Bioprospecting Potential of *Bacillus subtilis* for Access and Benefit Sharing

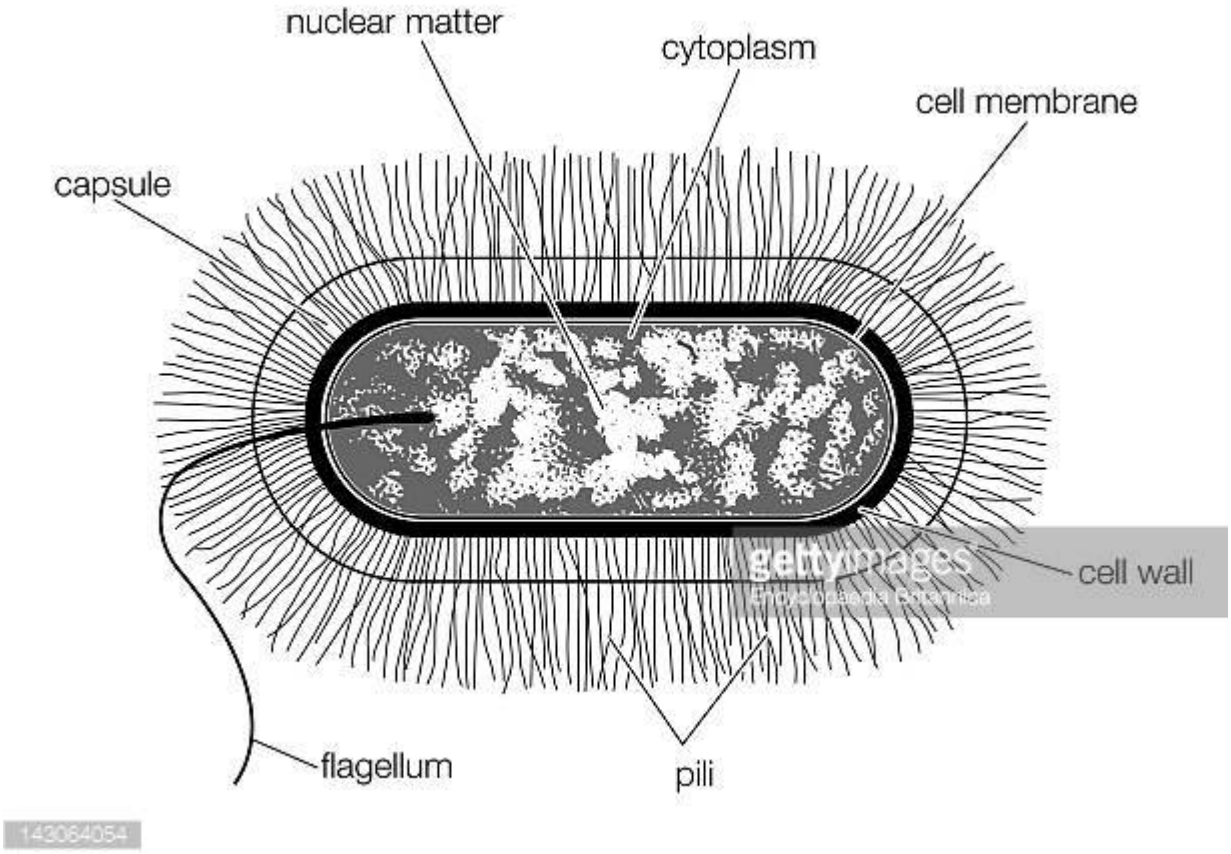

Image of *Bacillus subtilis* (Adopted from Encyclopaedia Britannica, 2014))

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1. Introduction

Ethiopia, a country endowed with rich biodiversity and traditional knowledge, promotes bioprospecting activities, which are important in the search for potentially valuable genetic resources and useful biochemical compounds in nature. However, like many other developing countries, the country lacks technical expertise and adequate monetary expenditures to explore the potential biological resources significantly. In order to explore and utilize the biological diversity strategically and wisely for commercial purposes, Ethiopia needs to collaborate with developed nations, local investors and any interested parties in the spheres of pharmaceuticals, cosmetics and other companies.

The Ethiopian Biodiversity Institute (EBI), acting as the National Competent Authority, has structured the Genetic Resources Access and Benefit Sharing Directorate, which plays a pivotal role in the implementation of the Nagoya Protocol on Access and Benefit Sharing of Genetic Resources and Associated Community Knowledge. Furthermore, through legal frameworks of the Proclamation No 482/2006 and Regulation 169/2009 (Access to Genetic Resources and Community Knowledge and Community Rights), Ethiopia has been exercising the implementation of the access and benefit sharing objectives of the Nagoya Protocol, that were emanated from Convention on Biological Diversity (CBD). The Proclamation and Regulation includes a range of issues for example: ownership, user rights, conditions for access, benefit sharing, and types of benefits, powers and responsibilities among others.

The objective of this review is to encourage any bioprospecting Company or interested individuals or groups to work on the genetic resources, *Bacillus subtilis* L. for industrial enzymatic activities in the production of many commercially useful products**.**

2. General feature

Bacillus subtilis belongs to the Domain-Bacteria, Phylum-Firmicutes, Class-Bacilli, Order-Bacillales, Family-Bacillaceae, Genus-*Bacillus*, and Species - *B. subtilis.* It was originally named *Vibrio subtilis* by Ehrenberg (1835) and renamed as *Bacillus subtilis* by Cohn (1872).

Bacillus subtilis is a Gram-positive bacterium and catalase positive. *B. subtilis* cells are typically rod-shaped, and are about 4-10 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of about 4.6 fL at stationary phase. Yu *et al.* (2014) stated that it can form an endospore like other members of the genus *Bacillus*, to survive extreme environmental conditions of temperature and desiccation. Madigan and Martinko (2005) stated *B. subtilis* as a facultative anaerobe. Nakano and Zuber (1998) considered it as an obligate aerobe until 1998. *B. subtilis* is heavily flagellated, which gives it the ability to move quickly in liquids. *Bacillus subtilis* has proven highly amenable to genetic manipulation, and has become widely adopted as a model organism for laboratory studies, especially of sporulation, which is a simplified example of cellular differentiation. In terms of popularity as a laboratory model organism, *B. subtilis* is often considered as the Gram-positive equivalent of *Escherichia coli*, an extensively studied Gram-negative bacterium.

3. Habitat

This species is commonly found in the upper layers of the soil. There is also an evidence that *B. subtilis* is a normal gut commensal in humans. Hong *et al.* (2009) compared the density of spores found in soil (about 10^6 spores per gram) to that found in human feces (about 10^4 spores per gram). The number of spores found in the human gut was too high to be attributed solely to consumption through food contamination (Hong *et al.,* 2009). *B. subtilis* has been linked to grow in higher elevations (Sudhagar *et al.,* 2017).

4. Significance in producing extracellular enzymes

The microorganisms of *Bacillus* genus are known to be one of the most important sources of enzymes and other biomolecules of industrial interest, being responsible for the supply of about 50% of the market for enzymes (Schallmey *et al.,* 2004). The world market for enzymes is estimated at 1.6 billion dollars, 29% for the food industry, 15% for animal feed and 56% in other applications (Outtrup *et al.,* 2002).

Bacillus subtilis produces a variety of extracellular enzymes including proteases, amylases and lipolytic enzymes of great importance in industrial processes such as pharmaceutical, leather, laundry, food and waste processing industries (Schallmey *et al.,* 2004).

The hydrolase enzymes are among the largest categories of industrial application, of which, the alpha-amylase and beta-galactosidase have received special attention (Konsoula and Linkopoulou-Kyriakides, 2006). These enzymes catalyze the hydrolysis of starch and are produced by a wide variety of microorganisms, however, for commercial applications they are basically derived from the genus *Bacillus* (Schallmey *et al.,* 2004). The major amylase produced by *Bacillus* is heat resistant, which is commercially interesting because many processes require high temperatures, so the thermo-sensibility ceases to be a limiting factor (Konsoula and Linkopoulou-Kyriakides, 2006).

The protease represents about 30% of the total sold worldwide enzymes. The thermostable proteases produced by *Bacillus* spp are among the most industrially important. Lipases which catalyze the hydrolysis of triacylglycerols are widely used in organic chemistry due to its high selectivity and specificity and, thus receive much attention because of its potential use in industrial processes (Lesuisse *et al.,* 1993). *Bacillus subtilis* secretes different types of lipases, which may vary according to different growth conditions, environmental factors such as pH and amino acid supply (Eggert *et al.,* 2003).

4.1. Role of extracellular enzymes in Pharmaceutical Industries

The amylases can be derived from several sources, such as plants, animals and microorganisms. The first enzyme produced industrially was an amylase from a fungal source in 1994, which was used for the treatment of digestive disorders (Crueger and Crueger, 1989). The enzymes from microbial sources especially of *B. subtilis* generally meet industrial due to their short growth period (Odee *et al.,* 1997; Ready *et al.,* 1999). Nowadays, *Bacillus subtilis* is considered to be the most important sources of industrial amylases (Pandey *et al.,* 2000; Gupta *et al.,* 2003 cited in Unakal *et al*., 2012).

Amylolytic enzymes / amylases produced from *Bacillus subtilis* degrade starch. They are the most important enzymes and of great significance in present day biotechnology. Rao *et al.* (1998) reported their significance in the enzyme market earlier. With the advent of new frontiers in biotechnology, the spectrum of amylase applications has expanded into many other fields such as clinical, medicinal and analytical chemistry (Pandey *et al.,* 2000) and other applications.

4.2. Role of extracellular enzymes in Leather Industries

Some of the important alkaline proteases having pH optima in the range of 8.0-11.0 are solanain, hurain and proteolytic enzymes of *Bacillus* (Hameed *et al.,* 1996; Lee *et al.,* 2002; Tang *et al*., 2004).

Alkaline proteases produced by *Bacillus subtilis* have extensive applications in leather industry. Alkaline proteases play a vital role in a tannery where a rawhide is subjected to a series of chemical treatments prior to tanning and finally converted to finished leather. They replace the hazardous chemicals which are especially involved in soaking, dehairing and bating (Puvankrishnan and Dhar, 1986).

Alkaline proteases produced by an Ethyl Methyl Acetate (EMS) mutant strain of *B. subtilis* play a vital role in leather processing starting from soaking of hides to finished products (Mukhtar and Haq, 2007). In advanced tanneries, soaking is usually performed with combination of proteolytic enzymes that are optimally active in the alkaline or neutral pH (Ward, 1985; Christner, 1995; Godfrey, 1996).

4.3. Role of extracellular enzymes in Laundry

Detergent enzymes, particularly proteases are well-established constituents of modern washing and cleaning products, allowing the removal of protein-containing soiling. Due to their stability at high pH and temperature and their tolerance towards elevated concentrations of denaturing agents such as detergents or oxidants, subtilases are mostly used in this field of application. Commercially relevant enzymes group into true and high-alkaline subtilisins (Family A), Thermitases (Family B) and Proteinase K-type enzymes (Family C). Family A comprises the widely used detergent proteases of *Bacillus licheniformis* / subtilisin Carlsberg (Smith *et al.,* 1968; Jacobs *et al.,* 1985), *Bacillus amyloliquefaciens* / subtilisin BPN' (Wells *et al.,* 1983) and *Bacillus lentus* / subtilisin BL (Goddette *et al.,* 1992). Many efforts have been made to identify new proteases and optimize existing ones to meet the specific requirements for very diverse laundry and cleaning processes (Herbots, 2007).

4.4. Role of extracellular enzymes in Food Industry

The world market for enzymes, estimated at 1.6 billion dollars which contributed for 29% for the food industry and 15% for animal feed, has been increasing from year to year (Outtrup *et al.,* 2002). Several enzymes important for food processing have been recently derived from recombinant strains of the Gram-positive bacteria *B. subtilis* and *B. licheniformis*. *Bacillus subtilis* has been used for several decades as a source of food processing and industrial enzymes, mainly -amylases and proteases. Of particular importance is *B. subtilis* strain 168, a well-known wild-type strain from which numerous strains widely used in research and industrial applications were developed. Strain 168 is the progenitor of many *B. subtilis* strains that have been used as sources of food-processing enzymes, primarily amylases (Nazina *et al.,* 2001).

4.5. Role of extracellular enzymes in Waste Processing Industry

Poultry and leather industry wastes, which are are rich in keratin wastes, are degraded by chemical and mechanical hydrolysis which is not eco-friendly. However, enzymatic degradation by using alkaline proteases is best method and eco-friendly. *Bacillus* species is the most widely reported bacterial source of keratinases for feather degradation, too(Kudrya and Simonenko, 1994).

5. Application of *Bacillus subtilis* to Biotechnology

Bacillus organisms, isolated by soil sprinkle technique, are responsible for producing antibiotics. The most antibiotic activity was seen in *Bacillus subtilis* MH-4. *Bacillus subtilis* form polymyxin, difficidin, subtilin, and mycobacillin. The antibiotic bacitracin was determined to be effective against Gram-positive bacteria only (Jamil *et al.,* 2007). Polymyxin is effective against Gram-negative bacteria, whereas difficidin has a broader spectrum (Todar, 2008-2012).

In the modern world, demand for the eco-friendly products resulted in replacement of chemical methods with enzymatic methods. Alkaline protease is one of the important groups used in various industries like leather, detergents, textile, food and feed etc. *Bacillus subtilis* is mostly used for production of alkaline proteases having industrial importance and responsible for producing antibiotics.

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