unveil novel B. cereus group isolates with distinctive physiology, genetics, and virulence [3].The Bacillus cereus group encompasses eight closely related species, namely Bacillus anthracis, Bacillus cytotoxicus, B. cereus, Bacillus mycoides, Bacillus thuringiensis, Bacillus weihenstephanensis, Bacillus pseudomycoides, and Bacillus toyonensis, all sharing a significant degree of phylogenetic similarity [4].

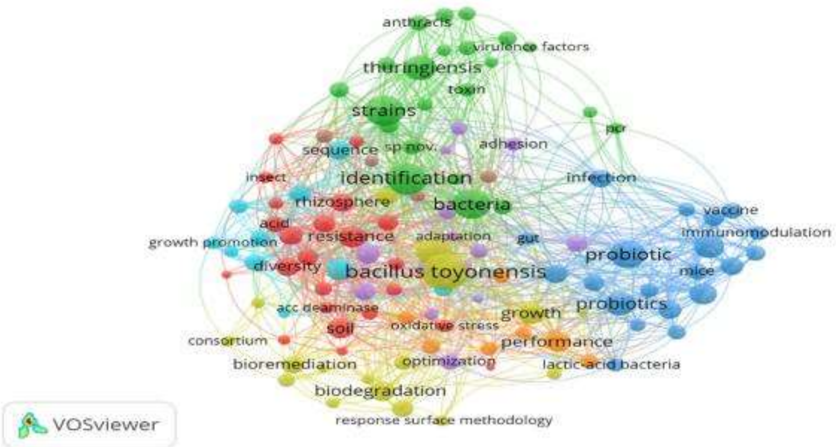
B. anthracis, a main causative agent for the zoonotic disease anthrax, can form cutaneous, gastrointestinal, and inhalation anthrax [5]. It causes anthrax outbreaks in cattle, especially in the A.Br.Aust94 sublineage, which has been found in Asian, European, African, and Australian countries[6]. B. cytotoxicus, a thermotolerant bacterium with varying pathogenicity potential, is widely found in food production sites, particularly potato goods, contaminating them. While the majority of strains are non-toxic, a few can cause extreme lethargy owing to enterotoxins[7, 8]. It is one of the endospore-forming bacteria that caused a food poisoning outbreak in France in 1988 [7]. They have cytotoxin K, a cytotoxic variation encoded by the cytk-1 gene with varied intra-species features [9]. It is more hazardous than the cytotoxic K-2 generated by other Bacillus strains. They are also engaged in bug and dried food-related items [10]. It is a thermotolerant species of the B. cereus group, while B. weihenstephanensis is a psychrotolerent species [9, 11].

Bacillus weihenstephanensis is known for its ability to induce food poisoning, yet the majority of strains are not cytotoxic. Some strains have the genetic makeup necessary to produce key pathogenicity components, and some demonstrate this ability in controlled laboratory settings [12]. This bacterium is distinguished from other species due to its ability to grow in a colder environment (7 °C) and the presence of specific strains in 16S RNA and other housekeeping genes[13]. Two of the strains, MC67 and MC118, generate cereulide, which is comparable to B. cereus. Both B. cereus and B. weihenstephanensis are psychrotolerent and provide a significant risk of food contamination [14]. B. mycoides, a halotolerant member of the B. cereus group, has the ability to promote plant development and reduce salt stress by producing antioxidant enzymes[15]. They can occur in different environmental conditions and have the ability to adapt quickly to new environments due to the presence of 83% of the predominant adaptive genes [16]. B. pseudomycoides differs from B. mycoides and B. cereus in fatty makeup. It is so termed because of the same phenotypic and unique genotype as B. mycoides [17].

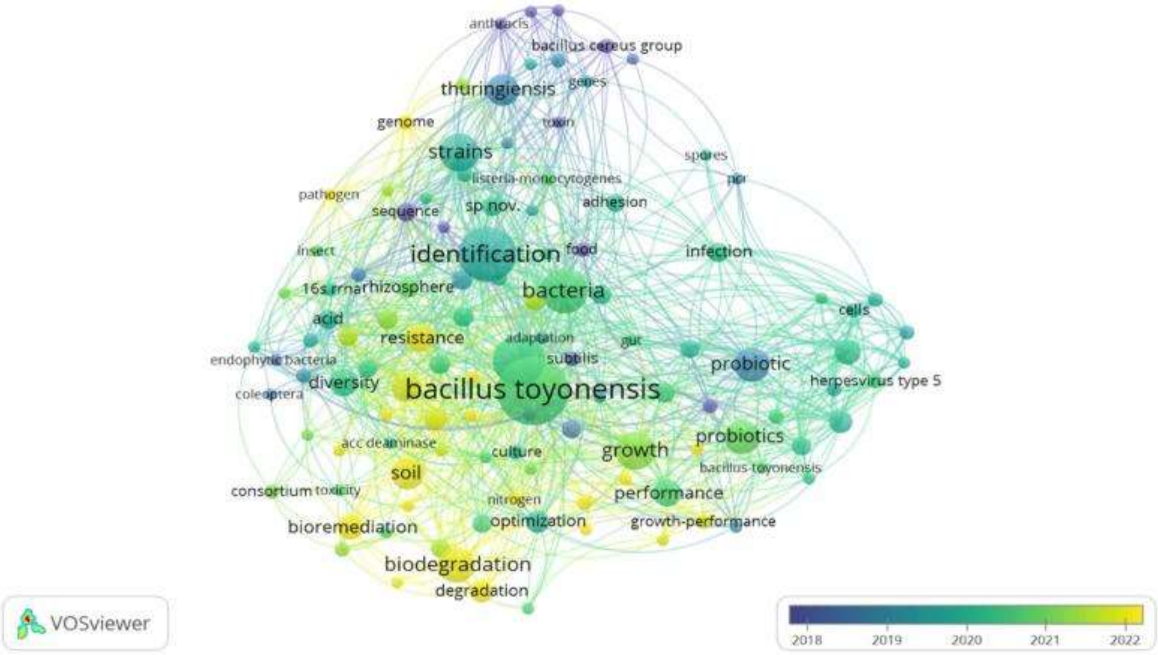
They have the ability to promote plant growth and drought tolerance in plants, particularly wheat. It can mute the MYB gene in wheat and express the Dreb1 gene and other genes that are responsible for wheat's drought resistance[18]. B. thuringiensis is well-known for its ability to control insects in agriculture. They were utilized as a microbial pesticide [19]. They have Cry and Cyt proteins that are toxic to insects, nematodes, and human cancer cells [20]. Bacillus toyonensisis a popular probiotic ingredient in livestock food [21]. It belongs to the Gram-positive bacteria strain in the Bacillus cereus group that is used as a probiotic or biocontrol weapon in some cases [22]. As a recently classified species, it exhibits genetic resemblances to Bacillus cereus and Bacillus thuringiensis [23]. This investigation delves into its positive attributes as well as its potential for pathogenicity, assessing whether its effects lean more towards the pathogenic or beneficial aspects.

2. METHODS

For the compilation of this review, we conducted an extensive search for relevant articles on Bacillus toyonensis across various online platforms. Utilizing keywords such as Bacillus toyonensis, we systematically explored databases including Web of Science (50 articles), PubMed (73 articles), Google Scholar (926 articles), BASE (128 articles), Research Gate (100+ articles), CORE (320 articles), Semantic Scholar (12,70,000 articles), Worldwide Science (575 articles), Refseek (100+ articles), JSTOR (7 articles), Academia (657 articles), Citeseer (109529 articles), Google Books (80 articles), DOAJ (58 articles), WorldCat (171 articles), and university library (114 articles). From these sources, we identified numerous articles encompassing research on B. toyonensis, Bacilluscereus, and other Bacillus species. In total, we gathered 58 articles specifically focused on findings related to B. toyonensis. Vast numbers of articles not aligned with the specified keywords were excluded from the study.



a



b

Fig 1. (a). A scientometric visualization was conducted the search criteria included "Bacillus toyonensis" Using the Web of Science Core Collection database, the visualization was generated using VOS viewer. In the visualization, each circle represents a keyword, and its size corresponds to the frequency of appearance in pairs of keywords within the publications. (b). The visualization represents the average publications from the year 2017with color coding indicating citation intensity based on a predefined scale.

1. Habitation

Bacillus toyonensis has been detected in the tree hollows of QuercusrotundifoliaLamark, particularly within the digestive tracts of Mallotadusmeti larvae, a vulnerable species [24]. Additionally, it has been observed in the blood-feeding red poultry adult mites [25]. B. toyonensis is found in a variety of environments, including the deep sea, where strain P18 was recently discovered [22].This bacterium is globally distributed and is commonly found in agricultural soil [26]. Recent molecular investigations in the Sucre Department mark the first documentation of B. toyonensis in the feces of developing zebra calf. It is well known for its use as a probiotic in animal feeding, but it also has a large presence and importance in a variety of ecological niches and agricultural settings [27].

2. Beneficial sides of B. toyonensis

2.1. Probiotics

The Bacillus toyonensis XIN-YC13 strain, identified through a laboratory-based screening approach, produces a novel leaderless bacteriocin named toyoncin. With a molecular mass of 7,817.1012 Da and a unique N-terminal amino acid sequence featuring formylmethionine, toyoncin exhibits robust pH and heat stability. This 70-amino-acid-long bacteriocin has unique antibacterial action against B. cereus and Listeria monocytogenes, functioning by inducing cell membrane damage and bactericidal effects. Toyoncin suppresses the growth of B. cereus spores and demonstrates significant promise as a biopreservative in the food industry, showcasing its ability to suppress or eliminateB. cereus and L. monocytogenes in pasteurized skim milk [28]. In post-weaning piglets, a feed supplement containing Bacillus toyonensis (Toyocerin®) has demonstrated protective effects against enteric pathogens when administered at the appropriate dosage. The provision of Toyocerin® at a rate of 1000 mg/kg of diet has been associated with increased average daily feed consumption, a decrease in specific infections of the gut, and elevated levels of lactate bacteria. However, the probiotic's potential influence on pigs of various maturities has yet to be examined [29]. The entire genome of strain 7112, a key part of the feed ingredient Toyocerin, has been decoded and examined to disclose the genetic properties of this friendly bacterium. This strain, initially categorized as Bacillus cereus var. toyoi, has been used safely in the feed industry for more than three decades. It serves as a key ingredient in TOYOCERIN®, a supplement employed in the nutrition of various animals, includingpigs, chickens, cows, bunnies, and fisheries [30].B. toyonensis BCT-7112T has been shown to lower proinflammatory cytokine gene activity, improve immunological function, and expand microbial populations in the ceca of meat-type ducks [31].

Moreover, incorporating B. toyonensis and other food additives, such as pomegranate peel powder, into the diets of roaster chickens has shown positive effects on serum component levels, immunological parameters, and the nutritional value of meat [32].In Japanese quails and koilers, dietary supplementation with BT (Bacillus thuringiensis) has been linked to increased body weight and weight gain, improved developmental outcomes, improved digestion, enhanced antioxidant levels, and favorable changes in ileal histomorphometry. Additionally, higher levels of BT supplementation in the diet of Japanese quails have resulted in reduced blood cholesterol levels and a delay in the growth of harmful microorganisms in both the food and the gut [33]. Its supplementation enhances productivity, egg quality, reproductive success, and gut health in laying Japanese quail [33].The inclusion of BT as a feed supplement not only enhances the efficiency of growth and quality ofanimal products but also mitigates the growth of harmful bacteria in their food and gut.

2.2. Bioremediation and biodegradation

Bacillus toyonensis demonstrated its potential for environmental applications, particularly in the remediation of lead-contaminated industrial area groundwater. The biomass of B. toyonensis efficiently accumulated lead metal ions, reaching a maximum uptake of 8 ppm to sustain its live biomass. The bacteria exhibited a capability to accumulate lead ions within the range of 180-190 nm, with an optimal pH for the highest accumulation identified at 7.2 over a period of 160 minutes. Through careful adjustment of variables such as incubation time, shaking, levels of pH, warmth, and interaction period, a consistent generation of homogenous nanoparticles from biological material can be achieved alongside effective bioremediation of lead ions [34]. In the realm of industrial wastewater treatment, it shows its remarkable flocculation capabilities, presenting a safer alternative to the toxic chemical flocculants presently employed in wastewater purification processes, operating through a bridging mechanism. The bacteria produced a bioflocculant, denoted as REG-6, consisting of polysaccharide and protein under optimal conditions where glucose and ammonium nitrate served solely as nitrogen and carbon sources. These conditions included a starter medium of pH 5, with Ca2+ selected as the preferred ion [34]. This highlights the potential of B. toyonensis in eco-friendly wastewater treatment practices, contributing to both effective flocculation and reduced reliance on harmful chemical agents.

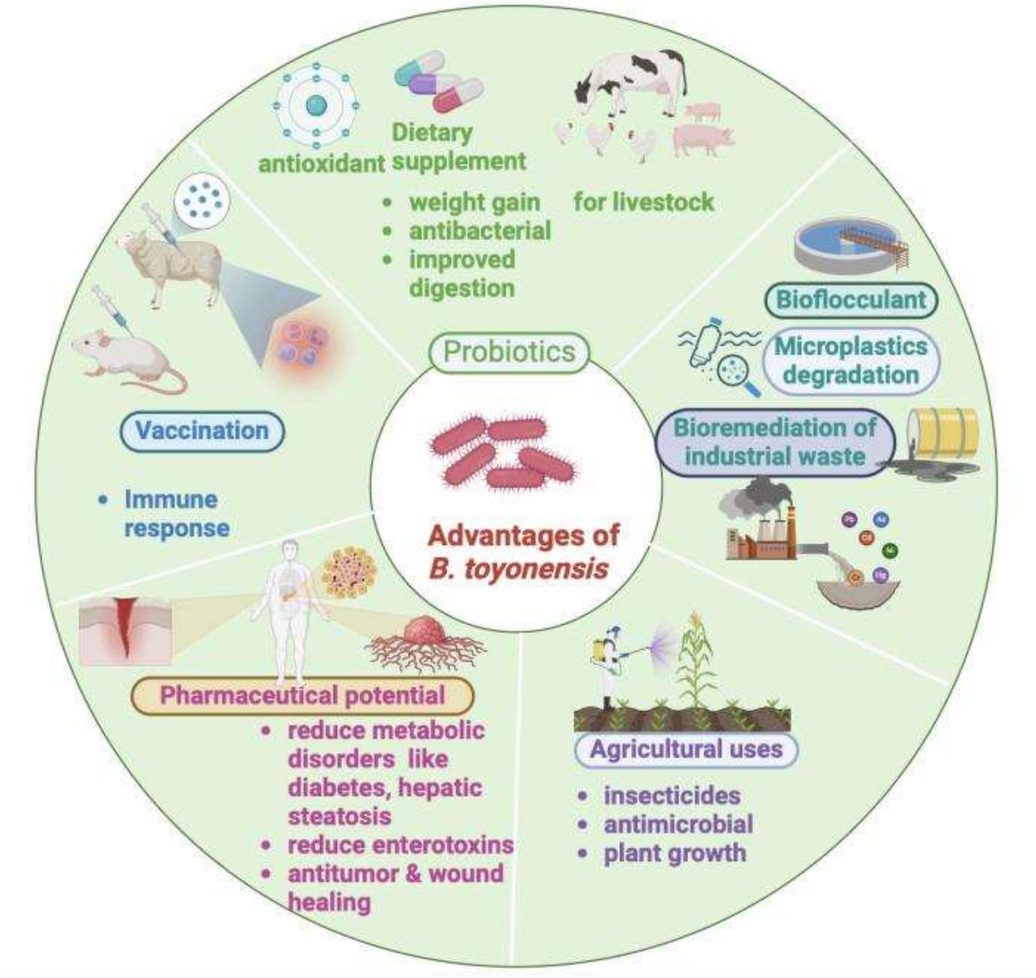


Fig 2.An illustration on advantages of B. toyonensis.

It proves to be a versatile and valuable resource in various environmental and industrial applications. When utilized as a bioremediation agent, it demonstrates effectiveness in removing chromium and phenol pollutants. In biohybrid membranes formed with polymeric nanofibers, the vehiculation of this bacterium results in hydrolysis-resistant, reusable systems that effectively and simultaneously remove contaminants for more than five cycles [35]. Moreover, B. toyonensis has been instrumental in the removal of wastewater containing common environmental platinum complexes, showcasing its ability to recover precious metals [36]. The bacterium's significance extends to diverse domains, such as its role in the generation of carbonates of calciumprecipitate in the Kashmir valley. This discovery implies its potential for remediating concrete structures and stabilizing beach sands [37].

Bacillus toyonensis strain PNTB1, with its CM-cellulase, lipase, and chitinase activities, holds promise for potential in biological remediation, agriculture, industry, and medicine [38]. The AM07 strain of B. toyonensis has demonstrated promising outcomes in petroleum breakdown assessments, suggesting its potential application in upcoming remediation strategies for environments impacted by hydrocarbon contamination [39]. Additionally, the presence of B. toyonensis in the gut of Chironomusriparius larvae and its partial bioremediation properties for microplastic degradation, including polyethylene, polyvinyl chloride, and polyamide, further underscore its diverse and impactful contributions [40].The incorporation of B. toyonensis AK2 along with B. albus AK3 has been found to expedite and enhance the efficiency of the biodegradation process in composts containing polylactide materials with tar.

These strains perform a vital function in altering the physicochemical characteristics of the films, facilitating the emergence of a biofilm on the film's surface and a reduction in the film's barrier properties. This alteration renders the materials more susceptible to biodegradation. The investigated films show promise for potential applications in the packaging industry and can intentionally undergo biodegradation processes, including bioaugmentation, after their initial use, utilizing these strains [41]. Tofu wastewater has been recognized as a promising alternative substrate for bacterial cultivation, specifically in the context of growing B. toyonensis. Hence, it is quite useful for the tofu wastewater [42]. Bacillus toyonensis strains, identified as top producers of highly active biosurfactants, have displayed stability under extreme conditions, including temperatures up to 120°C, pH levels as high as 12, and in the presence of 10% NaCl. The acknowledged biosurfactants, namely oleamide and decanoic acid, have proven successful in remediating wastewater contaminated with oil residues and pathogens.

Significantly, these biosurfactants exhibit potent antibacterial activity, surpassing the efficacy of conventional antimicrobial drugs. Moreover, they offer valuable contributions to enhancing bioremediation technologies in sites contaminated with petroleum oil residues, aligning with the principles of sustainable development [43].Polyhydroxybutyrate (PHB), known for its favorablephysico-chemical and biological properties, demonstrates ready biodegradability in river water and compost. Compost, with its richer biodiversity, serves as a more suitable environment for PHB biodegradation. However, the challenge arises when incorporating higher concentrations of PHMG derivatives into PHB, resulting in reduced biodegradability. Addressing this issue, applying biofilm-forming microorganisms to the surface of polyhydroxybutyrate (PHB) offers a solution and may even accelerate the overall biodegradation operation. Bacillus toyonensis HW1 isolates have been identified as particularly effective in enhancing the degradation of PHB composites [44].Bacillus toyonensis SFC 500-1E, part of the consortium SFC 500-1, demonstrates proficiency in Cr(VI) removal and great phenolic resistance. Proteomic analysis revealed 400 differentially expressed proteins, with 205 up-regulated in the context of Cr(VI)+phenol and 152 down-regulated under Cr(VI). The strain adapts by affecting key metabolic pathways, including carbohydrate, energetic, lipid, and amino acid metabolism. ABC transporters, iron-siderophore transporters, and stress-related responses play crucial roles. This study enhances recognition of B. toyonensis metabolism in bioremediation and contributes to optimizing its use in environmental cleanup [45].

2.3. Role in the vaccination

The incorporation of spores from Bacillus toyonensis shows significant promise as a key component in the development of vaccine formulations. Studies in mice have demonstrated that the adjuvanticity of BoHV-5 rgD was notably enhanced when B. toyonensis spores, whether live or inactivated, were combined with alum [46]. This probiotic holds potential for augmenting the reactions generated by recombinant vaccines, particularly those designed to enhance both cell- and antibody-mediated immune responses for optimal effectiveness [21].In the context of vaccination regimens, a simple five-day administration with B. toyonensis BCT-7112T prior to vaccination has been shown to dramatically improve the ewe humoral immune response to the C. perfringens recombinant ETX vaccine. This suggests a potential role for B. toyonensis BCT-7112T as an immune modulator, showing its ability to enhance immune responses with short-term probiotic supplementation [47]. Furthermore, subcutaneous injection of B. toyonensis BCT-7112T spores has demonstrated an augmented cellular and humoral immune response against BoHV-5 in mice. Splenocytes from mice administered B. toyonensis spores in the vaccine preparation exhibited elevated transcription levels of the cytokines IL-12, IL-4, and IFN-γ. Additionally, the activation of the macrophage-like cell line RAW264.7 with B. toyonensis spores significantly increased cell growth and mRNA transcription levels of IL-12 and IL-4 cytokines in such cells [48].The addition of B. toyonensis supplementation to enhance effectiveness against BoHV-5 has been implemented in sheep. This resulted in increased seroconversions and higher titres of neutralizing antibodies ($p> 0.05$) against BoHV-5 compared to non-supplemented animals.

After sixty-three days of investigation, enriched lambs showed greater mRNA transcription levels of the cytokines IL-17A and IL-10 in splenocytes [49]. These findings highlight the immunomodulatory potential of B. toyonensis spores in vaccine development, contributing to enhanced immune responses. Supplementing, with this bacteriahas shown promise in enhancing the immune response to canine parvovirus vaccines.The supplementation proved effective in promoting the production of specific IgG antibodies against parvovirus, with considerably greater titers compared to those in the control group. The increases were notable, reaching 2-fold at 7 days post-vaccination, 3-fold at 21 days, and 2.5-fold at 35 days post-vaccination. Moreover, T cells, B cells, and NK cells from dogs treated with B. toyonensis DNA, vegetative cells, and spores showed increased mRNA transcription for the cytokines IL-17, IL-4, and IFN-γ in response to these stimuli, indicating an enhanced immune response [50]. In a related context, brief-period feeding with both B. toyonensis and S. boulardii has been found to have an adjuvant effect on the immunological response to the C. chauvoei vaccination in sheep. Sheep receiving supplementation with S. boulardiiand B. toyonensis exhibited considerably elevated titters of specific IgG, IgG1, and IgG2, with approximately 14 and 24-fold increases in total IgG levels compared to the non-supplemented group. Additionally, T cells, B cells, and NK cells from the supplemented group displayed higher mRNA transcription levels of the IFN-γ, IL2, and Bcl6 genes, indicating an enhanced immune response [47]. These findings underscore the potential of B. toyonensis in boosting immune responses, offering implications for vaccine development in various animal species.

2.4. Pharmaceutics potential

The introduction of B. toyonensis SAU-19 has demonstrated significant positive effects in the context of type 2 diabetes mellitus (T2DM). This probiotic strain led to an enhancement in blood glucose levels, a reduction in liver insulin resistance, and positive structural changes in the liver. The improvements included a significant amelioration of dyslipidemia, enhancedglycogen formation, and improved levels of antioxidants($p < 0.05$). These findings collectively suggest that B. toyonensis SAU-19 possesses beneficial effects in mitigating hepatic insulin resistance associated with T2DM. Furthermore, this probiotic strain has been observed to exert an inhibitory effect on T2DM by not only mitigating insulin resistance but also enhancing levels of antioxidants and suppressing genes associated with the synthesis of glucose. These effects highlight the potential therapeutic applications of B. toyonensis SAU-19 in the treatment of diabetes and other disordersof metabolism [51]. The observed positive impacts on glucose metabolism, insulin resistance, and liver health emphasize the potential of this probiotic strain as a valuable asset in managing and addressing metabolic disorders, particularly type 2 diabetes mellitus.

B. toyonensis is identified as one of the endophytic bacteria that resides within host plant tissues without causing harm. This bacterial species shows potential antibiotic activities attributed to the synthesis of secondary metabolic products [52]. In evaluating the risk of using B. toyonensis as a probiotic, gene activity studies focused on enterotoxins, specifically haemolysin BL (Hbl) and the non-haemolytic enterotoxin (Nhe). The expression levels of enterotoxin genes (hblA, hblD, nheA, nheB, and nheC) in B. toyonensis BCT-7112T were found to be significantly lower compared to those in B. cereus reference strains. The expression of enterotoxins and protein levels in B. toyonensis were either absent or reduced, suggesting a diminished or less probable capacity to produce active enterotoxins compared to B. cereus reference varieties. This method of experimentation serves as a valuable tool for assessing the health, safety, and non-harmful nature of probiotic microorganisms [53].B. toyonensis VU-DES13 has exhibited high resistance to penicillin and demonstrated the ability to inhibit the growth of various pathogens. The genome study indicated an excess of genes that confer resistance specifically associated with beta-lactam antibiotics compared to the type isolate. Additionally, the analysis identified gene clusters responsible for the synthesis of secondary metabolites, includingbacteriocins, siderophores, and non-ribosomal peptide synthetases [54].

This resistance profile and the synthesis of bioactive compounds enhance the potential of B. toyonensis in various applications, including as a probiotic with reduced risks associated with enterotoxin production. The supernatant derived from cultures of Bacillus toyonensis BV-17 has demonstrated a remarkable ability to rapidly kill different tumour cell lines in vitro within moments. This cytotoxic effect was observed through mechanisms such as membrane rupture, blebbing, and cytoplasmic leakage. Analysis using mass spectrometry and Fast Protein Liquid Chromatography (FPLC) identified hemolysin BL (HBL) as the active molecule responsible for this cytotoxicity. This discovery presents a distinct mechanism compared to previous studies, and it's noteworthy that intra-tumour injection of small amounts of HBL hindered the development of both cured and unattended tumours in mice.

These ground-breaking findings indicate that HBL demonstrates anticancer activity and holds potential as a chemotherapeutic drug. Furthermore, there is potential for engineering HBL to selectively target tumour cells in future applications [55]. In a different context, B. toyonensis SAU-20 has been shown to mitigate resistance to insulin and liver steatosis by enhancing levels of lipids and antioxidantsand downregulating lipogenic genes. Additionally, it suppresses the expression of pro-inflammatory cytokines, contributing to its potential in addressing metabolic disorders such as insulin resistance and hepatic steatosis [56].Bacillus toyonensis HST13 is one of the Bacillus species strains that exhibit antimicrobial activity against plant pathogens and produces beneficial traits like chitinase and indole-3-acetic acid. Bacillus cereus and others stimulate garden cress growth, suggesting medicinal plants as promising sources for plant-beneficial bacteria to enhance plant growth and combat soil-borne pathogens [57].This microorganism was employed to generate chitosanase, which, in turn, facilitated the hydrolysis of chitosan. The resulting chitosanase exerted an important influence in the formation of chitooligosaccharides, contributing to their pharmaceutical efficacy in wound healing [58].These results emphasize the varied capabilities of Bacillus toyonensis in various therapeutic applications, ranging from potential chemotherapeutic agents to agents addressing metabolic disorders.

2.5. Agricultural uses

Bacillus toyonensis has been identified as a plant-promoting bacterium, and the strain COPE52, with a total of 5979 genes, including 5631 protein-coding genes, 81 tRNA genes, 11 rRNA genes, 5 ncRNAs, and 251 pseudogenes, is particularly noteworthy in this regard. This strain has been shown to boost biomass growth and chlorophyll levels in blueberries. The mechanisms underlying these effects involve the synthesis of indoleacetic acid, protease activity, and the release of volatiles such as 2,3-butanediol, acetoin, and dimethyl disulphide. Additionally, the strain exhibits antifungal activity against the phytopathogenic fungus Botrytis cinerea, commonly known as grey mold [59]. The study highlights the possibility of Bacillus toyonensis, specifically the COPE52 strain, in promoting plant growth, enhancing chlorophyll content, and providing protection against pathogenic fungi, thereby contributing to the overall health and vitality of the plants.

Bacillus toyonensis COPE52 has demonstrated the capability to change membrane phospholipids, suggesting a possible protective way that sustains plant growth-promoting (PGP) characteristics in saline soil conditions. When subjected to 100 and 200 mM NaCl, this strain exhibited an increased relative quantity of branched-chain fatty acids, including 15:0i and 16:1Δ9, and the accumulation of an unidentified membrane lipid. Conversely, phosphatidylethanolamine (PE) levels decreased during its growth in the presence of NaCl. In an indoor project involving tomato plants (LycopersiconesculentumSaladette), the inoculation with B. toyonensis COPE52 culminated in enhanced root and shoot length, increased dry biomass, and improvedlevels of chlorophyll. These effects were observed both in the absence of NaCl (0 mM) and under 100 mM NaCl conditions. This shows the potential of B. toyonensis COPE52 to positively influence plant growth and performance, even in saline soil conditions [60].

It is one of the probiotic bacteria that has partial capacity for the insecticide property against Spodopteraexigua that infects corn, where it was found in the gut of this insect in the larval stage [61]. It is present mainly in the roots of plants and deep soil environments, thereby promoting phosphorous solubilization [190.5 µg/mL of PO4− equivalents] in the soil. Hence, it has potential for soil recovery, especially in fire affected agricultural areas [62].B. toyonensis as a plant growth promoting bacteria was supported by the study of Robas Mora et al., (2022). It can reduce plant rhizosphere stress caused by high mercury (Hg) pressure and improve the tolerance of plants to such environmental situations [63]. Genomic analysis of B. tyonensis GM18 strain also showed the visibility of pesticide, nematicide, and anticancer proteins [23]. It is also the best producer of poly-γ-glutamic acid (γ-PGA), which is an amino acid polymer that offers an ecologically sound alternative to plastic products, which have significantly damaged the environment.

The optimum condition in the medium is glucose, and ammonium sulfate at 25°C with a pH of 5.5 and an incubation period of 48 hours. The highest yield can be obtained through the fermentation of agricultural waste such as cassava peels[64].B. toyonensis shows promise in its ability to generate a range of antimicrobial substances. The conducted analyses suggest that the endophytic strain BAC3151 has the potential to be instrumental in devising novel approaches for mitigating microbial diseases in plants, which are known to inflict significant harm on agricultural crops. Four Bacillus strains with complete genome sequences exhibited a shared set of 3811 protein-coding DNA sequences (CDSs). However, strain-specific CDSs, such as those responsible for producing antimicrobial compounds, were linked to distinct chromosomal regions and mobile genetic elements within the strains. B. toyonensis strains displayed a greater prevalence of potential bacteriocin gene clusters compared to the traditional Bacillus species employed for antimicrobial synthesis.

Moreover, BAC3151 contained gene clusters that could be engaged in the creation of new bacteriocins, as well as genes responsible for various other compounds, including non-ribosomal peptides, N-acyl homoserine lactonase, and chitinases. This extensive genetic repertoire suggests a greater capacity for antimicrobial synthesis in B. toyonensis strains than in other Bacillus strains that have proved effective against plant pathogens [65]. The study evaluated the positive impact of Bacillus toyonensis strain Bt04, isolated from the rhizosphere of the Algerian Sahara, on maize roots. The findings indicate that Bt04 produces indole-3-acetic acid (IAA) both from tryptophan (Trp) and endogenously. The strain also produces higher concentrations of another endogenous auxin, non-indole phenylacetic acid (PAA), compared to IAA. Furthermore, Bt04 has been shown to produce cytokinins (CKs). Inoculation with Bt04 led to the development of maize growth and increased root formation, particularly under conditions of aluminium (Al) toxicity. Importantly, Bt04 reduced the accumulation of aluminium in young maize roots. The study also demonstrated that Bt04 itself is tolerant to high concentrations of $AlCl_3$. The growth-promoting effect of Bt04 may be attributed, at least in part, to increased defence from oxidative damage, as bacterial treatment decreased lipid peroxidation in seedlings of maize exposed to Al toxicity [66].

3. Pathogenesis

Bacillus toyonensis exhibits diverse interactions with different organisms, with implications for plant health, insect toxicity, biofilm formation, and potential concerns for dairy plants. In the context of plant interactions, a study suggests that B. toyonensis may have pathogenic effects on the plant Sorocea, as it showed resistance to agry-gent antibiotics. This resistance has been linked with a decline in plant growth and a rise in bacterial growth by 19%, raising concerns about its potential negative impact on seedling performance [67]. In terms of insect toxicity, a newly discovered biovar, Bacillus toyonensis biovar Thuringiensis, displayed toxicity towards certain insects, such as Cydiapomonella and Anthonomus grandis. However, it exhibited relatively low toxicity against Aedesaegypti and Alphitobiusdiaperinus and showed no toxicity towards the free-living nematode Panagrellusredivivus [68]. Concerns also arise in the context of dairy plants, where Bacillus toyonensis has been found to form biofilms even on stainless steel surfaces, raising potential issues for hygiene and contamination in dairy production facilities [69]. Additionally, a pathogenic strain of B. toyonensis, specifically strain P18, has been identified in the deep sea environment. This strain contains 319 putative virulence genes and is closely related to strain 7112T. It has demonstrated survival in fish and mouse sera and possesses hemolytic activity, indicating potential pathogenicity in these environments[22].The strain BCT-7112, initially identified as Bacillus cereus var. toyoi, has been established as the type strain for the newly classified species Bacillus toyonensis within the B. cereus group[30]. The preliminary genome analysis of B. toyonensis strain 4HC1, collected from marine wastes, reveals the presence of numerous antibiotic resistance genes (ARGs) and virulence genes, indicating resistance to multiple drugs. The draft genome of strain 4HC1 is 6,156,259 base pairs in size, comprising 133 contigs, with a GC content of 34.95%. It encompasses 6089 protein-coding genes, 86 tRNAs, and 24 rRNAs. Strain 4HC1 demonstrates resistance to trimethoprim, cefotaxime, and ampicillin, harboring various antibiotic resistance genes, including an aminoglycoside 6-adenylyltransferaseβ-lactamase, a TetM family tetracycline resistance gene, two distinct tetracycline efflux pumps, and a bleomycin resistance gene. Additionally, the strain carries virulence genes associated with immune evasion, iron acquisition, and toxin production [70].

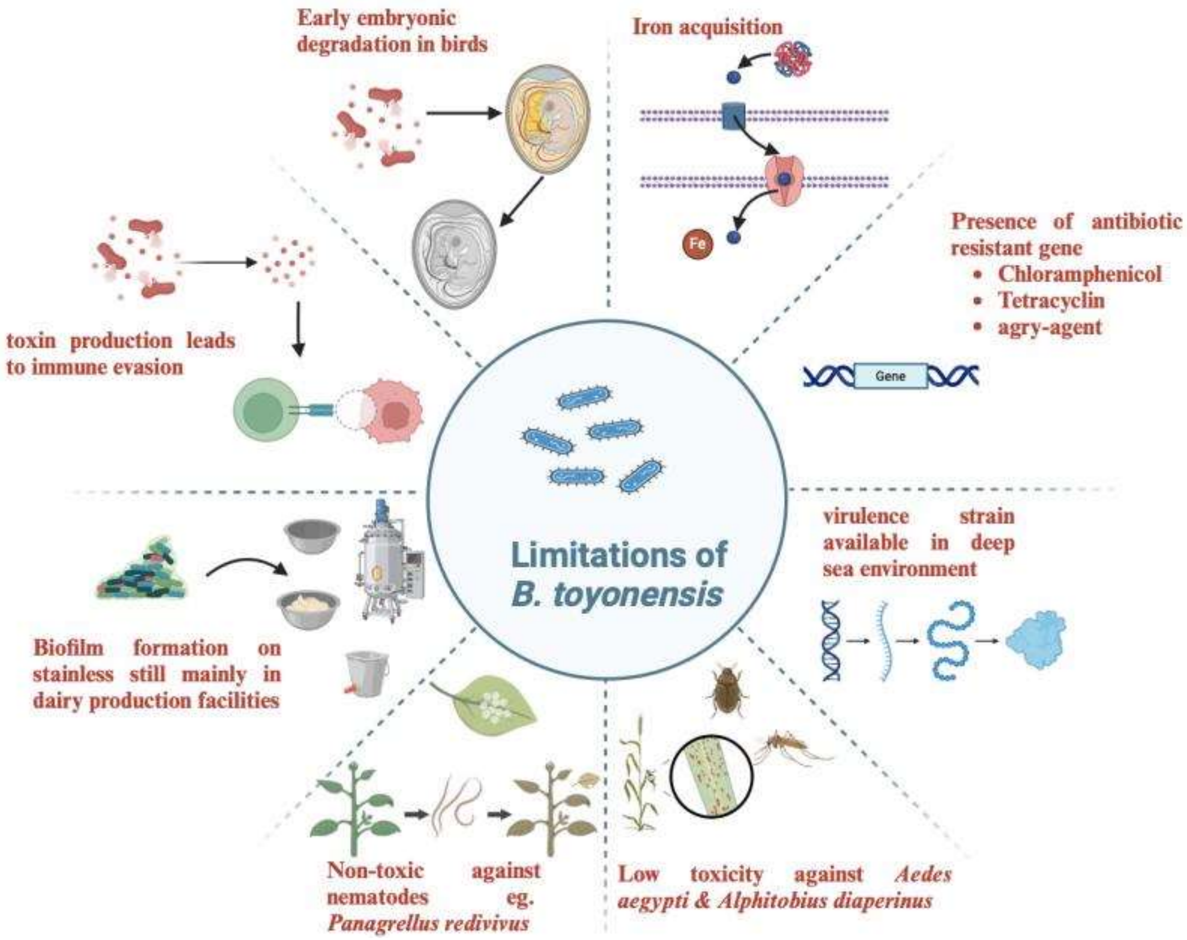


Fig 3.An illustration of the pathogenicity of B. toyonensis.

Bacillus toyonensis is identified as one of the bacteria in the microbiota found in microbial contamination of eggshells, potentially causing early embryonic stage degeneration and bird death [71]. Moreover, it has been shown to induce endophthalmitis, with the strain LY557 capable of causing complete retinal function loss similar to B. thuringiensis and B. cereus. The presence of molecular indicators of virulence and motility genes, disproportionately prevalent in intraocular isolates, suggests potential contributions to host adaptation [72].

Strain name of B.toyonensis	**Source of isolation**	**Identified activities**	**References**
BCT GM18	Agricultural soil, Mexico	Pesticide, nematicide and anticancer	[23]
BCT XIN-YC13	Soil, China	Toyoncin production, robust pH, heat stability, antibacterial against B. cerues, L. monocytogenes	[28]
BCT 7112T	Soil, Japan	Heamolytic activity in fish, Toyocerin production, decrease proinflammatory production, increase immunological function, improved immune response through vaccination against C. perfringens and BoHV-5 in mice, increase macrophage activity, increase IL-12, IL-4, IL17A and IL-10 cytokines, less enterotoxin genes	[22,30,31,47,48,49,53]
BCT PNTB1	Arabian sea, India	Bioremediation through CM-cellulase, lipase and chitinase activity	[38]
BCT AM07	Dike effluent of Urucu Petrol Basin, Amazonas-Brazil	Petroleum breakdown	[39]
BCT AK2	Compost, Poland	Biodegradation of tar	[41]
BCT HW1	Lake water, Poland	Degradation of polyhydroxy butyrate	[44]
BCT SFC500-1E	Sedimentation sludge, Argentina	Cr(VI) removal and phenolic resistance	[45]
BCT SAU-19	Ageretinaadenophra, China	Decrease type 2 diabetes mellitus, increase antioxidant level and decrease metabolic disorders	[51]
BCT VU-DES13	Folsomia candida	Penicillin resistance, genes for bacteriocins, sidephores, non-ribosomal peptide synthetase	[54]
BCT BV-17	Host Homo sapiens, China	Antitumor through membrane rupture, blebbing and cytoplasmic leakage by haemolysin production	[55]
BCT SAU-20	Ageretinaadenopohra, China	Decrease proinflammatory cytokines and insulin resistance and hepatic steatosis	[56]
BCT HST13	Iris pseudocarus L., Uzbekistan	Antimicrobial against phytopathogens and enhance plant growth	[57]
BCT COPE52	Root endosphere of Rubusulmifolius, Mexico	Increase biomass growth and chlorophyll level in blueberries, antifungal (Botrytis cinerea), sustain plant growth in saline conditions	[59,60]
BCT BAC3151	Leaves of Phaseolus vulgaris	Antimicrobial against phytopathogens through non-ribosomal peptides, N-acyl hemoserinelactonase and chitinase	[65]
BCT Bt04	Rhizosphere of Algerian Sahara, on maize	Indole-3-acetic acid production and enhance maize growth in Aluminium toxicity conditions	[66]
BCT 4HC1	Marine plastic, Norway	Antibiotic resistant genes against trimethoprim, cefotaxime, ampicillin, tetracycline, bleomycin and virulence genes	[70]
BCT LY557	Patients diagnosed with posttraumatic	Loss of retinal function	[72]

	endophthalmitis		

Table 1: List of identified strains of B. toyonensis along with their source of isolation and identified characteristics.

Toyocerin®, a feed additive originally classified as Bacillus cereus and later reassigned to Bacillus toyonensis, faced suspension of authorizations due to safety concerns raised by the EFSA. The applicant submitted additional data for re-evaluation, revealing that B. toyonensis carries resistance genes to chloramphenicol and tetracycline, posing a risk for antibiotic resistance spread. The strain also harbors enterotoxin genes, indicating a potential risk to human health. While less potent than positive controls, B. toyonensis can produce cytotoxic agents. Despite its potential to enhance piglet performance, caution is advised due to safety considerations. Compatibility issues with certain coccidiostats were noted, excluding salinomycin sodium and semduramycin sodium [73].Genome sequencing of Bacillus toyonensis in Toyocerin1, a feed additive, revealed tetM and cat genes associated with tetracycline and chloramphenicol resistance. tetM and cat genes are present in the commensal microbiota, and Toyocerin1 does not exacerbate resistance in the intestines beyond the introduced B. toyonensis spores [74].

4. Conclusion

In conclusion, Bacillus toyonensis is a versatile bacterium with various applications in diverse fields. It has probiotic properties in animal nutrition due to its positive effects on growth, immune function, and microbial communities. It also exhibits significant potential in bioremediation and biodegradation. However, it has potential risks, including pathogenicity and antibiotic resistance. Some strains have virulence genes and hemolytic activity, posing safety concerns. The versatility of Bacillus toyonensis is further emphasized by its role in vaccination, where it has shown promise as an adjuvant in enhancing immune responses. Additionally, the bacterium exhibits pharmaceutical potential, with strains demonstrating positive effects in the context of type 2 diabetes mellitus and showing cytotoxic effects against tumour cell lines. In agriculture, it promotes chlorophyll content, plant growth, and protection against pathogenic fungi. It also has insecticidal properties, aiding in soil recovery. Further research and careful evaluation are warranted to fully understand and harness the capabilities of Bacillus toyonensis across its diverse range of applications.

Conflicts of interest

The authors declare no conflicts of interest to disclose.

Acknowledgements

The authors would like to thank Annamalai University (www.annamalaiuniversity.ac.in), Chidambaram, India, and the University of Kashmir (www.kashmiruniversity.net) for their support in the present work.

REFERENCES

1. Turnbull P, Kramer J, Melling J. Bacillus In: Topley and Wilson Principles of Bacteriology. Virology and Immunity 8th ed Edward Arnold, London. 1990:185-210.
2. Turnbull PC. Bacillus in Medical Microbiology.(Baron, S.) Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 15.
3. Ehling-Schulz M, Lereclus D, Koehler TM. The Bacillus cereus group: Bacillus species with pathogenic potential. Microbiology spectrum. 2019 May 17;7(3):10-128. https://doi.org/10.1128/microbiolspec.gpp3-0032-2018
4. Sauka DH, Peralta C, Pérez MP, Onco MI, Fiodor A, Caballero J, Caballero P, Berry C, Del Valle EE, Palma L. Bacillus toyonensis biovar Thuringiensis: A novel entomopathogen with insecticidal activity against lepidopteran and coleopteran pests. Biological Control. 2022 Apr 1;167:104838. https://doi.org/10.1016/j.biocontrol.2022.104838

5. Spencer RC. Bacillus anthracis. Journal of clinical pathology. 2003 Mar 1;56(3):182-7.
6. Roonie A, Majumder S, Kingston JJ, Parida M. Molecular characterization of B. anthracis isolates from the anthrax outbreak among cattle in Karnataka, India. BMC microbiology. 2020 Dec;20:1-2.
7. Guinebretière MH, Auger S, Galleron N, Contzen M, De Sarrau B, De Buyser ML, Lamberet G, Fagerlund A, Granum PE, Lereclus D, De Vos P. Bacillus cytotoxicus sp. nov. is a novel thermotolerant species of the Bacillus cereus group occasionally associated with food poisoning. International journal of systematic and evolutionary microbiology. 2013 Jan;63(Pt_1):31-40.
8. Etter D, Biggel M, Greutmann M, Cernela N, Johler S. New insights into Bacillus cytotoxicus sources, screening, toxicity, and persistence in food production facilities. Food Microbiology. 2024 Feb 1;117:104399.
9. Fayad N, Koné KM, Gillis A, Mahillon J. Bacillus cytotoxicus genomics: chromosomal diversity and plasmidome versatility. Frontiers in Microbiology. 2021 Dec 9;12:789929.
10. Cairo J, Gherman I, Day A, Cook PE. Bacillus cytotoxicus—A potentially virulent food-associated microbe. Journal of Applied Microbiology. 2022 Jan 1;132(1):31-40.
11. Lechner S, MAYR R, Francis KP, PRÜß BM, KAPLAN T, Wießner-Gunkel EL, STEWART GS, SCHERER S. Bacillus weihenstephanensis sp. nov. is a new psychrotolerant species of the Bacillus cereus group. International journal of systematic and evolutionary microbiology. 1998 Oct;48(4):1373-82.
12. Stenfors LP, Mayr R, Scherer S, Granum PE. Pathogenic potential of fifty Bacillus weihenstephanensis strains. FEMS microbiology letters. 2002 Sep 1;215(1):47-51.
13. Soufiane B, Côté JC. Bacillus weihenstephanensis characteristics are present in Bacillus cereus and Bacillus mycoides strains. FEMS microbiology letters. 2013 Apr 1;341(2):127-37.
14. Thorsen L, Hansen BM, Nielsen KF, Hendriksen NB, Phipps RK, Budde BB. Characterization of emetic Bacillus weihenstephanensis, a new cereulide-producing bacterium. Applied and environmental microbiology. 2006 Jul;72(7):5118-21.
15. Ali B, Wang X, Saleem MH, Azeem MA, Afridi MS, Nadeem M, Ghazal M, Batool T, Qayyum A, Alatawi A, Ali S. Bacillus mycoides PM35 reinforces photosynthetic efficiency, antioxidant defense, expression of stress-responsive genes, and ameliorates the effects of salinity stress in maize. Life. 2022 Jan 30;12(2):219.
16. Fiedoruk K, Drewnowska JM, Mahillon J, Zambrzycka M, Swiecicka I. Pan-genome portrait of Bacillus mycoides provides insights into the species ecology and evolution. Microbiology Spectrum. 2021 Aug 31;9(1):10-128.
17. Nakamura LK. Bacillus pseudomycoides sp. nov. International Journal of Systematic and Evolutionary Microbiology. 1998 Jul;48(3):1031-5.
18. Paul GK, Mahmud S, Dutta AK, Sarkar S, Laboni AA, Hossain MS, Nagata A, Karmaker P, Razu MH, Kazi T, Uddin MS. Volatile compounds of Bacillus pseudomycoides induce growth and drought tolerance in wheat (Triticumaestivum L.). Scientific Reports. 2022 Nov 9;12(1):19137.
19. Ibrahim MA, Griko N, Junker M, Bulla LA. Bacillus thuringiensis: a genomics and proteomics perspective. Bioengineered bugs. 2010 Jan 1;1(1):31-50.
20. Palma L, Muñoz D, Berry C, Murillo J, Caballero P. Bacillus thuringiensis toxins: an overview of their biocidal activity. Toxins. 2014 Dec 11;6(12):3296-325.
21. Santos FD, Menegon YA, Piraine RE, Rodrigues PR, Cunha RC, Leite FL. Bacillus toyonensis improves immune response in the mice vaccinated with recombinant antigen of bovine herpesvirus type 5. Beneficial Microbes. 2018 Jan 29;9(1):133-42. https://doi.org/10.3920/BM2017.0021

22. Luo JC, Long H, Zhang J, Zhao Y, Sun L. Characterization of a deep sea Bacillus toyonensis isolate: genomic and pathogenic features. Frontiers in cellular and infection microbiology. 2021 Mar 10;11:629116. https://doi.org/10.3389/fcimb.2021.629116
23. Moreno-Peña CJ, García-García JH, Hernández-Vásquez CI, Galán-Wong LJ, Pereyra-Alférez B. Draft Genome Sequence of Bacillus toyonensis Strain GM18, Isolated from Agricultural Soil. Microbiology Resource Announcements. 2021 Mar 4;10(9):10-128. https://doi.org/10.1128/MRA.00008-21
24. Sánchez-Galván IR, Ferrer J, Galante E, Marcos-García MA. Bacteria and hoverflies (Diptera: Syrphidae) in tree hollows from the Iberian Mediterranean forest. Environmental entomology. 2017 Feb 1;46(1):137-42. https://doi.org/10.1093/ee/nvw158
25. Hubert J, Erban T, Kopecky J, Sopko B, Nesvorna M, Lichovnikova M, Schicht S, Strube C, Sparagano O. Comparison of microbiomes between red poultry mite populations (Dermanyssusgallinae): predominance of Bartonella-like bacteria. Microbial ecology. 2017 Nov;74:947-60. https://doi.org/10.1007/s00248-017-0993-z
26. Zervas A, Aggerbeck MR, Allaga H, Güzel M, Hendriks M, Jonuškienė I, Kedves O, Kupeli A, Lamovšek J, Mülner P, Munday D. Identification and characterization of 33 Bacillus cereus sensulato isolates from agricultural fields from eleven widely distributed countries by whole genome sequencing. Microorganisms. 2020 Dec 18;8(12):2028. https://doi.org/10.3390/microorganisms8122028
27. Bertel Mesa LM, BetancurHurtado CA, Oviedo Zumaque LE. Identificación de Bacillus toyonensis en hecesdeganadocebú en el Departamento de Sucre, Colombia. Revistacolombiana de Biotecnología. 2019 Dec;21(2):12-21. https://doi.org/10.15446/rev.colomb.biote.v21n2.69421
28. Wang J, Xu H, Liu S, Song B, Liu H, Li F, Deng S, Wang G, Zeng H, Zeng X, Xu D. Toyoncin, a novel leaderless bacteriocin that is produced by Bacillus toyonensis XIN-YC13 and specifically targets B. cereus and Listeria monocytogenes. Applied and Environmental Microbiology. 2021 May 26;87(12):e00185-21. https://doi.org/10.1128/AEM.00185-21
29. Kantas D, Papatsiros VG, Tassis PD, Giavasis I, Bouki P, Tzika ED. A feed additive containing Bacillus toyonensis (Toyocerin®) protects against enteric pathogens in postweaning piglets. Journal of applied microbiology. 2015 Mar 1;118(3):727-38. https://doi.org/10.1111/jam.12729
30. Jiménez G, Blanch AR, Tamames J, Rosselló-Mora R. Complete genome sequence of Bacillus toyonensis BCT-7112T, the active ingredient of the feed additive preparation Toyocerin. Genome announcements. 2013 Dec 26;1(6):10-128. https://doi.org/10.1128/genomeA.01080-13