

Extremophile Current Challenges and New Gate of Knowledge by Nanoparticles Pathways.

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Abstract: Extremophiles are a unique organisms that have ability to exist in critical environmental conditions such as temperatures, pH, saline and pressures. They are characterized by high efficiencies in growth and enzymes product that led them to be a candidate in industrial productions as detergents, brewing, cosmetics, dairy products, bakery, textiles, and as degradation materials. More information concerning the behavior of extremophiles is still required. Recently, several studies are conducted to detect deep information about extremophiles using the advantages of nanoparticles. For instances, gold (Au) and silver (Ag) nanoparticles open a new gate of knowledge for researcher particularly for study different pathways of extremophiles. In this review we first concerns with extremophiles definition, history and applications then we reflects general idea about the environmental conditions taking in account the uses of nanoparticles.

Keywords: Extremophiles, microorganisms, applications, nanoparticles.

I. Definition

In nature, there are several kind of environmental conditions and resources such as temperature, pH, pressure, salinities, desiccation, radiation and metal. These can effect survival ratio of living organisms, plants and animals. Due to the different values of physico-chemical and survival conditions, the relationships between environmental situations are definitely complicated. To understand that, we have to know the normal range for every conditions and resources including the efficiency of a variety lives. However, abnormal range of conditions and resources could be optimum environments for different organisms to survive. These kind of organisms are known as extremophiles or briefly, the organisms which have ability to be active in abnormal environments [1].

II. Background

Farlow was the owner for first extremophile species isolated from salted fish which described as a salted-loving organisms (halophilic) [2]. In 1936, up to 34% salted concentration resistance strains have been isolated from the Dead-sea by Elezari Volcani [3]. He showed the improper appellation of that kind in water body. Volcani is considered as historical leader due to his efforts since he got the starting point in extremophilic field and focused in halophilic microbes. Since halophilic organisms start to be interesting for researchers and had more attentions until they revealed that the majority of them follow the third sphere of existence which have been identified as archaea [4]. During that decade, hyperthermophilic and thermophilic microbes were isolated at 60 to 80°C. Furthermore, in 1969, *Thermus aquaticus* thermophilic bacterium was isolated by Thomas Brock from Yellowstone National Park hot spring in the United States [5]. After one year, *Sulfolobus acidocaldarius* was the first hyperthermophile isolated which able to raising in low pH (1-5) and up to 85°C [6]. Accordingly, this was a large discovering for biologists because its changed all the previous concepts, there are no organisms can resist higher than 80 °C [7]. These discoveries were the first extremophile approaches that develops the diversity of microbiology. In the following section, we discuss more details about the extremophile environmental conditions.

III. Different Environmental Conditions And Enzymes

3.1 Temperature

The environmental temperature is essential factor for organisms to survive and growth. There are minimum, optimum and maximum temperatures, and each organism has its characteristic temperature for growth and survival.

3.1.1 High Temperature

Organisms that able to survive and grow into thermal area are called thermophilic. according to their resistance to temperature, thermophilic organisms are divided into three groups: 1) mesophilic has an optimum between 50 to 60°C, 2) thermophilic has an optimum higher than 70°C, and 3) hyperthermophilic which has an optimum temperature more than 80°C [8]. It was found that prokaryotic (Bacteria and Archaea) can grow in much higher temperature (113°C) than eukaryotic (not over 60°C) [9]. Thermophilic bacteria such as *Thermoanaerobacteriales*, *Bacillales*, and *Clostridiales* were isolated from a variety of environmental and also ordered as alkaliphiles, neutrophils and acidophiles; strict anaerobes, selective anaerobes, and aerobes; and chemolithotrophs and chemoorganotrophs [10].

High percentage of hyperthermophilic isolated from terrestrial and marine thermal area [11]. Hyperthermophiles are very diversity in physiological properties and phylogeny. Now, novel taxonomy has been recognized and characterized by using 16S rRNA sequenced-based of prokaryotes. Further, to acquiring more efficiency resolution of taxonomy by using several kinds of traditional taxonomic features like physiological features, morphology, DNA-DNA homology and GC content DNA [12]. Phylogenetic tree of the 16S rRNA-based presented that, it has three domains, the eukarya, bacteria, and archaea (the former 'archaeobacterial') [13]. In the branches root cluster, hyperthermophiles appear as a short deep phylogenetic branches in the domains of bacteria and archaea. In fact, the highest growth temperatures are the members of the shortest and deepest lineages [14]. Accordingly, the eukaryotic has no short deep lineage, that mean it cannot survive as hyperthermophilic due to the limitation of membrane system adaptation [15]. In hyperthermophilic bacteria, there are 10 orders including 29 genera, and more than 70 species [16]. Some examples of such bacteria are *Thermocrinis rubber*, *T. neapolitana*, *Thermotoga maritima*, *Fervidobacterium pennavorans*, *A. profundus*, and *Aquifl xpyrophilus* [17]. Among these species, *T. maritima* and *A. pyrophilus* shows the highest growth temperatures (90 and 95°C, respectively). In Archaea, which represent the majority of hyperthermophiles the genera *Desulfofococcus*, *Thermococcus*, *Thermoproteus*, *Sulfolobus*, *Pyrobolus*, *Pyrococcus*, *Pyrodictium*, *Pyrobaculum*, *Ferroglobus*, and *Archaeoglobus* are grown above 80 °C. However, *P. fumari* and *P. occultum* recorded the highest temperature (113 and 121 °C, respectively) [18].

Thermophilic enzymes have gotten much attention than others. Lipases, polymer-degrading enzymes (amylase, cellulose, and chitinases), and proteases, were a particular thermophilic enzymes. Thus, they display as industrial application enzymes [19]. For an examples, proteases can be used in detergents, brewing, hydrolysis in feed and food, and baking. Glycosyl hydrolysis (e.g. xylanases, cellulases, glucosidases, glucoamylases, pullulanase, and amylases) using for textiles, pectin, chitin, cellulose, starch, and processing. Chitin modification for food and health products, have been produced by chitinases, and also paper bleaching by xylanases. Stereo-specific reactions (e.g. trans-esterification, organic biosynthesis) and detergents were produced by lipases and esterases [20]. Furthermore, DNA polymerase (e.g. *Taq* polymerase) produced by *Thermophilusaquaticus* could be classified as thermophilic enzymes [21]. Researchers were exploiting thermal enzymes that are active and stable at elevated temperature due to the solubility of reaction components and reduction of contamination. It was concluded that, in structural studies of thermophilic enzymes the stability was accomplished by a combination of replacement of uncovering 'thermolabile' amino acids, increased the hydrophobicity of protein core, and increased the charge of surface [22-24].

3.1.2 Low temperature

Low temperature can be considered as a risk for all organism's activities and occurrences. Organisms which growth in cold environment could be inhabit in mountainous zones and Polar Regions [25]. The most part of the earth which has low temperature is ocean due to it is covering 3 quarter of the earth surface. The average of ocean's temperature is 5°C but the depth average is 1-2°C. Especially when it start freezing due to the lower temperature. In such environmental condition the growth is suspended, and then the intracellular become as water solidifies. The freezing point could be lowered until the values become below 0°C by the occurrence of great concentrations of solutes, which behave as antifreeze solutions. The freezing point has an ability to inhibit organisms and a part of continents can be characterized as low temperature. Accordingly, psychrophilic organisms could be reported in the permafrost of Siberia and Antarctica [26]. Microorganisms can be survive and adapted the cold conditions but it is very hard to distinguish that microorganisms live as psychrophiles or psychrotolerant in cold environment [27]. The snow algae and eukaryotic can be colonized in the ice such as the color of red, orange, or green snow due to the pigment of algae. For example, *Chlamydomonas nivalis* which presented its color at 1°C. On the other hand, a large number of bacteria is often live in sea ice (exactly, in ice pocket liquid) [28]. Cowan *et al*, found that in permanently cold (the ratio of temperature at -0.5 to 3.8°C) Antarctic soils, the living bacteria can be ascertained 3×10^6 and 2×10^9 by the determination of bioluminescent ATP. Higher numbers of bacteria appearing well in permafrost soils [29]. More surprising activities in permafrost by the communities of microbes are that, the lipid fractions which have been incorporation by ^{14}C -labeled acetate enabled the appraisal of the community lower doubling

periods for 160 days at -20 °C, for 20 days at -10°C, and for 1 day at 5°C [30]. Irgens *et al*, have been found that in Antarctic marine water is the most popular place for psychrophilic microorganism, a part of the γ -Proteobacteria called *Polaromonas vacuolata*, which can grow up in the range of 0-12°C and the optimum temperature is 4°C [31]. Also psychrophilic enzymes have appeared in industrial field as lipases, proteases, amylases and cellulases. These enzymes participate in different manufacturing such as dairy products, bakery, and textiles or may shares in detergents and cosmetics. In structural studies, there is assumption that psychrophilic enzymes decreased the stability and correlated with increased flexibility [27].

3.2 pH

A life of microbial is achievable by a wide values of pH. Organisms which being in low pH environment called acidophiles, while the organisms living in high pH environments are termed alkaliphiles.

3.2.1 Acidity

The acidity of environments can be increased by biological factors or abiotically (volcanic area has the solfatar fields). For instance, the chemoautotrophic bacteria (*Thiobacillus ferrooxidans* or *Thiobacillus thiooxidans*) has ability to oxidize pyrite, elemental sulfur, sulfide, and other chemical compounds leading to the pH reduction by accumulation concomitant with sulfuric acid. Acidity production could also result from organic acids during fermentation or ferrous iron oxidation. For example, *Thiobacillus ferrooxidans* generates 380 acids that precipitates in protons release such as $\text{Fe}(\text{OH})_3$ through the oxidation of Fe^{2+} to Fe^{3+} .

Acidophilic microorganisms can be found in three domains: Eukarya, Bacteria, and Archaea. Eukaryotes have heterotrophic and phototrophic, extremely acid-tolerant and/or acidophilic. *Dunaliella acidophila* as a unicellular green alga needs acidic conditions between pH 0 to 3 and the optimum pH is 1.0 [32]. The cluster of acidophilic and thermophilic are often occurs through different microorganisms particularly in algae [33]. The thermoacidophilic of a unicellular alga (e.g., *Cyanidium caldarium*) can tolerate the acidity of pH (0.2) and the optimum growth in the range of pH 2-3 [34, 35]. This kind of acidophilic alga can tolerate 1 N sulfuric acid. A few number of fungi may growing at pH values near zero e.g., species of the genera *Trichosporon*, *Cephalosporium*, and *Acontium*. The bacterial domain as mentioned previously (*Leptospirillum ferrooxidans* and *Thiobacillus ferrooxidans*) could be grown at pH 2-4. Furthermore, *Thiobacillus thiooxidans* could be grown at lower pH 0.5.

Among the microorganisms, archaeal domain is the most acid-tolerant and acidophilic. Up to date, *Picrophilus torridus* and *Picrophilusoshimae* (*hyperacidophilic* species) were able to grow in the lowest reported pH, that is -0.06, the optimum pH at 0.5 and the cells lyses above pH 4. In addition, the archaea require high temperature and 60°C is considered as an optimum temperature for growing. An aerobic heterotrophs of *Picrophilus*, isolated from Japanese solfatar, can grow on yeast extract as energy and carbon sources [36-38]. Also numerous of thermoacidophilic archaea such as *Thermoplasma acidophilum* has an optimum pH for growth at 1.8-2 and pH 0.4 is the minimum value for growth [39]. Also several biocatalysis enzymes are active at low pH such as glucosidases, glucoamylases, pullulanases, oxidases, cellulases, proteases and amylases. These enzymes have been used in different applications including starch processing, desulfurization of coal, and feed component [40, 41].

3.2.2 Alkalies

Alkaliphilic bacteria, growing at pH 9-10 and above, can be isolated from soil or from other environmental resources. For example, *Bacillus calcalophilus* is the popular organism that used to study the adaptation of alkali values (pH exceeding ten) depending on bioenergetics aspects. In soda lakes, more extreme alkaliphiles can be found at pH 12. Many distinct kinds of metabolism that support elements in complete cycling such as sulfur, nitrogen, and carbon have been expressed by existence of alkaliphiles [42, 43]. In archaeal domain, there are some species have obligate alkaliphiles such as haloalkaliphilic genera *Natronococcus* and *Natronobacterium*, that used hypersaline soda lakes as a characteristic inhabitants (e.g. the lakes of Wadi Natrun in Egypt, Tibet and Certain in China, and Magadi in Kenya) [44]. These archaeal could grow approximately at pH 12, the greatest pH of all living organisms.

Microorganism enzymes, which can survive under alkalis have efficiency for many applications such as the production of detergents [45]. Internally, the striking of alkaliphilic properties of microorganisms are to maintain a neutral pH, so the adaptation to extreme growing conditions for the intracellular enzymes is not required. This advantage is not including the extracellular proteins. Alkaliphiles enzymes have ability to resistant high concentration of detergents and showing their optimum activity at high pH such as lipases, amylases, and proteases. Based on PCR homology combinations, the alkaline proteases have been isolated from hot environments by collecting thermoacidophilic bacteria and archaea [46].

3.3 High saline

Salt is a common requirement in marine bacteria which stay in environmental salts (30-35 g/l). Microorganisms of extremely halophilic could be survived at higher salt concentrations that equal to the saturation of NaCl (300 g/l). For example, the inhabitant of halophilic microorganisms in the Dead Sea (between Jordan and Palestine) which has about 340 g/l salt concentration, with 0.4 M Ca^{2+} and more than 1.9 mM Mg^{2+} . Haloalkaliphilic microorganisms, *Natronococcus* and *Natronobacterium*, are extreme halophilic that found in soda lakes. The lakes of salt such as hypersaline alkaline lakes, Great Salt Lake (Utah), the Dead Sea, and the crystallizer ponds of NaCl-saturated for solar salters production are probably red color. The appearance of reddish color indicate the presence of rhodopsins and carotenoids with high density of the halophilic Archaea communities of Halobacteriaceae family [44, 47, 48]. Moreover, halophilic archaea could be useful in fermentation of some foods like kimchi (a popular Korean dish)[49]. Some species of unicellular green algae Chlorophyta (genus of *Dunaliella*) could participate in brine coloration. The β -carotene of massive accumulation that produced by algae could lead to red-orange color. Bacterial domain has extremely halotolerant or halophilic species, including sulfate reducers, fermentative species, aerobic heterotrophs, as well as the bacterial of anoxygenic photosynthetic sulfur (*Halochromatium*, *Halorhodospira*), and oxygenic phototrophs (cyanobacteria) [44].

Also, in hypersaline habitats the halophiles can be survived by maintaining of osmotic balance as compatible solute production and biofilm formation [50]. Compatible solution including proline, glutamate, carnitine, betaine, glycine, ectoine, and exotic compatible solutes as 2-sulfotrehalose [51, 52]. In environmental isotonic, the accumulation of salts like KCl or NaCl are in equal concentrations. As a results, the concentration of high salts have been copied by halophilic proteins (e.g. concentrations of NaCl and KCl, going to be >5M and 4M respectively) [53, 54]. Halophilic enzymes can degrade some chemicals in high salt concentration such as phenols [55]. In environmental pressure, the enzymes adapted to prevent precipitation by earning a large number of relatively negative charge amino acids on their surfaces. Therefore, the application of halophilic enzymes are probably very poor and limited due to lower solubility in salt concentration [56]. However, the advantage of this property could be carrying by using halophilic enzymes in nonaqueous and aqueous/organic media [57]. *Halobacterium halobium* extracellular protease exploiting to synthesize efficient peptide in N'-N'-dimethylformamide [58]. In very lower concentrations of salt, the reversed micelles of enzyme entrapped by using *Halobacterium salinarum* p-nitrophenylphosphate phosphatase (p-NPPase) [59]. In same conditions, the stability and activity of p-NPPase is same as halophilic malate dehydrogenase [60]. The advantage of halophilic enzymes with reversed micelles are promising to develop novel applications of enzymes [61].

3.4 Pressure

Since the oceans depth is probably in average of 4 to 11 km as a maximum, the high pressure degree is observed. One atmosphere (0.1MPa) of hydrostatic pressure has been increased for every ten meter depth. In near Philippines (Mariana Trench), the deepest sea point pressure is about 110 MPa. The presence of halophilic, alkaliphilic, acidophilic, and thermophilic microorganisms in the deep sea, may help in finding a little relative about inhabiting the bacterial pressure loving (piezophiles or barophiles). In the last decade, the first bacterial truth piezophile (generally polyextremophiles) has been isolated and characterized. In the deep sea, they found piezophilic bacteria and in the same time being psychrophilic or thermophilic bacteria [62, 63]. The truly piezophilic bacteria (rather than piezotolerant) need to growth at least on 0.1 MPa as minimum and 10-50 MPa as optimum pressure or more. Accordingly, the γ -branch of the Proteobacteria in the genera of *Shewanella* and *Moritella* which obtained from the Mariana Trench in 11 km depth have optimum growth pressures 70 and 80 MPa, respectively, however, they could not grow up below 50 MPa [64]. In the deep sea of Philippine and Japan Trench, the optimum growth ratio of piezophilic strains isolated from the group of *Shewanellais* 50-70 MPa at 10 °C [65]. In the archaeal domain, there are piezophiles in the hydrothermal vent of Mid-Atlantic Ridge found as a hyperthermal *Thermococcus barophilus*. For growing up they required high pressure with optimum temperature 100°C [66]. Yayanos *et al*, isolated most of barophilic strains (probably MT41) from the Mariana Trench under highest pressure 130MPa [67, 68]. Several barophiles needing a complex system and devices such as complex gas systems and hydraulic pump to maintain the optimum pressure [69].

The major selectivity force of the pressure dose not exert in protein function [70]. There is assumption that if the pressure exceed 400 MPa it will induce a single chain in proteins denaturation. In fact, the above assumption it seems impossible because the microorganisms could not exceeding 400 MPa even that living in the deep sea (exceeding at most 120 MPa) [70]. Accordingly, their enzymes does not requiring a specific pressure related adaptation. However, some examples using high pressure for the stabilization of specific protein [71, 72]. In food production, the application of food material sterilization and processing being in high pressure that is why protein pressure resistant can be used [73]. In the previous decade, the opportunities of

piezophiles biotechnology have been reviewed [74]. Piezophiles with extremely temperature have adaptation number as a primarily deal, but they may contain unique adaptation numbers which have high pressure conditions such as multimeric protein propensities as well as dense hydrophobic cores. All of these adaptations are already studied [75]. On the other hand, barophiles have cell membranes adaptation uniquely to adapt the pressure [76]. There is nothing reported in barophilic NPs [77].

IV. Nanoparticles Synthesis

Nanoparticles (NPs) are a promising at the last decade that can be using for energy production, environmental sectors, electronics, and biomedical applications [78-81]. Different materials produce NPs engineering that could be have many characteristics of chemicals e.g. silica, carbon, multipolymers and other metals or biological origins e.g. chitosan, dextran, lactic acids, phospholipids, lipids, and proteins [82]. NPs utilized for therapy and diagnosis in medicine that according to their special characterizes (e.g. relatively large surface area, high number concentration, and ultra-small size) which support the researchers to passing all limitation of therapeutic agent and traditional diagnosis [83-85]. These special characterizes can help to finding the presenting of novel biomarker by new devices development.

Extreme microorganisms can be as a factory to synthesize inorganic nanoparticles (NPs) such as gold (Au) and silver (Ag) NPs. For example, *Geobacillus* sp. ID17 provides a special pathways and could be considered as a novel organism that has ability to synthesize NPs by NADH- dependent mechanism. It should be mentioned that this kind of bacteria synthesize AuNP under high temperatures and involving four capacities of unknown enzymes. Moreover, intracellular quasi-hexagonal NPs (5-50nm) have been produced by ID17 when challenged in potassium phosphate buffer (20mM) and hydrogen tetrachloroaurate (1mM) [86].

Due to stability of this thermophilic organism (at 65 °C), the NPs can be designed as capped proteins.

Thermotolerant fungus such as *Kluyveromyces marxianus* synthesized complete extracellular spherical Ag-NPs (3-12 nm), which characterized by anti-fungal activities and stability of proteins capping [87]. Compared with *Streptomyces* sp., *Aspergillus fumigatus* shows high efficiency in stability of Ag-NPs production and size (range between 15-45 nm). Further information, the capping of NPs reduce Ag by passing the mechanism of NADH-reductase [88]. *Cryptococcus laurentii* producing an extracellular stable Ag-NPs by the supernatant of cell-free, however, the psychrotrophic yeast such as *Yarrowia lipolytica* NCYC 789 producing an intracellular Ag-NPs under submerged condition in the medium containing yeast nitrogen base glucose [89, 90] *Bacillus* NARW11 was able to produce titanium oxide at pH 2 with a tolerate temperature at 50°C that used for fluoride adsorption [91]. *Alcaligenes faecalis* has efficient ability to produce antimicrobial Ag-NPs under condition of alkali tolerant and non-nitrate reduction [92, 93]. The synthesis process of cell-free supernatant was optimized at 60 °C and at pH 10. Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) was used for determination of the existence of stabilizing NPs in capping protein [93]. The organism of nitrate lacking reduction provide a novel way for producing stable Ag-NPs [94].

The halophilic marine fungus *Aspergillus sydowii* (recognized as bio-factory) has capability to produce gold NPs in highly specific pattern [95, 96]. While, *H. salifodinae* BK3 of halo-archaea categorized as Ag-NPs biosynthesis [97]. This kind of halo-archaea maintain the surrounding osmotic balance by increasing the concentration of K⁺ within their cells. Spherical morphology of crystalline structured Ag-NPs have been identified as intracellular production, e.g. *H. salifodinae* increasing the growth of microorganism over time and develop silver tolerance [97].

V. Conclusion

This study interested to present from arise of extremophiles to new gate of extremophile nanoparticles synthesized pathways to explain some of extremophiles conditions such as temperatures, pH, saline, pressures, and in combination of several conditions. Also it shed light on the unique organisms which could be a promise candidate in different application of industrials. Finally, some of metallic NPs provided effective extremophiles synthesized pathway.

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