Biological Forum – An International Journal **13(2): 83-94(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

A Review on the Therapeutic Roles of Selected Thermophilic Secondary Metabolites

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(Received 25 March 2021, Accepted 01 June 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Thermophiles are gaining immense attention due to the extensive roles of thermophilic secondary metabolites in therapeutics. Thermophiles have presented a new age of novel molecules as they are able to adjust their metabolic pathways to unfavorable physicochemical conditions. This has widened the scope of application of thermophilic secondary metabolites in industries including pharmaceutical industries, medicine, and other life-saving activities. A good understanding of the secondary metabolites and/or factors that bestow these characteristics on thermophiles can lead to the discovery of new drugs. Even though mesophilic and psychrophilic microorganisms have wide ranges of therapeutic applications, they are unable to optimize productivity due to their inability to withstand high-temperature conditions. Thermophilic microorganisms offer a convenient, stable, and cost-efficient means of increasing productivity. Over the decades, the exploitation of thermophiles has been hindered due to the difficulties in accessing their habitats, detailed understanding of the mechanisms of thermostability, etc. This review, therefore, seeks to highlight some of the major roles of thermophiles in therapeutics.

Keywords: Bacteriocins, Colonization, Coprostanol, Diketopiperazines, Plasminogen and Thermolytes

INTRODUCTION

Microorganisms with optimal temperature ranges between 60 to 80°C can be classified as thermophiles (Santos and Da Costa, 2002). Competition for space and nutrients in the aquatic environment constitute a selective force leading to the development and production of multiple thermolytes viz. secondary metabolites produced by thermophilic systems to adapt to the different environments (Dalmaso *et al.*, 2015). These thermolytes are thermostable and have a wide spectrum of industrial applications compared to mesophilic and psychrophilic secondary metabolites due to their potential of withstanding extremely harsh industrial conditions. Thermolytes such as thermozymes are active and efficient under high temperatures, extreme pH values, high substrate concentrations, and high pressure (González-Siso, 2019). Due to the thermostable property of thermophilic microorganisms, thermolytes produced by these microorganisms are able to withstand agents that can cause protein denaturation, hence, the rate of reactions catalyzed by thermophilic secondary metabolites are rapid and easily separable from proteins and other solvents during purification processes (Sarmiento *et al.*, 2015). These characteristics make thermolytes economically relevant. Different thermolytes isolated from different thermophilic microorganism impact greatly on bio-industrial activities, for instance, the pharmaceutical industries use lipases in manufacturing compounds that are impregnated with nitrogen which in turn can be used to produce amines and amides. Pharmaceutical industries use gelatinase in designing new drugs due to their function in embryonic development, morphogenesis, reproduction, tissue remodeling in addition to their role in diseases including cardiovascular diseases, arthritis and neurological diseases (Hmidet *et al.*, 2009). Thermophilic microorganisms and hyperthermophiles do not only produce metabolites that are of industrial relevance but they also produce essential bioactive compounds which are of clinical relevance, for example, antibiotic products, antiviral products, anti-inflammatory agents as well as enzyme inhibitors (Shivlata *et al.*, 2015).

Thermophilic microorganisms have several habitats that can be divided into two large groups namely the aquatic habitats and the terrestrial habitats. Aquatic habitats comprise environments that are bordered with water

forms such as lakes, rivers, ponds, wetlands, oceans, and seas, while terrestrial habitats are those found predominantly on land, for example, rainforests, deserts, grasslands, deciduous forests, etc. The aquatic habitats support more lives due to their stability, diversity, and their nutrient components compared to the terrestrial habitats. Terrestrial habitats such as hot springs are very important habitats of thermophilic microorganisms which form crucial interfaces within parts of the earth crust, linking water, its related matter, and energy at different levels ranging from the microscopic to macroscopic level (Reiss and Chifflard, 2017). The biodiversity of thermophiles is shown by the characteristics of the hot springs they inhabit, for example, acidic hot springs support acidophilic microorganisms such as Sulfulobus while thermophilic microorganisms such as *Thermoproteus, Pyrobaculum, Methanothermus, Desulforococcus, and Thermofilum* exist in neutral hot springs (Huber and Stetter, 1998). According to Delong and Yayanos (1987), microorganisms harbor several molecules or metabolites which are relevant in fields such as agriculture, medicine, pharmacy, and physiological studies. The adaptation of thermophiles to high-temperature conditions due to their ability to modify their structural features and metabolic pathways to suit their environments besides their ability to inhibit the growth or survival of other organisms that surround them make them suitable for therapeutic use (Horikoshi *et al.*, 2011). Table 1 present a list of some microbes and their antimicrobial activity.

Table 1: Some microbes and their antimicrobial activity.

Thermophiles	Enzymes	Activity	Organism/ Target	References
Pseudomonas aeruginosa	metalloprotease-alkaline protease	Antibacterial and antifungal	Aspergillus niger Escherichia coli	Andrejko and Siemińska (2016)
Bacillus subtilis BP-36	alkaline protease	Antibacterial and antifungal	*Aspergillus niger* *Escherichia coli*	Mazar *et al.*, 2012; Andrejko and Siemińska (2016)
XylariapsidiiKT3	Serine protease	Antibacterial	*Staphylococcus aureus* ATCC 6538 *Bacillus subtilis* ATCC 19659	Indarmawan *et al.*, 2016 Budiarto *et al.*, 2014
Bacillus subtilis strain M6 KC315773	protease	Antibacterial	*Klebsiella pneumoniae* MTCC 3384, *Staphylococcus aureus* KU2, *Bacillus pumilus*, *Arthrobacter* sp. and *Micrococcus luteus* MTCC105	Rachanamol *et al.*, 2017.
Actinomycetes	Lipase, caseinase, gelatinase, cellulase and amylase,	Antibacterial and antifungal	*E. coli, P. aeruginosa, S. epidermidis, B. subtilis and C. albicans,*	Ramesh and Mathivanan, (2009)

A. Diversity of Environments Inhabited by Thermophiles

Thermophile can be found in several habitats that favor their growth and development. These habitats can be acidic, alkaline, saline, high temperature, high pressure, and/or a combination of these conditions. Examples of these habitats include the marine habitat, Hot spring habitat, and the desert habitat. Fig. 1 shows a pictorial view of the above mentioned habitats.

Marine Habitats. The marine habitat covers over 70% of the earth which harbor different biological entities, accounting for over 95% of the biosphere.

(a) **(b)** **(c)**

Fig. 1. Habitats of thermophiles. (a) Hydrothermal Vent of the Marine Habitat (Niederberger, 2016). (b) hot spring in Yellowstone National Park (Bodaker *et al.*, 2010). (c) The Sahara desert is the world's largest hot desert (Ross, 2016).

The marine habitat can be categorized into two broad groups known as the coastal marine environments and the open ocean environments which have the capacity to support a wide diversity of life in the marine world (Dalmaso *et al.,* 2015). According to Bull and his colleagues (2000), about 10% of prokaryotes in the marine environment have been characterized. Extreme conditions in the marine environments combine different physical parameters including radiation, pH, pressure, salinity, temperature, chemicals, oxidative stress, and metal (Dalmaso *et al.,* 2015). Thermophiles are able to adapt to these parameters despite their toxicity because they have flexible metabolic pathways which allow them to thrive in these conditions (Nath and Bharathi, 2011).

The huge diversity of the marine habitat has led to the production of several secondary metabolites such as novel hydrolases with unique characteristics, specificities, and the capacity to withstand harsh conditions in industries (Samuel *et al.,* 2012). Zhan and his associates (2014) also reported that, a cysteine peptidase isolated from marine thermophiles was the first allosteric biocatalyst that has negative cooperativity with chloride ions which was essential for pharmaceutical activities.

Hot spring habitat. The main habitat of thermophiles are geothermal areas where hot springs represent excellent habitats for their survival (Khalil *et al.,* 2011). These geothermal environments have certain uncommon ecological characteristics which include their occurrence in groups as well as their existence in limited parts of the world in isolation where conditions are **favorable** for their growth. As a result of these features, hot springs are found in only a few locations of the world (Mohammad *et al.,* 2017). According to literature, the most studied and recognized hot springs are located in New Zealand, Central America, the United States, Central Africa, Iceland, Indonesia, Japan, and Italy (Baltaci *et al.,* 2017). Most striking features of hot spring water bodies include the diversity of its ecology as well as the formidability of the bonds that hold their molecules (Genc *et al.,* 2015). Examples of thermophiles found in hot spring areas are summarized in Table 2.

Table 2: Examples of hot spring thermopiles.

Organism	Maximum Temperature	Reference
Sulfolobus acidocaldarius GG12	80.4 °C	Anderson *et al.,* 2017
Hydrogenobacter thermophilus TK-6^{T}	78 °C	Zeytum *et al.,* 2011
Thermocrinisruber	89°C	Huber *et al.,* 1998
Thermophilic blue-green algae	55°C	Jackson and Castenholz (1975)

Desert habitat. The desert has remained one of the reservoirs of thermophiles that has not been exploited to its full potential because much investigation has not been conducted on their occurrence and distribution. Aanniz and his associates (2015) were the first to identify, isolate, and characterize culturable thermophilic bacteria form the seven desert soils in Morocco which comprises of *Bacillus licheniformis*, *Bacillus aerius*, *Bacillus sonorensis*, *Bacillus subtilis* and *Bacillus amyloliquefaciens*. A comparative study conducted among thermophilic bacteria isolated from hot springs, salt marshes, and desert soils in Morocco showed that, the desert soils recorded more isolates (108 isolates) compared to the hot spring water (79 isolates) and the salt marshes (53 isolates), and this the result was attributed to the high-temperature conditions in the desert lands (temperatures between 34 °C to 57 °C). *Bacillus subtilis* and *Bacillus pumilus* were also reported from extreme arid Atacama (Lester *et al.,* 2007). Palmisano and his associates (2001) projected that, *Bacillus sonorensis* is mostly seen in arid areas including Mojave, Gobi Deserts, Sahara, and Sonoran.

B. Mechanisms of Thermostability in Thermophiles

Research has shown that, the stability of thermophilic protein varies between thermophiles, mesophiles, psychrophiles, etc.

At extremely high temperatures, thermophiles have different factors and/or mechanisms of remodeling proteins in order to maintain their functions and structures (Reed *et al.,* 2013). According to Tomazic and Klibanov (1988), proteins that are deficient in these adaptive mechanisms are subjected to a permanent change in their protein structures and functions due to the exposure of their inner hydrophobic residues, etc., leading to aggregation. Fig. 2 shows a summarized diagram of some mechanisms involved in thermostability.

Salt-Bridging. In a contrasting study conducted, Karshikoff and Ladenstein (2001) stated that salt-bridging is predominant among thermophiles as compared to mesophiles whiles Hendsch and Tidor (1994) indicated that salt-bridging may disrupt proteins in mesophiles. According to Chan and his associates (2011), hydrophobic residues resulting from the mutations of charged residues that are involved in salt-bridging increased heat capacity change of unfolding (ΔCp).

Reducing the ΔCp could enhance the maintenance of a stable temperature by ensuring that proteins maintain their natively folded states. Hence, the interaction of salt bridges potentially enhances thermostability (Chan *et al.,* 2011).

Surface Charges. Studies conducted by Fukuchi and Nishikawa (2001) showed that, every thermostable protein is influenced by high charged residues on the proteins' surface through the replacement of uncharged polar surface residue with the charged residues.

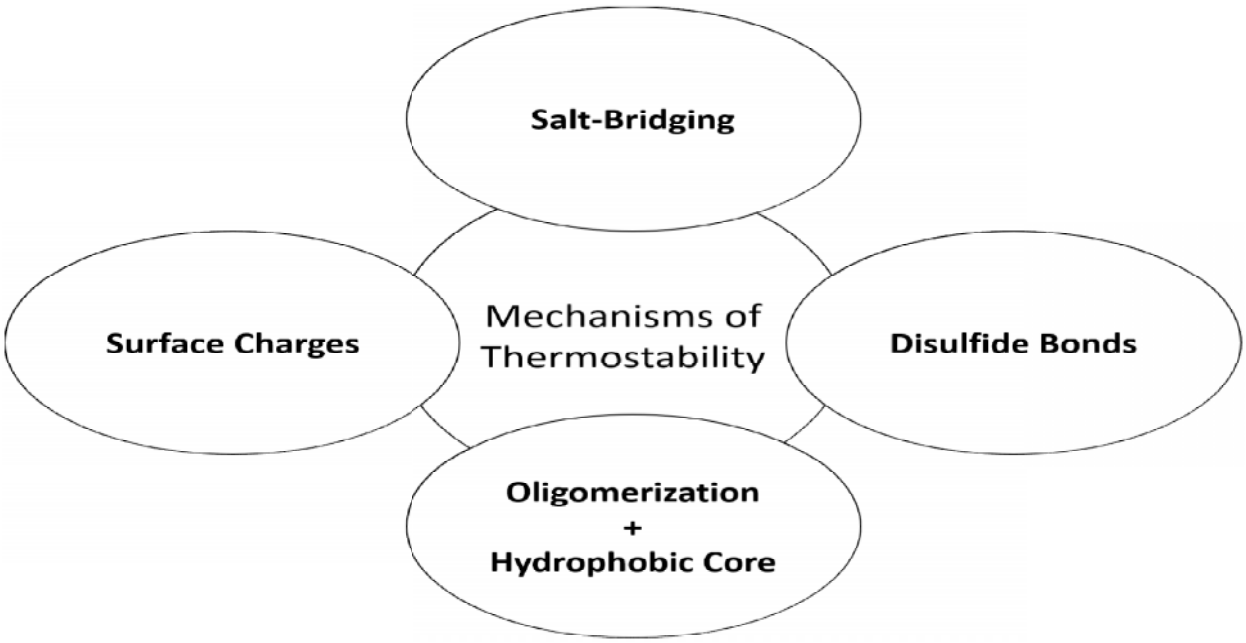

Fig. 1. Summary of the mechanisms of thermostability.

Thermolabile residues such as asparagine and glutamine which potentially cause a decrease in stability through deamination can be replaced to increase charge interactions in order to avoid denaturation caused by heat (Fukuchi and Nishikawa 2001). Research on *Methanothermobacter thermautotrophicus* (MTH10b) showed that salt increases the thermal capacity of proteins by offsetting repulsive forces that disrupt the protein structure (Liu *et al.*, 2012). In contrast, Mamat *et al.,* (2002) pointed out that, highly charged surface residues can also result in the disruption of protein structure despite it can avert aggregation at higher temperatures. The role of salt in the functionality and thermostability of proteins obtained from *M. kandleri* suggests that charged surface protein may require other factors to meet its thermal capacity (Reed *et al.,* 2013).

Disulfide Bonds. Disulfide bond between cysteine residues is an important determinant of a complete protein structure as these bonds have been known to increase the thermostability of proteins (Boutz *et al.*, 2007). In a study by Boutz and his associates (2007), cyclized protein chains interlocked by two subunits (monomeric) of a homodimer using disulfide bonds of citrate synthetase isolated from *Pyrobaculum aerophilum* has shown thermostability by preventing the separation of distinct subunits. Research by Cacciapuoti and his team demonstrated the role of disulfide bonds in maintaining protein stability using 5 -deoxy-5 methylthioadenosine phosphorylase II (Cacciapuoti *et al.,* 2012). The study showed that hexameric protein was reversibly broken into their monomeric states while alteration in the protein shape caused by heat and chemicals remained permanent. The researchers also observed that, the native protein denatured at a higher temperature compared to the double mutants which indicate that disulfide bonding gives the protein the property of thermal adaptation since, the mutations in the residues reduced the thermostability.

Oligomerization and Hydrophobic Core. *Pyrobaculum aerophilum* and *Ignicoccus hospitalis* have shown an oligomeric state contrary to their mesophilic counterparts while their enzymes form octamer and monomer/homodimer respectively (Mayer *et al.,* 2012). Hydrophobic influence is necessary for the maintenance of protein folding and stability while the temperature rises as shown in phosphotriesterase isolated from *Sulfolobus solfataricus* which was compactly packed at the dimer interface (Del Vecchio *et al.,* 2009). Even though *Pyrococcus furiosus* is deficient in transferring activity, a new domain on the N-terminus allows its amylase to show activity in the monomeric state. The N-terminus bacteria have a loop that covers the active site which stabilizes some particular substrates including maltose in contrast to amylase from *Pyrococcus furiosus. Pyrococcus furiosus* changes substrate specificity due to the lack of N-terminal loop and quaternary structure which indicates the necessity of the changes in the protein stability (Vihinen, 1987).

C. Biochemical Compounds Produced by Extremophiles

Cyclic dipeptides. Cyclic dipeptides (which can also be referred to as diketopiperazines) are very important compounds that are usually synthesized by fungi from the genera *Penicillia* and are composed of mevalonic acid and two amino acids. Diketopiperazines include meleagrine, glandicolines A and B, oxalin, roquefortine, and other alkaloids which are associated with these diketopiperazines are synthesized by

histidine, tryptophan, and mevalonic acid **(**Kumar *et al.*, 2018). Diketopiperazines such as rugulosuvines and brevianamides have been found to have clinical properties such as antiviral, antimicrobial, antitumor, antifungal as well as impact blood clotting. These compounds are found in halophiles such as *Natronococcus occultus* and *Naloterrigena hispanica* (Charlesworth and Burns, 2015).

Carotenoids. Carotenoids are pigments that occur in nature which plays vital roles in the protection of organisms such as plants and animals against the harmful effects of air, light and sensitizer pigments. According to Schiraldi and his associates (2002), halophilic archaea and algae are the major sources of carotenoids among extremophiles. However, most carotenoids synthesized by plants and/or extracted from them do not meet industrial requirements but β-carotene is an exception (Chandi and Gill, 2011). Among extremophiles, *Dunaliella salina* is an excellent source of β-carotene and as a result of its wide range of solubility, it serves as an outstanding source of food supplement (DasSarma *et al.,* 2009). α-Carotene, β-carotene, and β-cryptoxanthin can be broken down into retinol, hence, they are good sources of provitamin A. Carotenoids are embedded in the matrices of plants and are made available in abundance for absorption when cooked and taken with a meal supplemented with 3 to 5 grams of fat, even though the minimum requirements may vary from one carotenoid to the other (Priyadarshani, 2017).

D. Thermozymes

The thermostable nature of thermozymes makes these biocatalysts suitable for diverse applications in industries including Fat and Oil industries, Dairy industries, Food industries, Bakery industries, pharmaceutical industries, meat industries, etc. Several investigations have revealed that, the defense mechanism adopted by extremophiles can be used in the pharmaceutical industry in the development of novel therapeutic drugs. For example, sunscreens, antioxidants, and anticancer drugs were identified based on the survival mechanism of extremophiles that are resistant to radiation (Gabani and Singh 2013).

Lipases. Even though Polyunsaturated Fatty Acids (PUFAs) such as Omega-3 and Omega-6 play important roles in the central nervous system, they become lethal to our health when oxidized. Lipases isolated from thermophilic microorganisms can be used to enrich Polyunsaturated Fatty Acids and can also be used for the extraction of lipids from both plants and animal sources while their acyl glycerides (mono and di) have relevance in the pharmaceutical industries. For example, in the medical fields, liposomes are used as carriers for drugs such as amphotericin B (used for treating fungal infections) which convey the drugs to target zones as well as preventing wastage and anatomical barriers. (Linko and Yan Wu, 1996). This is achieved by the enzymatic degradation of liposomes by microbial biocatalysts such as lipases. Immobilized lipases isolated from *Lactobacillus ruteri* have been found to be potent in the manufacture of nutraceuticals (products derived from food sources with extra health benefits apart from their nutritional value) such as antioxidants, dietary supplements, fortified dairy products, and citrus products, vitamin products, minerals, herbal products, and other cereal products (Linko and Yan Wu 1996). Pandey and his associates (1999) also reported that lipases can be used to speed up reactions used in manufacturing drugs and can also be used in the manufacture of anti-inflammatory drugs such as Aspirin, Ibuprofen, Naproxen, Indomethacin, Meloxicam, Celecoxib, kinetic resolution, antibiotics, potential anti-viral agent (lamivudine), vitamins, anti-arteriosclerotic drugs, anti-tumor and anti-allergic compounds.

Streptokinase. Streptokinase is another biocatalyst with a wide range of applications in the pharmaceutical industry. In 1952, Johnson and Tillett synthetically induced a thrombus (cloth) in the marginal ear of a rabbit and successfully used Streptokinase as a thrombolytic or a fibrinolytic agent to dissolve blood cloth. Its mode of operation is converting plasminogen into plasmin by binding with the plasminogen and forming active complexes with protease to catalyze the conversion process (McClintock *et al.*, 1971). Investigation during clinical trials have shown that, intravenous injection of streptokinase has proven potent compared to placebos in both long- and short-time casualties in patients suffering from decreases or stops in blood flow to parts of the heart, causing damage to the heart muscle (myocardial infarction or heart attach) (Kumar et al., 2012). Despite the fact that pharmaceutical products developed by using streptokinase have side effects such as nausea, headache, dizziness, low blood pressure, mild fever, bleeding from wounds or gums, rashes, and itching, its patronage is still high because it is less expensive, effective in its role, and stress-free to obtain (Kumar *et al.*, 2012).

Amidases and Nitrilases. Amidases and nitrilases are nitrile degrading enzymes. Amidases and nitrilases speed up the breakdown of nitrile compounds to the corresponding carboxylic acid and ammonia. These two biocatalysts form the building block for synthesizing intermediates in pharmaceutical and other bio-industries. A nitrile is basically an organic chemical that contains a cyano functional group or subunit (CN^-) in which the carbon and nitrogen atoms have a triple bond (that is $C{\equiv}N^-$). The catalytic reactions involved in the synthesis of these corresponding intermediates take place at high temperatures. This makes amidases and nitrilases vital biocatalyst in the pharmaceutical industry. These enzymes have different substrates, for example, aromatic amidases and aliphatic amidase catalyzes the hydrolysis of aromatic substrates and aliphatic

substrates respectively (Panda *et al.,* 2013). Research has shown that amidases were found in thermophilic microbial species of *Pyrodoccus yayanosii* (Fu *et al.,* 2014) and *Sulfolobus tokodaii* strain 7 (Suzuki *et al.,* 2006) while nitrilases were found in *Pyrococcus abyssi* (Mueller *et al.,* 2006) and new *Pyrococcus* sp. from Antarctica (Cabrera *et al.,* 2017) even though these enzymes can be identified in some species of plants, fungi, and archaea. According to Panda (2013), amidases are potentially used to hydrolyze long stretches of nitriles while nitrilases are used to hydrolyze a single step nitrile to their corresponding carboxylic acids and ammonia.

Chitinases. Chitinases are hydrolytic enzymes that break down glycosidic bonds in chitin. Chitin is a linear polymer of β-1, 4-N-acetylglucosamine (GlcNAC) that is hydrophobic and crystalline in nature. After cellulose, chitin is the next most abundant biopolymer in nature and it is usually found in the exoskeleton of fungi, algae, yeasts, lobsters, crabs, and within the internal membrane of invertebrates (Bhattacharya *et al.,* 2007). Chitinases have been found effective in the pharmaceutical industry for designing anti-inflammatory drugs that can be valuable in the treatment of disorders such as osteoarthritis, ulcerative colitis, and other gastrointestinal inflammations. Chitinases can be used to disintegrate chitin and the product of the breakdown can be used by a wide range of industries in manufacturing surgical stitches, production of artificial skin, anti-fungal creams, wound healing, lotions, and dietary fiber. Products derived from chitin are biodegradable, non-allergic, non-toxic, and biocompatible (Cabrera *et al.,* 2018). These properties make the derivatives of chitinase an important element for making scaffolds on which stem cells are seeded.

Glucocerebrosidase. Glucocerebrosidase is a lysosomal glycoside hydrolase that cleaves glycolipid glucosylceramide. Gaucher's disease is the resultant disease of insufficiencies in the production of these enzymes to cleave the glycolipid glucosylceramide leading to the accumulation of glucosylceramide (GlcCer). Mueller (2006) reported that Gaucher's disease is a pan-ethnic disease that affects at least 1 in 50,000 or 1 in 100,000 across the globe. Gaucher's disease is becoming a growing global concern but Glucocerebrosidase has the potential to curtail the menace of this disease and other emanating disorders.

Proteases. Research has shown that, the major producers of alkaline proteases for industrial purposes belong to the genera, "*Bacillus*". Proteases are very crucial elements in the pharmaceutical industry for drug discovery due to their role in the life cycles and spread of infectious diseases. The involvement of proteases in pathophysiological processes may induce the production of potent therapeutic agents that can combat lethal diseases such as AIDS and cancer (Rawlings *et al.,* 2004). Due to the diversity of microbial proteases, researchers prefer them to those produced by other organisms such as plants and animals (Palsaniya *et al.,* 2012). The extracellular and intracellular proteases produced by these microorganisms are imperative to protein hydrolysis, hormone regulation, cellular protein pool, protein turnover, and differentiation (Adrio and Demain, 2014).

It has been reported that 2% of human genes are encoded by proteases which are characterized by complex roles than one could imagine. Some of these roles include the regulation of chemokines, growth factors, cellular receptors, and cytokines via toggling (activation and inactivation) of the genes involved, resulting in downstream intracellular signaling and gene regulation while up-regulation can be linked to different cancers and tumor metastasis, growth, and invasion (Rodríguez *et al.,* 2010). "Pancreatic enzyme replacement therapy" is an effective treatment developed to restore normal absorption or improvement of malabsorption using a blend of proteases, amylases, and lipases obtained from microbial sources. For example, Eurand (a specialty pharmaceutical company offering a range of products to cystic fibrosis and gastrointestinal patients in the U.S.) has received approval from the FDA for pancrelipase (Zenpep®) to be marketed and has been proven to be effective in nitrogen and fat (Wooldridge *et al.,* 2009). Sollid (2002), suggested that, the ability of protease to hydrolyze the peptide bonds in gluten makes it a potential agent for the treatment of coeliac disease which emanates from gluten hypersensitivity, leading to intestinal inflammation.

E. Medical Applications of Thermophilic Secondary Metabolites

Thermophilic secondary metabolites are attractive sources of therapeutic agents as they have a wide range of chemical diversity within species and strains. Exploitation of these metabolites can lead to the discovery of novel drugs to treat various diseases like cancer, high cholesterol, diarrhea, obesity, diabetes, anemia, etc. Long ago, microbes have been used as bio-chemicals in many fields such as medicine for the treatment of diseases; food processing industry as probiotics, etc., and in agriculture for breeding purposes. Thermophilic secondary metabolites have also been found to be good sources of vitamins, enzymes, and their inhibitors, as well as antibiotics.

Reduction of Cholesterol. Cholesterol is an oily substance naturally found in the body as well as foods containing fat/oil which is required by the body to carry out certain biochemical functions. The body is able to synthesize the quantity of cholesterol it needs to carry out its activities. Cholesterol plays a major role in the formation of hormones for cell signaling and regulation of membrane fluidity, serves as a major component of the cell membrane, a source of bile acid and vitamin D; and a precursor for the synthesis of female and male hormones viz. estrogen and progesterone respectively

(Li and Chiang, 2014). Unfortunately, cholesterol in high concentrations can expose the body to lethal diseases such as stroke, coronary artery diseases, and atherosclerosis. The biotransformation of cholesterol to Coprostanol by Coprostanoligenic Bacteria offers a novel means of controlling cholesterol levels in the body.

Management of obesity. According to Ohtani and his colleagues (2014), Type II diabetes, hypertension, coronary heart disease and some cancers can be attributed to obesity. When the number of triglycerides stored in adipocytes exceed the energy usage of the body as a result of excess fat of energy consumption results in obesity (Liou *et al.,* 2013). Obesity can be acquired as a result of age, physical activity, developmental stage, and genes (Kohli *et al.,* 2020). Research has shown that the ability to modify the ecosystem of bacteria in the gut by administering probiotics will provide a new means of treating or preventing obesity (Kohli *et al.,* 2020). Szilágyi and Závodszky (2000) also reported that, *Lactobacillus ssp.* (such as *Lactobacillus acidophilus* NCFB 1748 and *Lactobacillus sporogenes* and bacteria belonging to the genus *Bifidobacterium* are potential candidates for controlling obesity and overweight in both humans and experimental models. Kohli and his colleagues (2020) also projected that, probiotics producing bacteria such as *Lactobacillus ssp.* have been used as natural medication in the management of weight loss while prolonging both satiation and satiety as well as reducing fat deposition and food consumption.

Production of Probiotics. Probiotics are live microorganisms that confer benefits when consumed or applied to the body. These microbes can be obtained from several sources including fermented foods, dietary supplements etc. Gut microbiota such as *Firmicutes, Proteobacteria, Actinobacteria* are some of the major phyla of microbes that inhabit the human intestine while being involved in a variety of roles, for instance, regulation of host metabolism and immune system homeostasis (Shahbazi *et al.,* 2020). Modern lifestyle or various diseases as well as exposure to certain toxins and chemicals may lead to an imbalance of the intestinal flora and this can result in dysbiosis which can lead to several health conditions such as obesity, metabolic syndrome, non-alcoholic fatty liver disease, type 1 and 2 diabetes etc. (Sharifi-Rad *et al.,* 2020).

Several studies have focused on the reduction of human associated pathogens such as *Salmonella typhi* and *E. coli* and this has made some probiotic metabolites appear to play a role in the modulation of diverse signaling and metabolic pathways in various cells. Several components of probiotic metabolome (organic acids, bacteriocins, hydrogen peroxide, etc.) have been reported to interact with multiple targets in some metabolic pathways that regulate cellular proliferation, differentiation, apoptosis, inflammation, angiogenesis, and metastasis (Plaza-Diaz *et al.,* 2019). Plaza-Diaz and his associates (2019) indicated that, *Bifidobacteria* and *Lactobacilli* have shown over 20 distinct enzymatic activities with *β*-galactosidase having the highest enzymatic activity. It was added that, bacterial *β*-glucuronidase found in the intestine hydrolyses glucuronidated metabolites to their toxic forms, leading to impairment of the intestine. The above-mentioned bacteria also exhibit colonization resistance as probiotics by producing antimicrobial peptides such as bacteriocins which prevent the proliferation of some pathogens or kill enteric pathogens.

Kim and Jin (2001) projected that, decreased activity of *β*-glucuronidase in fecal matter was linked to an up-rise in the quantity of colonic lumen carcinogenic substances. The addition of *Bifidobacterium longum* may contribute significantly to intestinal microbiota thus, decreasing the activity of *β*-glucuronidase which inhibit the formation of an aberrant crypt and early preneoplastic marker for malignant potential in the process of colon carcinogenesis (Plaza-Diaz *et al.,* 2019).

Among the therapies for treating COVID-19, amelioration of inflammation can be used as an extra supplement due to its potential records, for instance, therapeutic supplementation of probiotics can be an effective means of boosting immunity as it was shown to be effective in the treatment of viral diseases including influenzas (Libertucci, 2019). As a result, the National Administration of Traditional Chinese Medicine and the National Health Commission of China have opted for probiotics as one of the therapies for COVID-19 (He, 2020). Probiotics can be used for the modulation of dysbiosis that is being caused by viruses, thus, can be used as a potential treatment against COVID-19 besides the attenuation of gastrointestinal tract indications (Mathew *et al.,* 2019). According to research, probiotics aids in balancing microbiome in the digestive tract in order to reduce the intensity of diseases that may result positively in gut–lung axis communication (Wypych *et al.,* 2019).

Production of Antibiotics. Antimicrobial resistance arises when potent antimicrobial derivatives lose effectiveness against microbes, thus, permitting microbes to thrive upon subsequent application of antimicrobials. This calls for research into alternative classes of antimicrobials from varying sources.

Studies have shown the production of bacteriocins from extremophiles including thermophilic *Bacillus* spp. and thermophilic *Geobacillus* spp. (Ravot *et al.,* 2006). In a study conducted by Alkhalili and his colleagues (2016), a protein extract isolated from Thermophilic *Geobacillus* sp. strain ZGt-1 showed an antibacterial activity against both mesophilic and thermophilic bacteria comprising Salmonella typhimurium, *Bacillus subtilis*, and *G. stearothermophilus.* The research disclosed that, the protein extract ZGt-1 was isolated at a temperature of 60°C and denatured when subjected to

protease. Research conducted by Rachanamol and his associates (2017) demonstrated that, mesophilic bacterium associated with the marine sponge *Callyspongia diffusa* was shown antibacterial activity against fish pathogens which include *Vibrio vulnifucus, Vibrio anguillarum,* and *Vibrio fluvialis* as well as *staphylococcus aureus* of human origin. The associated bacterium was confirmed to be *Bacillus subtilis* through 16S rRNA gene sequencing with maximum protease activity recorded at pH 9 and 30°C (Rachanamol, *et al.*, 2017). According to Andrejko and Siemińska (2016), metalloprotease-alkaline protease isolated from *Pseudomonas aeruginosa* has both antibacterial and antifungal activities. When sublethal doses of the alkaline protease isolate were introduced into the hemolymph of *Galleria mellonella* larvae, antimicrobial activity was observed. Independent research conducted by Indarmawan *et al.,* (2016) on a fungal species known as *Xylaria psidii* KT30 which was sourced from *Kappaphycus alvarezii* pointed out that, protease is a potent source of antibacterial agent. It was proven that *Xylariapsidii* KT30 displayed antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* while the highest antibacterial activity was at a value of 2.33 ± 0.19 U/mL on fungal extract fifteen days upon culturing.

Patents on thermophilic secondary metabolites. Thompson and his associates (2020) have patented Thermophilic and thermoacidophilic Metabolism Genes and Enzymes from *Alicyclobacillus acidocaldarius* and Related Organisms. Polypeptides and nucleic acid sequences encoding polypeptides from *Alicyclobacillus acidocaldarius* were isolated and/or purified. Methods for modulating or altering metabolism in a cell is also provided, using isolated and/or purified polypeptides and nucleic acid sequences from *Alicyclobacillus acidocaldarius.* Enzymes have a great deal of potential for the production of useful chemicals in industrial processes. However, industrial processes typically occur at extremes of temperature, pH, salt, etc., to which most of the well-studied enzymes and organisms are not well suited, hence, *Alicyclobacillus acidocaldarius* is a potential candidate for therapeutic and biotechnological processes.

Puri and his colleagues (2006) also acquired a patent on "Novel endophytic camptothecin and camptothecinoid producing fungi and process of producing the same". This invention is associated with a novel microorganism source for the production of Camptothecin and related camptothecinoids. The invention also reveals its isolation, screening for Camptothecin production, growth, fermentation requirements, and Camptothecin chemical analysis (camptothecinoids). Camptothecin (CPT), 4-(S)-4-ethyl-4-hydroxy-1-H-pyrano, [3,4,6,7]-indolizino-(1,2b) quinoline-3,14 (4H,12H) dione} belongs to a class of anticancer agents that work by interfering with eukaryotic DNA. CPT and minor camptothecinoids were extracted in high concentrations from the Indian tree *Nothapodytes foetida*.

Eyberger and his associates (2006) patented endophyte fungal isolates from *Podophyllum peltatum* which produced podophyllotoxin. The present invention is about podophyllotoxin derived from natural sources. The invention is specifically concerned with endophytic fungi that are capable of producing podophyllotoxin, methods of isolating such endophytic fungi, and the production of podophyllotoxin by the fungi. Podophyllotoxin is a plant product that is used to treat a variety of cancers. The compound serves as a precursor in the semi-synthesis of the anticancer drugs, etoposide, teniposide, and etoposide phosphate (Canel *et al.,* 2000). These drugs are in high demand for the treatment of a wide range of cancers, either alone or in combination with other anticancer agents.

Challenges that hamper the exploitation of thermophiles and some advances made

Researcher have not been able to exploit thermophiles to their full potentials due to constraints including the ability to get access to thermophilic habitats, proper understanding of the mechanisms that confer the capacity of thermophiles to withstand high thermal conditions, and challenges for developing robust genetic tools and systems as well as Growth requirement constraints that are needed for the genetic manipulation of thermophiles (Straub *et al.,* 2018).

At the hydrothermal vents, chimneys release fluids at high temperatures exceeding 100°C which cannot support human lives, making it impossible to access thermophiles in the past. A thorough investigation conducted on the vents using sophisticated equipment which includes remotely operated vehicles (ROVs) accompanied with manipulator arms, lights, and cameras have improved the accessibility of deep-sea hydrothermal vents and their inhabitants (Natural History Museum, 2018).

Despite the mechanisms of thermostability have not been understood in details, the little understanding of the mechanisms of salt bridging, surface charges, disulfide bonding, oligomeric and hydrophobic core as briefly described above have contributed to the exploitation of thermophiles. For example, with the understanding of the mechanism of surface charges, thermolabile (uncharged polar surface) residues such as asparagine and glutamine can be replaced by thermostable residues (highly charged) to increase thermostability (Fukuchi and Nishikawa 2001).

FUTURE SCOPE

Engineers and researchers are working hard to obtain novel and robust microbial secondary metabolites for commercial and industrial processes. The exploitation of thermophilic secondary metabolites for therapeutic purposes presents a new age for the treatment of emanating diseases. In the future, there is a high tendency that thermophilic secondary metabolites will

play a primary role in the production of effective drugs with novel characteristics. Advanced techniques in molecular biology, genetic engineering, bioinformatics and the related fields are a prospective tool for generating improved strains of thermophiles for therapeutic uses. This can be attributed to the fact that, microbial strains with desirable traits offer an increase in yield, improved substrate specificity, increased thermostability, etc.

CONCLUSION

Several research reports have demonstrated the role of microbial (thermophilic) secondary metabolites in the management of health conditions such as the conversion of cholesterol to coprostanol, drug delivery, detoxification, production of scaffolds materials for stem cell culture etc. despite most of the mechanisms and metabolic pathways of the microbes are not properly understood. Limited knowledge in this regard has restricted the exploitation of these secondary metabolites to their full potentials. Antimicrobial agents such as probiotics, antibiotics, drugs etc. produced by thermophilic microorganisms have greater industrial potential due to their thermostability. Therefore, further investigation into the features of thermophilic secondary metabolites is a prospective avenue for drug discovery and other medical remedies.

Acknowledgement. The authors would like to express special thanks of gratitude to the government of Ghana through the Ghana Scholarship Secretariat for making this mission possible. Special thanks to Lovely professional university (LPU) for their educative support. The authors are also grateful to faculty members of Lovely professional university and friends who contributed to this article directly and indirectly.

Conflicts of Interest. The authors declare no conflict of interest.

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How to cite this article: Ameho, S. and Christina, E. (2021). A Review on the Therapeutic Roles of Selected Thermophilic Secondary Metabolites. *Biological Forum – An International Journal*, *13*(2): 83-94.