POPULAR ARTICLE

Thermophilic Bacteria: Applications in Agriculture

Shivani Sankla[1], Asha Sahu[2*], Sudeshna Bhattacharjya[3], Nisha Sahu[4] and A B Singh[5]

[1]M.Sc.(Ag.) Student, [2]Senior Scientist, [3]Scientist, [4]Senior Scientist, [5]Director
[12345]ICAR-Indian Institute of Soil Science, Bhopal, Madhya Pradesh (462038) India

***Corresponding Author:**
Asha Sahu, Senior Scientist, ICAR-IISS, Bhopal
Email: ashaars.iiss@gmail.com; asha.sahu@icar.gov.in

Submitted: 22.01.2023 Accepted: 18.04.2023 Published: 19.05.2023

KEYWORDS: Thermophilic bacteria, composting, agriculture

SUMMARY

Thermophilic bacteria are common in soil and volcanic habitats and have a limited species composition. They are inhabitants of various environments, such as deep-sea hydrothermal vents, terrestrial hot springs, and other extreme sites, including volcanic region, tectonically active faults, and processing waste residues, like compost piles and deep organic landfill. Yet they possess all the major nutritional categories and metabolize the same substrates as mesophilic bacteria. The ability to proliferate at growth temperature optima well above 60°C is associated with extremely thermally stable macromolecules. Thermophiles have attracted considerable attention because they present specific features with biotechnological and industrial interest, such as the production of different biomolecules (exopolysaccharide, antimicrobial, biosurfactant) and thermostable enzymes (amylases, cellulases, chitinases, pectinases, xylanases, proteases, lipase, and DNA polymerases), for biotechnological applications in medical, industrial, and agriculture processes. Many thermophilic bacteria possess properties suitable for biotechnological and commercial use. There is, indeed, a considerable demand for a new generation of stable enzymes that are able to withstand severe conditions in agriculture processes by replacing or supplementing traditional chemical processes. Thermophilic bacteria have application in chemical feedstock and fuel production, bioconversion of wastes, enzyme technology, and immobilization of heavy metal. This article reviews the fundamental and applied aspects of thermophilic bacteria that are of potential agriculture interest.

INTRODUCTION

The word “bacteria” is often associated with disease, but only a few kinds of bacteria cause problem for human. The other thousands of bacteria although all simple organisms, play a complex role in earth ecosystem. Thermophilic bacteria are microbes that mostly inhabits hot spring, live and survive in high temperature above 70°C. Acidophiles and alkaliphiles are the class of thermophiles bacteria that can survive under extreme pH condition optimum growth of acidophiles is at pH near 3.0 or below and alkaline are between 8 and 10.5. Some thermophiles can also be classified as bacteria, which consists of one biological cell, there are twenty-eight bacterial culture that can be classified as thermophiles these thermophiles bacteria live in hotsprings, bacteria namely **Methanopyrus kandleri** is the most extreme thermophile able to withstand temperature as high as 121°C [1,2].

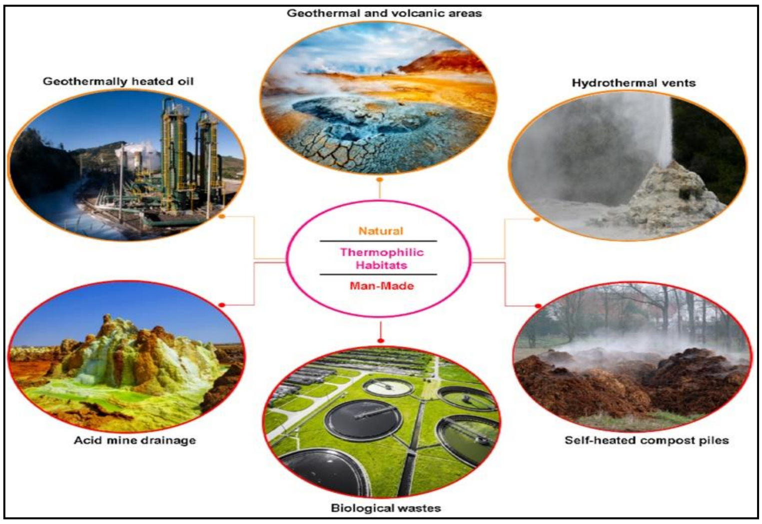

Figure 1: Thermophiles Habitats (Natural and Man-Made) [3]

Thermophiles are heat loving organism that exhibit optimal growth at a temperature above 60°C they are in habitats of various ecological niches like deep hydrothermal vent, terrestrial hot springs. Thermophiles are classified into obligate and facultative thermophiles. Obligate thermophiles (also called extreme thermophiles) require high temperature for growth and whereas facultative thermophiles (also called moderate thermophiles) can thrive at high temperature but also low temperature (below 50°C). These thermophilic bacteria can be used for various purposes in agriculture, some of the applications are listed below:

1. Enhancing the conversion of organic waste into biofertilizer

Three bacterial species namely ***Bacillus licheniformis***, ***Gordonia terrae***, and ***Virgibacillus halophilus*** were isolated from a composting aquaculture waste mixture bulked with sawdust, all were thermophilic bacteria with cellulase activity. Inoculation with bacteria shortened the composting time by one third compared with the uninoculated condition [4].[5] suggest that the fast conversion of aquaculture food waste into biofertilizer by mean of inoculation with thermophilic bacteria is a potential technologies. Inoculation of thermotolerant and thermophilic bacteria to agriculture waste for biofertilizer preparation enhance the rate of maturity and improves the quality of the resulting biofertilizer [6,7]. [8,9] reported that the Bacillus licheniformis identified and explored as the potential PGPR strain to be developed as multifunctional biofertilizer for multiple crop production [10,11].

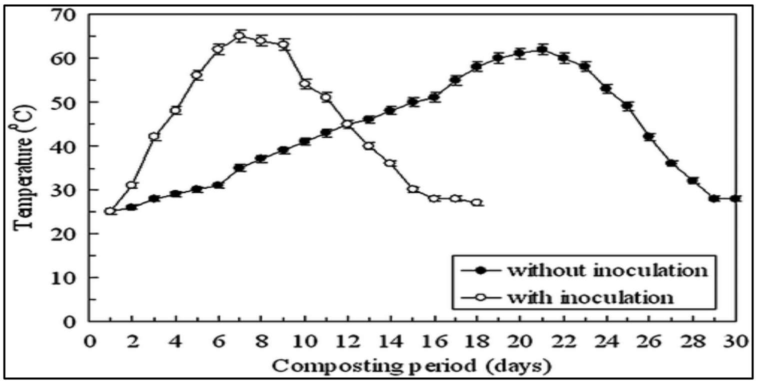

Figure 2: Composting Periods (with inoculation and without inoculation) [11]

2. Immobilization of heavy metal from soil

Thermophilic microorganisms, owing to their natural ability to survive and flourish under elevated temperatures along with other stressful environmental conditions including high concentrations of heavy metals, have developed various adaptation strategies to cope with harsh environments, which may offer enormous opportunities for bioremediation of heavy metals at higher temperatures. Thermophilic microorganisms, being common in geological and anthropogenic thermal environments with high concentrations of dissolved metal, possess unique cell wall structures and metabolic and enzymatic properties that may contribute in metals–thermophiles interactions. Biosorption/bioaccumulation of metals is most effective and widely used approach for the bioremediation. The nature and extent of metal biosorption onto thermophilic bacteria may differ greatly from the mesophilic organisms. Microbial transformation of metal through oxidation/reduction reactions plays a critical role in metal speciation, distribution, and thus altered toxicity in the ecosystems, which may be implemented in metal recovery and remediation. Both sulfate- and metal-reducing bacteria have profound application in metal bioremediation. Thermophilic bacteria with higher metal tolerance and metabolic characteristics at high temperature may exhibit enhanced metal solubilization through sulfur- or iron-oxidizing processes. Thermophilic microbial community can perform both degradative and productive functions through coupling of metal reduction with oxidation of a variety of organic and inorganic substrates. Thermophilic bacteria are also able to reduce a wide spectrum of metals including Mn (IV), Cr (VI), U (VI), Tc (VII), Co (III), Mo (VI), Au (I, III), and Hg (II) which can be used for the immobilization of toxic metals/radionuclides during bioremediation of hot wastewater of disposal sites of radioactive wastes having temperature range favorable for thermophiles for a long period of time [12, 13].

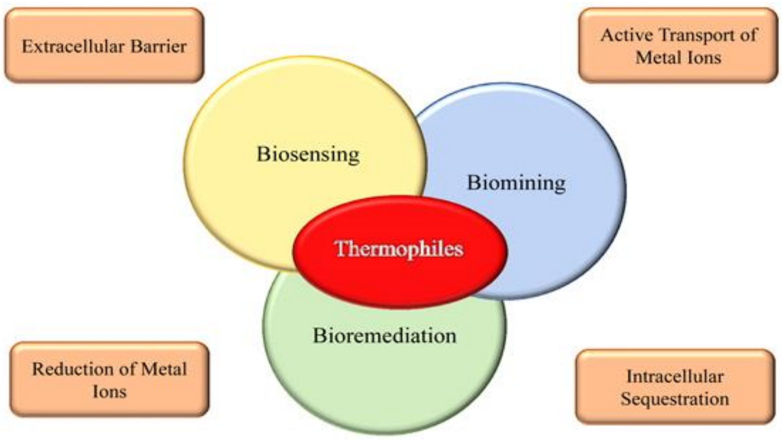

Figure 3: Schematic representation of potential applications in biotechnology of metal tolerant thermophile [13]

3. Hygiene indicator in dairy processing

Thermophilic bacilli are used as hygiene indicators of processed product, within the dairy processing context. This is because of the ability of these strains to form endospores and biofilms. The thermophilic bacilli, such as ***Anoxybacillus flavithermus* and *Geobacillus spp.*,** are an important group of contaminants in the dairy industry. Although these bacilli are generally not pathogenic, their presence in dairy products is an indicator of poor hygiene and high numbers are unacceptable to customers. In addition, their growth may result in milk product defects caused by the production of acids or enzymes, potentially leading to off-flavors [14]. Many strains of genera *Lactobacillus* and *Bifidobacterium*, as well as some enterococci and yeasts, have been shown to possess probiotic properties with potential for prophylaxis and treatment of a range of gastrointestinal disorders.

4. Role of thermophilic bacteria to suppress disease caused by plant pathogen

B. subtilis is a gram-positive bacterium that forms biofilms on inert surfaces and possesses many transcriptional factors. Different strains of *B. subtilis* synthesize a variety of hydrolytic enzymes, including i.e. cellulases, proteases, and β-glucanases. [15] suggested that *B. subtilis* has the ability to secrete antibiotics and hydrolytic enzymes, it can modify its' environment in a self-beneficial manner and also produce resistant endospores to sustain itself under adverse conditions. The ability of *B. subtilis* to exhibit biocontrol activity is dependent upon three factors: (1). host vulnerability; (2). pathogen virulence; and (3) the environment.

Bacillus species produce a variety of compounds that can be used for the management of a broad range of plant pests [16]. Stable growth and development of biological agent under field conditions is still a problem due to adverse environmental conditions. Formulation of active product with perfect stabilizer that can optimize its activity under field conditions is an alternative and more effective strategy for the management of plant pest instead of using living bacteria directly. The bacterial-based formulated products, when used with other synthetic chemical pesticides, can be harmful for the living bacteria. The formulation that can enhance the shelf-life of bacterial product during storage, transportation and also during field application is also important. Proper knowledge and understanding of the bacterial active compound is necessary for a stable and efficient formulation.

5. Plant Growth Promoting Properties of Thermophilic *Bacillus* Strains

Bacillus licheniformis was isolated and molecularly characterized by [17,18,19] which show the ability of multiple plant growth promoting characteristics such as ammonia production, indole acetic acid production, phosphate solubilization, catalase production, heavy metal tolerance and ACC deaminase activity. isolate is a potential PGPR candidate for enhancing sustainable agriculture. [20] reported that the B. pumilus and B. licheniformis have been documented to produce gibberellins. [21] reported that B. licheniformis, B. cereus, B. circulans, B. subtilis and B. thuringiensis found as potential biocontrol agents having chitinolytic activities.

6. Saccharificationof agriculture residue

High-efficiency saccharification technology is one of the bottlenecks of cellulosic bio-hydrogen production. Cellulosic feedstocks saccharification currently performed by commercial cellulase, which is composed of different fungal cellulase. Compared with fungi, thermo-cellulosic bacteria represented **by *Ruminiclostridium thermocellum*** have a complete cellulase system, and a higher cellulase catalytic efficiency than fungi; however, ***R. thermocellum*** is susceptible to feedback inhibition by cellobiose, which limits the application of *R. thermocellum* on cellulosic bio-hydrogen production. In this study, a strain named ***R. thermocellum* M3**, which is not subject to feedback inhibition by cellobiose, was used in the bio-hydrogen production of cellulosic agricultural waste feedstocks to explore the feasibility of bacterial saccharification of cellulosic substrates for biological hydrogen production. Thermo-anaerobic bacteria ***R. thermocellum* M3** enhanced the hydrogen production of the consolidated bioprocessing (CBP) of raw lignocellulosic agricultural wastes [22] .

7. Contribution in carbon, nitrogen and sulphur cycle

During high temperature condition soil thermophilic bacteria can develop and contribute to soil ecosystem functioning. In soils of medium and low latitude, their process would occur with a temporal basis necessary to the prolification of soil thermophilic bacterial communities [23]. The largest fraction of nitrogen and sulphur in the soil are in the form of organic matter. The mineralization of these organic compound contribute to CO_2 release to atmosphere and to making inorganic N and S readily available. The recent research has demonstrated that thermophiles gram positive bacteria of the firmicutes phylum mainly high genera ***Geobaccillus, Ureibaccillus and Breribaccillus*** were able to release significant quantities of sulphate under high temperature condition as a product of their metabolism. [24] which suggests that soil thermophilic bacteria can be actively involved in C and S cycling in soil upper layers and refutes the prevailing view of bacteria as poor S-mineralizers (Eriksen 1996).

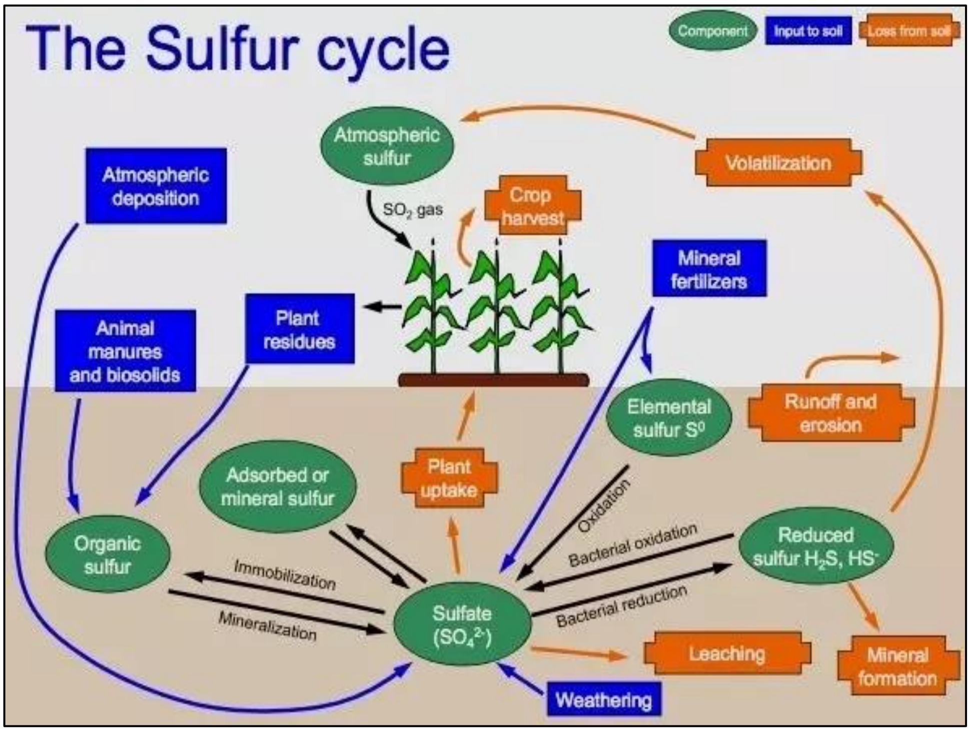

Figure- 4. Thermophilic bacteria support in oxidation process [25]

The oxidation process supports the population of chemolithotrophs like ***Beggiatoa, thiothrix, thermothrix (thermophile)*** recent findings suggest that during high temperature conditions soil thermophilic bacteria can develop and contribute to soil ecosystem functioning. In soils medium and low latitudes, this process would occur within a temporal basis necessary to the proliferation of soil thermophilic bacterial communities. This ecological shift of responses by complex and diverse soil microbial communities complements the functional role of the microbiota in processing soil organic matter and ultimately in the cycling and mineralization of essential nutrients (C, S and N) for plant growth.

REFERENCE

1.Mehta R, Singhal P, Singh H, Damle D, Sharma AK. Insight into thermophiles and their wide-spectrum application. 3 Biotech. 2016 Jun;6(1):81.

2.Thermophilic bacteria – yellow stone national park (U.S National Park Service)

Thermophilic Bacteria - Yellowstone National Park (U.S. National Park Service) (nps.gov)

3.Vavitsas, Konstantinos, Panayiotis D. Glekas, and Dimitris G. Hatzinikolaou. "Synthetic Biology of Thermophiles: Taking Bioengineering to the Extremes?." Applied Microbiology 2.1 (2022): 165-174.

4. Finore, I., Feola, A., Russo, L. *et al.* Thermophilic bacteria and their thermozymes in composting processes: a review. *Chem. Biol. Technol. Agric.* **10**, 7 (2023).

https://doi.org/10.1186/s40538-023-00381-z

5.Chen, C.H., Mei, H.C., Cheng, C.Y., Lin, J. and Chung.Y.C. (2012) Enhancing the Conversion of Organic Waste into Biofertilizer with Thermophilic Bacteria. Environmental Engineering Science,726-73.

6. Sahu Asha, Manna, MC, Bhattacharjya S, Thakur JK, Mandal A, Rahman MM, Singh UB, Bhargav VK, Srivastava S, Patra AK, Chaudhari SK and Khanna SS (2019) Thermophilic Ligno-Cellulolytic Fungi: The future of Efficient and Rapid Bio-Waste Management. Journal of Environmental Management.244:144-153

7. Sahu Asha, Manna MC, Bhattacharjya S, Rahman MM, Mandal A, Thakur JK, Sahu Kamlesh, Bhargav VK, Singh UB, Sahu KP, Patra AK (2020) Dynamics of maturity and stability indices during decomposition of biodegradable city waste using rapo-compost technology. Applied Soil Ecology. 155:103670

8.Kayasth, M., Kumar, V., and Gera, R. (2013). Exploring the potential of PGPR strain bacillus licheniformis to be developed as multifunctional biofertilizer. Cent. Eur. J. Biol. 2, 12–17.

9. Awasthi SK, Kumar M, Sarsaiya S, Ahluwalia V, Chen H, Kaur G, Sirohi R, Sindhu R, Binod P, Pandey A, Rathour R, Kumar S, Singh L, Zhang Z, Taherzadeh MJ, Awasthi MK. Multi-criteria research lines on livestock manure biorefnery development towards a circular economy: from the perspective of a life cycle assessment and business models strategies. J Clean Prod. 2022;341:1–20. https://doi.org/10.1016/j.jclepro.2022.130862.

10. Papale M, Romano I, Finore I, Lo Giudice A, Piccolo A, Cangemi S, Di Meo Nicolaus B, Poli A. Prokaryotic diversity of the composting thermophilic phase: the case of ground cofee compost. Microorganisms. 2021;9:1–19. https://doi.org/10.3390/microorganisms9020218

11. Chen, Chih-Yu, et al. "Enhancing the conversion of organic waste into biofertilizer with thermophilic bacteria." Environmental Engineering Science 29.7 (2012): 726-730.

12. Sar, Pinaki & Kazy, Sufia & Paul, Dhiraj & Sarkar, Angana. (2013). Metal Bioremediation by Thermophilic Microorganisms. 10.1007/978-94-007-5899-5_6.

13. Gallo, Giovanni, et al. "Metal-Tolerant Thermophiles: From the Analysis of Resistance Mechanisms to their Biotechnological Exploitation."2018, 12, 149-160

14.Burgress, S.A., Lindsay, D., Flint, S.H. (2010) thermophilic bacilli and their importance in dairy processing, International Journal of food microbial ,144(2)215-25.

15.Cazorla F., Romero D., Pérez-García A., Lugtenberg B., Vicente A.D., Bloemberg G. Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. *J. Appl. Microbiol.* 2007;103(5):1950–1959.

16.jamil Shafi, Hui Tian & Mingshan Ji (2017) *Bacillus* species as versatile weapons for plant pathogens: a review, Biotechnology & Biotechnological Equipment, 31:3, 44645

18. Patil R, Satpute R, Nalage D. Plant microbiomes and their role in plant health. Microenviron Microecol Res. 2023;5(1):2. doi: 10.53388/MMR2023002

19. Sahu Asha, Kaur Pardeep, Bhattacharjya Sudeshna, Sahu Nisha, Bharti K., Tilwari Anita, Singh AB (2022) Thermophilic bacteria isolated from Anhoni Hotsprings of Central India for Plant Growth-Promoting Traits and effect on Pigeon Pea (Cajanus Cajan) seedling. Scientist. 1(3): 4744-4759.

20.Gutiérrez-Manero, F. J., Ramos-Solano, B., Probanza, A., Mehouachi, J., R Tadeo, F., and Talon, M. (2001). The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiol. Plant.* 111, 206–211.

21.Sadfi, N., Cherif, M., Fliss, I., Boudabbous, A., and Antoun, H. (2001). Evaluation of bacterial isolates from salty soils and bacillus thuringiensis strains for the biocontrol of fusarium dry rot of potato tubers. *J. Plant Pathol.* 101–117.

22. Sheng, T., Meng, Q., Wen, X., Sun, C., Yang, L. and Li, L. (2021), Bioaugmentation with *Ruminiclostridium thermocellum* M3 to enhance thermophilic hydrogen production from agricultural solid waste. J Chem Technol Biotechnol, 96: 1623-1631.

23. Sahu, Asha, Bhattacharjya, S., Mandal, A., Thakur, J.K., Atoliya, N., Sahu, Nisha, Manna, M.C., Patra, A.K. 2018. Microbes: a sustainable approach for enhancing nutrient availability in agricultural soils. In: Role of Rhizospheric Microbes in Soil. Meena V.S. (Ed.) Springer publication. pp. 47-75. https://doi.org/10.1007/978-981-13-0044-8_2.

24.Gonzalez JM, Portillo MC, Pineiro-Vidal M. Latitude-dependent ˜ under estimation of microbial extracellular enzyme activity in soils. Int J Environ Sci Technol 2015; 12:2427–34.

25.https://stormwater.pca.state.mn.us/images/0/08/Sulfur_cycle.jpg