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Review Article

Current Status of Research on Ultraviolet Mutagenesis of Bacillus Subtilis

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Abstract

Bacillus subtilis is an active substance capable of producing a variety of antimicrobials, enzymes and promoting the growth of plants and animals, which is widely used in modern agricultural production due to its clear research background, wide range of application scenarios, rich industrial use time, non-toxicity and harmlessness to human beings, and its ability to produce spores to tolerate adverse environments. If Bacillus subtilis is further mutated, it can enhance the production of antimicrobials and enzymes, and improve the economic benefits and work efficiency, UV mutagenesis as a safer and widely used mutation technique has been chosen by many scientists, this paper summarizes the current status of Bacillus subtilis after UV mutagenesis in various applications in recent years, and elucidates the future application prospects and research hotspots of Bacillus subtilis in the field of agriculture. The paper summarizes the current status of the application of Bacillus subtilis after UV mutagenesis in various aspects in recent years, and elucidates the future prospects of Bacillus subtilis in the field of agriculture and research hotspots.

Keywords: Bacillus subtilis, UV mutagenesis.

Bacillus subtilis, widely used in the late twentieth century, is a rod-shaped Gram-positive aerobic bacteria, monolayer membrane, flagellum-assisted locomotion, no pods, because it is easy to grow and reproduce in the sap of the withered grass, extreme conditions, can be induced to produce endogenous spores resistant to high temperatures of 80 degrees Celsius, in order to achieve high temperatures and high pressures, such as adverse environments and anti-reversal purposes, hence the name of bacillus subtilis; whether in nature, plants and animals or organic matter in decay or soil can be seen in the figure, but also a common plant endophyte harmless to humans and animals. Whether in the natural world of plants and animals, organic matter in the process of corruption or soil can be seen in the figure of bacillus, but also a common non-toxic and harmless plant endophytes for humans and animals. Due to the fast growth rate of Bacillus subtilis, simple nutritional requirements, easy to grow and reproduce, not pathogenic to humans, and can secrete a variety of enzymes such as cellulase, protease, amylase, antibiotics and a variety of sugars, does not contaminate the environment, is often used as a biological type of fungicide, inhibit the growth of harmful bacteria [1-3].

The use of ultraviolet light as a physical mutagenic factor for mutagenesis of industrial strains, the changes produced by such mutagenesis include DNA strand breakage, DNA intramolecular and intermolecular cross-linking, cross-linking of nucleic acids and proteins, pyrimidine hydrate and pyrimidine dimer production, etc., especially the production of pyrimidine dimer for the changes in the DNA to play a major role in the [4]. UV mutagenesis has been used in biology for a long time, and up to now, nearly 80% of the high yielding bacteria obtained after treatment with various mutagenic factors are obtained by UV mutagenesis [5].

1. CURRENT STATUS OF APPLICATIONS

1.1 Chemical Applications

Produce a variety of active substances, enzymes and vitamins, such as can produce chytridiomycin, polymyxin, mycoplasma, short peptide and other active substances, play a role in inhibiting the role of pathogenic bacteria or cause endogenous infections of conditional pathogens; can stimulate the plant body to produce disease-resistant factors in order to improve the immunity of the plant; but also can be synthesized by the α-amylase, proteases, lipases, cellulases and other enzymes Enzymes [6], synergize with enzymes

produced by other plants; Promote plant growth and development by synthesizing a variety of B vitamins such as B1, B2, B6, niacin, etc. [4].

1.2 Physical Aspects

Bacillus is quick to consume the unused oxygen in the environment, turning the surroundings into a low-oxygen or even anaerobic environment, which promotes the growth of beneficial anaerobic bacteria while indirectly inhibiting the growth of pathogenic aerobic bacteria [7].

2. MECHANISM OF ACTION

The mechanisms and modes of action of Bacillus subtilis are not homogeneous but diverse, including: competition, antagonism, bacteriolysis, induction of plant disease resistance and promotion of plant growth [8]. For the genus Bacillus, synthesis of antimicrobial substances is one of the most important biocontrol mechanisms, followed by competition for nutrients and spatial aspects, in addition to the indirect induction of plant systemic resistance.

2.1 The Role of Competition

It can be divided into two kinds of nutritional and site competition, which is manifested as space, nutrients, oxygen, etc. in the same bacterial environment are jointly competed by two and more microorganisms, and the growth and development of the inferior organisms are inhibited, so as to achieve the prevention and control of harmful species [9], e.g., Lei Jingjing *et al.*, [10] constructed the inhibition rate of Bacillus subtilis B2-GFP against tobacco blight mold can reach 48.30%, and wang *et al.*, [11] enhanced the manipulator SPOVE, which showed higher inhibitory activity against sweet potato black spot pathogen because of the improved attachment topping ability.

2.2 Antagonism

The main manifestation is that when more than or equal to two kinds of microorganisms grow together, the surrounding growth environment will be changed because one kind of microorganism produces one or several kinds of specific secondary metabolites, and assimilation occurs, and inhibits or even kills the other kind of microorganisms [12], Li Yan *et al.*, [13] isolated a strain of endophytic bacterium B. subtilis C1R32, which has a stabilizing antagonistic effect on the pathogen of mulberry botrytis, and the supernatant of fermentation could significantly inhibit the growth of S. sclerotiorumPZ-2, and damage the cell structure of the pathogen. The fermentation supernatant could significantly inhibit the growth of S. sclerotiorumPZ-2 and destroy the cell structure of the pathogen; Wang *et al.*, [14] isolated an endophytic bacterium, B. subtilis BQ-33, which had a good antagonistic effect on B. subtilis of kiwifruit, and it could destroy the cell wall of the pathogenic bacterial mycelium and make a large amount of inorganic salts, proteins and nucleic acids exuded.

2.3 Inducing Resistance

Inducing the plant's own disease resistance potential such as: secretion of antibiotics, nutrient and biological ecosystem competition, indirectly enhancing the plant's disease resistance [15], Samaras *et al.*, [16] applied Bacillus subtilis BsMBI1600 to pathogenic tomato plants for defense gene analysis and found that B. subtilis activated the molecular mechanisms related to tomato defense and induced the development of systemic resistance (ISR). Amin *et al.*, [17] found that B. subtilis EMCCN 1211 could effectively inhibit the accumulation level of potato Y virus and alleviate the damage of potato. found that Bacillus subtilis EMCCN 1211 could effectively inhibit the accumulation level of potato Y virus and reduce potato damage.

2.4 Promotion of plant growth

Promote the growth of plant roots [18] and plants, indirectly enhance the ability of plant disease resistance [19] and reduce the occurrence of plant disease phenomenon, Li Chun *et al.*, [20] found that the application of Bacillus subtilis alone has obvious promotion effect on the growth of cucumber seedlings. Zhao *et al.*, [21] found that microalgae with the application of Bacillus subtilis can effectively improve soil nutrients, regulate the change of nutrient patterns, significantly increase the diversity of soil bacteria, optimize the structure of soil bacterial communities, and promote the interaction between bacterial communities.

3. MUTAGENESIS

3.1 Classification of mutagenesis

Mutagenesis mainly has: physical method, chemical method, space technology method, composite method and other kinds of mutagenesis methods.

3.2 Characterization of ultraviolet light

Located between x-rays and visible light waves, as an ultraviolet radiation, it is present in both sunlight and man-made light sources.

3.3 Mechanism of ultraviolet light

The use of ultraviolet rays with wavelengths between 200 and 400 as non-ionizing radiation on the irradiated object causes the inner electrons in the molecules of the irradiated substance to be excited, leading to physical and chemical changes in the molecules, thus realizing the purpose of mutagenesis [5].

3.4 Use of ultraviolet light

There are direct and indirect methods to determine the dose of UV light. Laboratory generally choose 15W UV lamp, distance control in thirty inside or so, lethality 80% ~ 90% of the time between the radiation for the optimal irradiation time, in this optimal irradiation time for the purpose of mutagenesis strains of

UV irradiation, irradiation is carried out when the liquid can not be selected to the composition of the complexity of the liquid, such as broth occurs, in order to prevent the occurrence of unanticipated chemical reactions to produce interference; at the same time, in the bacterial liquid concentration is too large, At the same time, in the bacterial liquid concentration is too large, the bacterium or spores and other large or heavy, in the mutation of the liquid is prone to sedimentation, to add electromagnetic stirrer and other equipment that can make the solution uniform to ensure uniform irradiation, mutation before the ultraviolet lamp should be warmed up in advance for ten to twenty minutes to ensure the stability of the light wave [22].

4. REASONS FOR UV MUTAGENESIS OF BACILLUS SUBTILIS

If the wild strains are isolated and screened directly from nature, the metabolic synthesis capacity, both in terms of yield and quality, is difficult to meet the needs of industrialized production, so the selection and breeding of microorganisms is extremely important [23]. At present, the selection and breeding of microorganisms include direct screening, mutagenesis, transcription, particle flow and plasma, etc. Among them, UV mutagenesis is widely used in the scientific community as a kind of mutagenesis selection method with simple and easy-to-obtain equipments, safe and simple operation, and low cost [24]; Zeng Guohong *et al.,* [25] carried out UV, UV-chemical compound mutagenesis and plasma mutagenesis on Bacillus subtilis HS-A38. A strain of Staphylococcus aureus mutHS-301 was obtained, and its bacteriostatic activity was increased by 19.7%, and its bacteriostatic activity against Vibrio canis was increased by 17.4%, which is of great significance to the marine aquaculture industry. Pang Guangwu *et al.,* [26] UV mutagenesis of Bacillus subtilis LC6-1 resulted in a highly productive and stable strain PW6-3. Fibrinolytic enzyme production was increased by 72.53% compared with that of the strain LC6-1 before mutagenesis, which provides an important reference for the future industrial fermentation of fibrinolytic enzyme production. Wang Song *et al.,* [27] sieved a Bacillus subtilis mutant strain 11-1 by UV mutagenesis. The protease activity was increased by 1.66 times compared with that of the original strain, which was 35.301 U/mL. The rate of change of the enzyme activity in the successive generation test was 4.8%, which was small and the genetic performance was stable. It can be used for the production of fermented corn protein powder feed, which lays the foundation for the production of corn protein powder feed by microbial fermentation. Wang Xiangyu *et al.,* [28] After screening and UV mutagenesis, we obtained a high-yielding mutant UBS-A29, with a bacterial inhibition effect 1.7 times that of the original strain of Bacillus subtilis β-BS01, which is genetically stable, and it has been pilot tested in the experimental field, and it is suitable for industrialized production. Ma Yimeng *et al.,* [29] performed UV-chemical complex mutagenesis on Bacillus subtilis HS-301 and screened mutHS-407, a mutant strain with high yield of antimicrobial lipopeptide, which had a significantly higher inhibitory effect on Aspergillus flavus than the common antimicrobial agents. Ozalpar, Busra *et al.,* [30] performed UV mutagenesis on the wild-type Bacillus subtilis E6-5, and the mutant strain (1096 IU) was genetically stable and suitable for industrial production. The mutant strain (1096 IU/mL) showed 18.2-fold higher yield than wild type (60 IU/mL) in modified medium. The protease obtained from the ATA38 mutant strain may have great potential in industry for different purposes. Wang *et al.,* [31] UV mutagenesis of Bacillus subtilis S1-4 screened for a high-producing protease mutant (UMU4), which showed 2.5-fold higher extracellular caseinolytic activity than that of the wild-type strain, and was able to degrade chicken feathers more efficiently, releasing soluble proteins from the feathers, in addition to this protease was not sensitive to heavy metal ions, surfactants or oxidizing reagents. The UMU4 mutant of Bacillus subtilis and its serine protease may be used in a variety of industries. Zhang *et al.,* [32] used tempeh-fibrinolytic enzyme-producing Bacillus subtilis DC-1 as a starter strain, and used ultraviolet mutation to select a high tempeh-fibrinolytic enzyme-producing and stably hereditary strain, and screened out a high tempeh-fibrinolytic enzyme-producing and stably hereditary mutant strain DC-V5, 1.74 times more potent than that of the pre-optimization starter strain DC-1. 1.74 times. LU *et al.,* [33], used UV laser random mutagenesis to treat Bacillus subtilis and found that LA-UV had the highest positive mutation rate. Two mutants, UV-1-84 and LA-UV-1-11, were screened and showed excellent genetic stability and protease activity. And the established high-temperature superconductivity process can be easily transferred to other similar high-yielding strains. Yaderets, Vera *et al.,* [34] UV mutagenesis was used to obtain a 77% increase in the inhibitory ability of Bacillus subtilis RBT-7/32 against Escherichia coli compared with the parental strains; and an 80% increase in the inhibitory ability against Staphylococcus aureus. The application prospect in feed additives is good.

5. SAFETY STUDIES OF BACILLUS SUBTILIS

Bacillus subtilis is often used as a model strain for bacterial genetics and cellular metabolism research, mainly because the sequence of the strain genome and gene-related information is relatively clear [35]. Due to its clear physiological and biochemical characteristics, easy genetic manipulation, strong secretion and expression ability, and convenient culture and fermentation, it has also been transformed into a microbial cell factory as an excellent chassis cell [36] for the production of industrial enzymes, vitamins, functional sugars, nutraceuticals and drug precursors, and other target products, which has allowed scientists to see its strong industrial production application, and at the same time, it has been recognized as a recognized safe

strain by the Food and Drug Administration (FDA) of the United States. A recognized safe strain [37].

6. SUMMARY

Although there are still flaws in the application process of Bacillus subtilis, the fungicides made by Bacillus subtilis have the advantages of relative harmlessness to humans and animals, excellent environmental compatibility, and low probability of resistance, etc. [38], which is more compatible with the current social demand for agricultural production and integrated pest control, and at the same time, researchers have accumulated rich research experience in the isolation and purification of Bacillus subtilis antimicrobial substances, mechanism of the molecular effects of antimicrobial, amplification and expression regulation of antagonistic genes, etc. Therefore, it is of great guidance and production value for the utilization of B subtilis in various aspects. At the same time, researchers have accumulated rich experience in the isolation and purification of antimicrobial substances, the mechanism of antimicrobial molecules, the amplification of antagonist genes and the regulation of their expression, and so on, so that the utilization of Bacillus subtilis in various aspects has an important guidance and production value [39]. In the future development, with people's increased concern for the safety of animal products, improved awareness of their own health care and urgent requirements for the improvement of their own living environment, the degree of attention to microecological preparations represented by Bacillus subtilis will be further strengthened.

7. OUTLOOK

In the future development and research, new technologies should be combined to optimize the production process and reduce the cost of the products, and at the same time promote them in all aspects; further optimize the development and utilization of Bacillus subtilis to enhance its competitiveness as an industrial strain, which includes: the continuous improvement of metabolism level of the genome, transcriptome, etc., the improvement of synthetic pathways and the creation of new enzymes [40]. It is hoped that one day, instead of relying on traditional synthetic routes, we can realize the production and synthesis of target products by using new development pathways that are simpler and with higher thermodynamic tendency, and realize the optimization of product production and conversion rate, and some of the problems that limit the application of Bacillus subtilis and other micro-ecological preparations will be solved one by one, and the micro-ecological preparations represented by Bacillus subtilis will be applied to more fields in a more extensive and in-depth manner. Microecological agents represented by Bacillus subtilis will also be more widely and deeply applied to more fields.

REFERENCES

1. Bian, Y. Q. (2017). Analyzing the research progress and application of Bacillus subtilis [J]. *Science and Technology Innovation*, *30*, 33-34. DOI: 10.3969/j.issn.1673-1328.2017.30.019.
2. Li, J., Chen, M., … & Lai, Z. (2024). Studies on cellulase production by Bacillus spp.[J]. *Guangdong Chemical Industry*, *51*(12), 45-46,44. DOI: 10.3969/j.issn.1007-1865.2024.012.015.
3. Pan, J., Kou, F., Liu, R., Wang, Y., & Zhao, H. (2020). Selection of β-farnesene-producing strains by atmospheric pressure room temperature plasma-ultraviolet complex mutagenesis. *Microbiology Bulletin*, *47*(2), 542-551.
4. Shi, Q., & Wu, S. eds. (2009). Industrial Microbial Breeding. 3rd edition [M]. *Science Press*, 377.
5. Zhou, J., Luan, J., & Feng, X. (2020). Effects and mechanism of action of probiotics on meat quality of livestock and poultry[J]. *Feed Research*, *43*(11), 124-127.
6. Yang, D., Zhou, C., & Huang, L. (2020). Research progress of Bacillus endophyticus on plant growth and development and disease control [J]. *Anhui Agricultural Science*, *48*(4), 11-14. DOI: 10.3969/j.issn.0517-6611.2020.04.003.
7. Zhou, Y. (2010). Characterization of Bacillus subtilis and its application [J]. *Journal of Qiannan National Medical College*, *23*(2), 84-88. DOI: 10.3969/j.issn.1008-4983.2010.02.003.
8. Sun, A., Sun, B., & Zhao, C. (2011). Study on the mutagenic effect of ultraviolet light on Bacillus subtilis[J]. *China Dairy Industry*, *39*(7), 12-14. DOI: 10.3969/j.issn.1001-2230.2011.07.003.
9. Zhang, W., Ren, L., & Feng, H. (2024). Progress of biocontrol mechanism of Bacillus subtilis with dosage form processing and conjugation[J]. *Pesticide*.
10. Lei, J., Kang, Y., & Miao. (2022). Construction of Bacillus subtilis B2-GFP strain and its colonization in soil[J]. *Journal of Fujian University of Agriculture and Forestry*, *51*(5),577-582.
11. Wang, T., Wang, X., Han, M., Song, X., Yang, D., Wang, S., ... & Shi, X. (2021). Enhanced spoVF operon increases host attachment and biocontrol ability of Bacillus subtilis for the management of Ceratocystis fimbriata in sweet potato. *Biological Control*, *161*, 104651.
12. Li, Y., Ou, T., & Jiao, W. (2024). Isolation and characterization of endophytic Bacillus subtilis and its biocontrol mechanism against mulberry botrytis[J]. *Journal of Microbiology*. DOI: 10.13343/j.cnki.wsxb.20240100.
13. Jiao, Y. (2015). Research on the antagonistic effect and mechanism of Bacillus subtilis on root cancer disease of Du Pear and grape[D]. *Hebei: Hebei Agricultural University*.
14. Wang, B. C., Lei, X., Chen, J., LiI, W. Z., Long, Y. H., & Wang, W. Z. (2022). Antifungal activities of Bacillus mojavensis BQ-33 towards the kiwifruit

black spot disease caused by the fungal pathogen Didymella glomerata[J]. *Microorganisms*, *10*(10), 2085.
15. Fan, Z., Feng, J., & Zheng, L. (2024). Control of cucumber wilt disease by Bacillus subtilis B579 and its induced resistance[J]. *Biotechnology Bulletin*. DOI: 10.13560/j.cnki.biotech.bull.1985.2024-0160.
16. Samaras, A., Roumeliotis, E., Ntasiou, P., & Karaoglanidis, G. (2021). Bacillus subtilis MBI600 promotes growth of tomato plants and induces systemic resistance contributing to the control of soilborne pathogens. *Plants*, *10*(6), 1113.
17. Amin, H. A., El Kammar, H. F., Saied, S. M., & Soliman, A. M. (2023). Effect of Bacillus subtilis on potato virus Y (PVY) disease resistance and growth promotion in potato plants. *European Journal of Plant Pathology*, *167*(4), 743-758.
18. Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., & Fira, D. (2022). Plant-associated Bacillus and Pseudomonas antimicrobial activities in plant disease suppression via biological control mechanisms-A review. *Physiological and Molecular Plant Pathology*, *117*, 101754.
19. Hashem, A., Tabassum, B., & Abd_Allah, E. F. (2019). Bacillus subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi journal of biological sciences*, *26*(6), 1291-1297. DOI: 10.1016/j.sjbs.2019.05.004.
20. Li, C., Pan, Y., & Xu, H. (2024). Effects of Bacillus subtilis and Aspergillus oryzae on root conformation and growth of cucumber seedlings[J]. *Journal of Lanzhou University (Natural Science Edition)*, *60*(2), 187-194. DOI: 10.13885/j.issn.0455-2059.2024.02.007.
21. Zhao, A., Wang, Z., & Yang, L. (2024). Effects of microalgae with Bacillus subtilis on soil chemical properties and bacterial communities in tobacco fields[J]. *Journal of Jiangxi Agricultural University*, *46*(1), 241-250. DOI:10.3724/aauj.2024023.
22. Xu, C., & Gao, M. (2013). Mutagenesis screening method for microbial strains and its application[J]. *Journal of Changjiang University (Autonomous Science Edition) (Mid)*, *10*(12), 56-60. DOI: 10.16772/j.cnki.1673-1409.2013.35.003.
23. Liu, Y., Mu, Q., Shi, Y., & Yu, B. (2021). Metabolic regulation in constructing microbial cell factories. *Sheng wu Gong Cheng xue bao= Chinese Journal of Biotechnology*, *37*(5), 1541-1563.
24. The Writing Group of Microbial Mutagenesis Breeding, ed. Microbial Mutagenesis Breeding [M]. *Science Press*,1973:83.
25. Zeng, G., Cong, L., & Bi N. (2019). Characterization of antimicrobial lipopeptide production by mutant strains of Bacillus subtilis[J]. *Journal of Dalian University of Technology*, *38*(4), 235-238. DOI: 10.19670/j.cnki.dlgydxxb.2019.0401.
26. Pang, G., & Liang, Z. (2022). Mutagenesis breeding and fermentation process optimization for the production of fibrinolytic enzymes by marine Bacillus subtilis[J]. *Chinese Journal of Bioengineering*, *42*(12), 27-36. DOI: 10.13523/j.cb.2207056.
27. Wu, W. T., Liu, Z. T., & Gao, G. L. (2023). Effects of Bacillus subtilis on the physiological characteristics of seed germination and seedling growth of sand plants[J]. *China Soil and Water Conservation Science (in Chinese and English)*.
28. Wang, S., Jiang, C., & Zheng, X. (2019). Ultraviolet mutagenesis selection of Bacillus subtilis for fermented corn protein meal feed[J]. *China Feed*, 3, 23-25,29. DOI: 10.15906/j.cnki.cn11-2975/s.20190305.
29. Wang, X., Hu, M., & Wu, Y. (2018). Suppression of common fungal diseases in northeastern greenhouses by β-BS01 mutant strain of Bacillus subtilis[J]. *Biohazard Science*, *41*(1), 16-19. DOI: 10.3969/j.issn.2095-3704.2018.01.05.
30. Ma, Y. (2019). Screening of Bacillus subtilis mutant strain mutHS-407 and purification and application of the produced active substances[D]. *Dalian University of Technology*. Doi: 10.26992/d.cnki.gdlqc.2019.000142.
31. Özalpar, B., Demirkan, E., & Sevgi, T. (2024). Obtaining Efficient Mutant from the Wild Type Bacillus subtilis E6-5 by Physical and Chemical Mutagenesis for High Efficiency Protease Production, Optimizing the Mutant's Culture Medium. *Gazi University Journal of Science*, 1-1. doi:10.35378/gujs.1191006.
32. Zhang, W.-Y., Zhang, H.-H., & Wang, X.-L. (2024). Selection of high-yielding tempeh fibrillase strains by ultraviolet mutagenesis and optimization of their enzyme production conditions[J]. *China Brewing*, *43*(3), 177-181. DOI: 10.11882/j.issn.0254-5071.2024.03.028.
33. Lu, F., Chao, J., Zhao, X., Betchem, G., Ding, Y., Yang, X., ... & Ma, H. (2022). Enhancing protease activity of Bacillus subtilis using UV-laser random mutagenesis and high-throughput screening. *Process Biochemistry*, *119*, 119-127. DOI: 10.1016/j.procbio.2022.05.018.
34. Yaderets, V., Karpova, N., Glagoleva, E., Shibaeva, A., & Dzhavakhiya, V. (2023). Bacillus subtilis RBT-7/32 and Bacillus licheniformis RBT-11/17 as New Promising Strains for Use in Probiotic Feed Additives. *Microorganisms*, *11*(11), 2729. DOI:10.3390/microorganisms11112729.
35. Zhang, X., Tang, W., & Zhang, L. (2007). Control of plant diseases and promotion of plant growth by Bacillus subtilis B931[J]. *Journal of Crops*, *33*(2), 236-241. DOI: 10.3321/j.issn:0496-3490.2007.02.010.
36. Huang, X., XU, L., & Huang, R. (2010). Research progress of Bacillus subtilis in suppressing plant pathogenic bacteria[J]. *Biotechnology Bulletin*, *1*, 24-29.
37. Kang, Q., Xiang, M. J., & Zhang, D. W. (2021).

Research progress and industrial application of Bacillus subtilis in systematic and synthetic biotechnology. *Chinese Journal of Biotechnology*, *37*(3), 923-938.

38. Wang, J., Wang, C., Du, Y., Xu, J., & Ban, R. (2021). Advances in heterologous protein expression and secretion of Bacillus subtilis[J]. *Microbiology China*, *48*(8), 2815-2826.
39. Wang, X. C., Zhao, H. Y., Liu, G., Cheng, X. J., & Feng, H. (2016). Improving production of extracellular proteases by random mutagenesis and biochemical characterization of a serine protease in Bacillus subtilis S1-4. *Genet Mol Res*, *15*(2), 1-11. DOI:10.4238/gmr.15027831.
40. Lü, X. Q., Wu, Y. K., & Lin, L. (2021). Strategies and tools for metabolic engineering in Bacillus subtilis. *Chinese Journal of Biotechnology*, *37*(5), 1619- 1636.