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Exploration of extremophiles for high temperature biotechnological processes

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Industrial processes often take place under harsh conditions that are hostile to microorganisms and their biocatalysts. Microorganisms surviving at temperatures above 60 °C represent a chest of biotechnological treasures for high-temperature bioprocesses by producing a large portfolio of biocatalysts (thermozymes). Due to the unique requirements to cultivate thermophilic (60–80 °C) and hyperthermophilic (80–110 °C) Bacteria and Archaea, less than 5% are cultivable in the laboratory. Therefore, other approaches including sequence-based screenings and metagenomics have been successful in providing novel thermozymes. In particular, polysaccharide-degrading enzymes (amylolytic enzymes, hemicellulases, cellulases, pectinases and chitinases), lipolytic enzymes and proteases from thermophiles have attracted interest due to their potential for versatile applications in pharmaceutical, chemical, food, textile, paper, leather and feed industries as well as in biorefineries.

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Introduction

Extremely hot terrestrial and marine environments are hostile for most living organisms on earth, but these niches represent excellent settings to be inhabited by specialized microorganisms. Many Bacteria and Archaea populate such harsh environments and tolerate high or low temperatures, extremes of pH or high concentrations of salts. This group of microbes is called extremophiles and can be further subdivided with regard to their specific needs [1]. Psychrophiles and mesophiles thrive at cold or moderate conditions, while thermophilic microorganisms optimally survive at elevated temperatures (60–80 °C), and hyperthermophiles prefer extremely hot conditions (80–110 °C). Since Thomas Brock's ground-breaking discovery of microorganisms in the hot springs of Yellowstone National Park in the 1960s, various thermophiles were isolated from the most extraordinary places on the planet [2]. Such habitats are considered extreme from a human point of view and they include deep-sea hydrothermal vents, volcanic islands, composts and deserts [3].

Heterotrophic (hyper-)thermophilic prokaryotes are capable to utilize various polymeric substrates as carbon sources. A resourceful enzyme repertoire that is stable at specific extreme environmental conditions facilitates the efficient degradation of complex natural polymers including starch, lignocellulose, chitin as well as proteins and fats. In this context, the designation extremozymes covers all enzymes derived from extremophiles. Cold-active and heat-active enzymes are designated as psychrozymes and thermozymes [4–6]. These biocatalysts are usually not only superior over their mesophilic counterparts because of their thermal adaptation, but they often provide further benefits such as solvent-tolerance, substrate selectivity and stability. Due to these unique properties, thermozymes are of tremendous importance for biotechnological applications and therefore, screening for novel biocatalysts from extremophiles represents a valuable alternative to elaborative engineering procedures for the optimization of available enzymes from mesophiles (Figure 1, Box 1).

This short mini-review covers biochemical properties of recently described thermozymes and their potential applications in various industries. However, this text does not claim to be complete, fo example highly relevant thermostable phytases that are applicable in feed industry or algal biomass-degrading enzymes including laminarinases or fucoidanases and others were omitted due to space limitations.

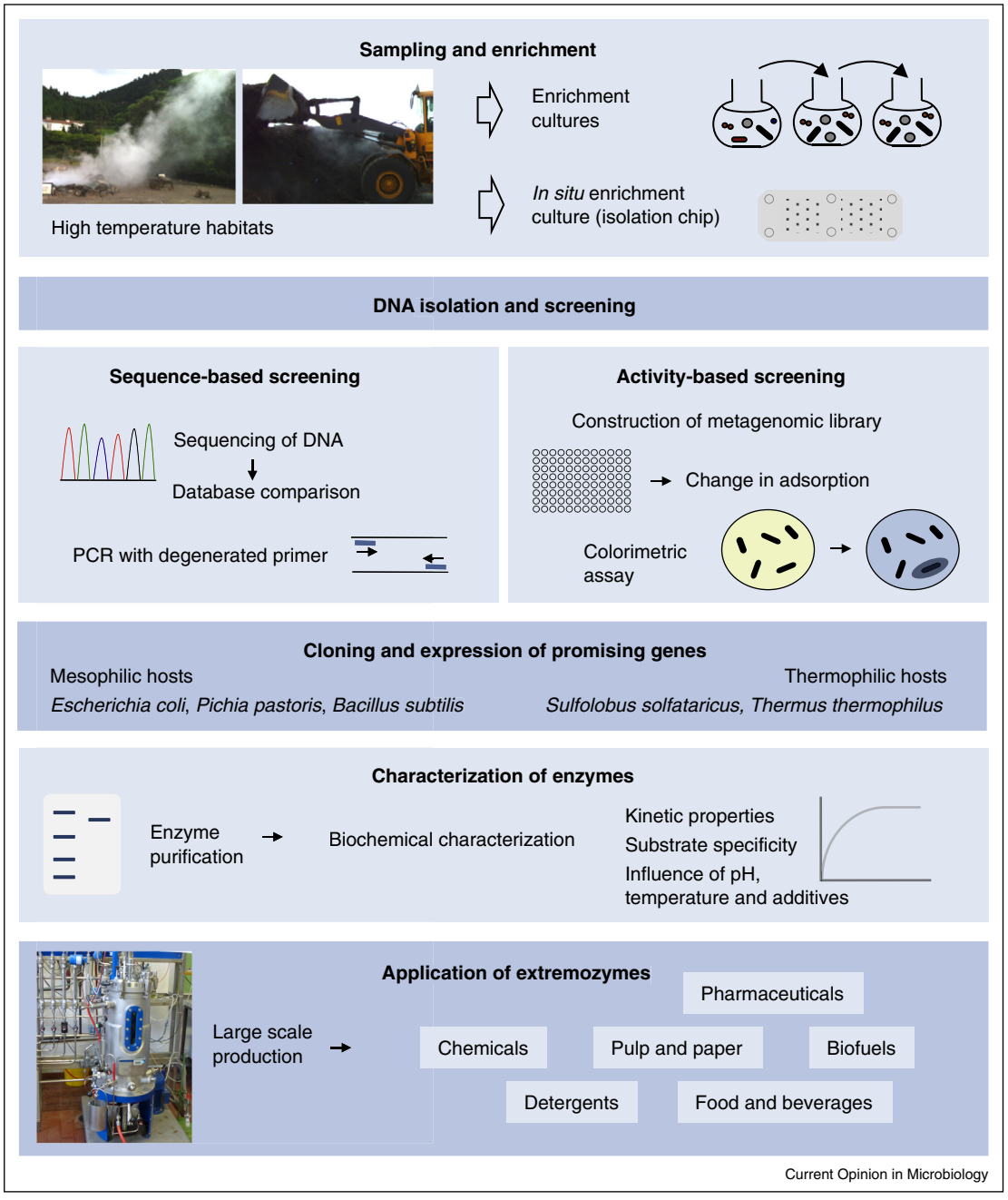
Polysaccharide degrading enzymes

Carbohydrates are the main form of energy and carbon reserve in living organisms. Due to the versatile structure of diverse polymers such as starch, cellulose, xylan, mannan, pectin, chitin and others, biocatalysts (mainly glycoside hydrolases) were evolved to specifically target different linkages of glycosidic backbones and substituents.

Starch-hydrolyzing enzymes

Starch is a polysaccharide with a heterogeneous nature composed of the linear insoluble compound amylose

Figure 1



Schematic representation of high-throughput screenings for genes encoding thermozymes, cloning and expression followed by biochemical characterization of biocatalysts and subsequent large scale production for suitable industrial applications.

(α-1,4-glycosidic bonds) and the branched soluble polymer amylopectin (α-1,4-glycosidic linkages and α-1,6-glycosidic linkages). Due to the complex structure of starch, various enzymes are required for efficient degradation including α-amylases (EC 3.2.1.1), β-amylases (EC 3.2.1.2), glucoamylases (EC 3.2.1.3), α-glucosidases (EC 3.2.1.20) and pullulanases (EC 3.2.1.41) [7].

Starch is of relevance in food industry as a source for glucose and fructose (high glucose–fructose syrup) and is conventionally converted by a liquefaction step followed by enzymatic saccharification [8]. Typical enzymes that are nowadays used for saccharification are a bacterial α-amylase in combination with a fungal glucoamylase to act on the α-1,4-linked backbone of the starch molecules.