

Biotechnological Versatility of *Bacillus* spp.

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The genus *Bacillus* represents a diverse group of Gram-positive, endospore-forming bacterial species with the well-deserved fame of being potent, versatile, and one of the most promising industrial microorganisms yet discovered. They have an average genome size between 3.4 and 6.0 Mbp. Genetically modified *B. subtilis* and, to a lesser extent, *B. licheniformis*, *B. amyloliquefaciens*, and *B. megaterium* have been used for the heterologous expression of numerous proteins (enzymes, vaccine components, growth factors), platform chemicals, and other organic compounds of industrial importance.

Bacillus

enzymes

growth factors

vitamins

amino acids

antimicrobial peptide

1. Introduction

The genus *Bacillus* represents a diverse group of Gram-positive, endospore-forming bacterial species with the well-deserved fame of being potent, versatile, and one of the most promising industrial microorganisms yet discovered. They have an average genome size between 3.4 and 6.0 Mbp [1] and a low GC% content ranging from ~35% in *B. thuringiensis* to 43.5–46.4% in *B. subtilis*, *B. licheniformis*, and *B. velezensis* [2][3][4][5]. The distinct advantages of *Bacillus* spp. used as microbial cell factories include a short fermentation cycle (around 48 h), ease of cultivation, and robust growth; non-pathogenic Generally Regarded as Safe (GRAS) status; the ability to secrete recombinant proteins in the medium; and the lack of external and endotoxins [1][6]. In recent decades, genetically modified *B. subtilis* and other *Bacillus* spp., notably *B. licheniformis*, *B. amyloliquefaciens*, and *B. megaterium*, have been used prodigiously for the heterologous production of anything from pharmaceutical proteins (antibody fragments, interferons and interleukins, growth factors, hormone precursors, and antimicrobial peptides) to industrial and food-grade enzymes. Large-scale genetic engineering has made possible the redirection of whole metabolic pathways toward valuable non-protein products such as organic acids, alcohols, and vitamins. Compared to *Escherichia coli*, its chief rival among recombinant bacteria [7], *Bacillus* spp. used to have some limitations in the past associated with the relatively smaller number of suitable regulatory regions for gene expression, peculiarities in the secretion of recombinant proteins, and the need for the selection of domesticated host strains [8]. This situation has been rapidly changed with the development of increasingly diverse and versatile vectors, novel genome editing tools such as the CRISPR/Cas9 system, and the construction of convenient strains deficient in multiple proteases.

An enormous variety of enzymes, growth factors, vitamins, peptides, amino acids, and low-MW compounds have been expressed in recombinant *Bacillus* spp. (**Figure 1**), often on a scale with industrial promise. Through the

optimization of expression systems and developments in the field of bioengineering and the use of recombinant *Bacillus* strains, the highest values of industrially important target products have been achieved (**Table 1**).

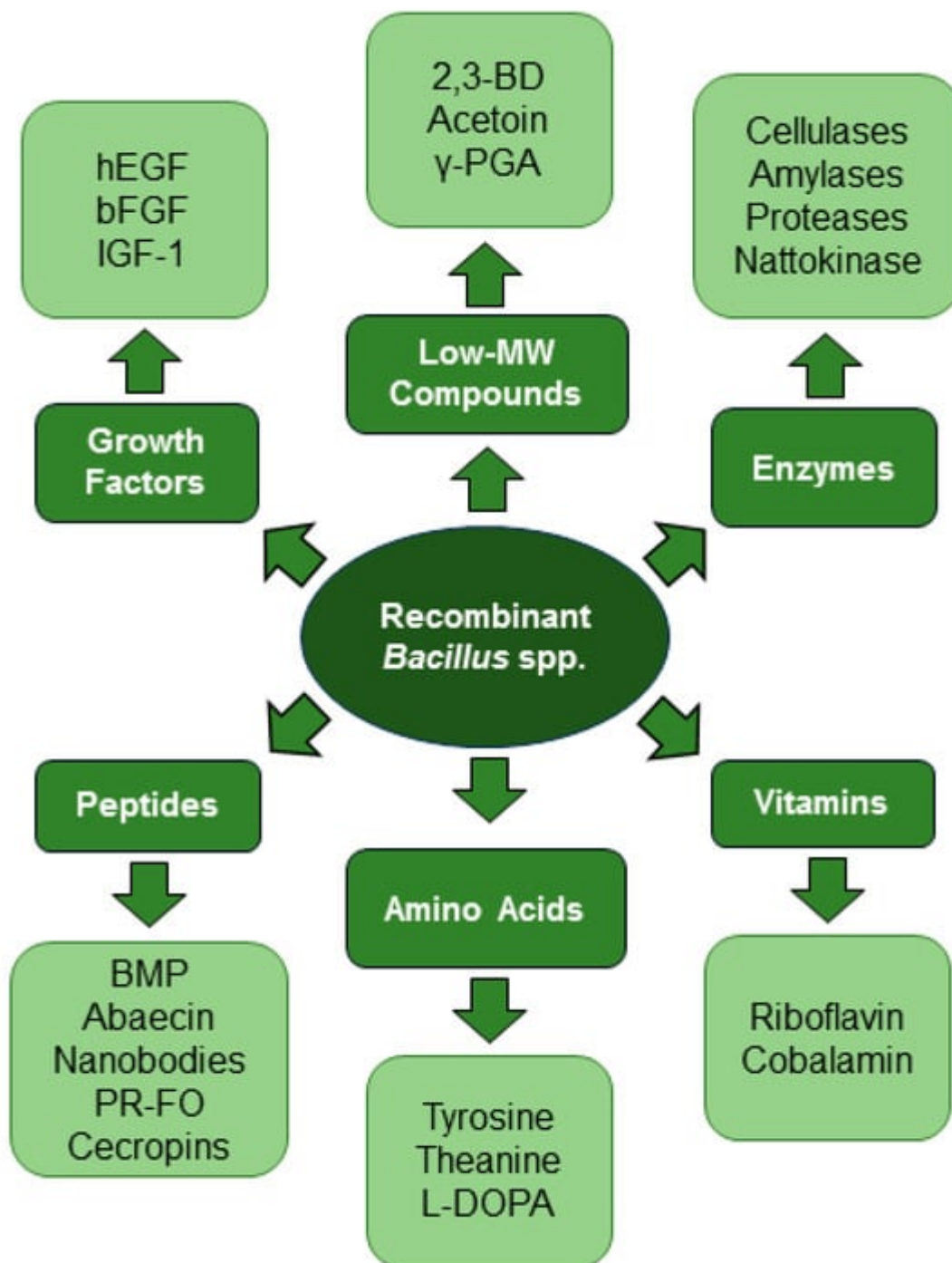


Figure 1. Biotechnological versatility of *Bacillus* spp.

2. Enzymes

Enzymes with applications in the food industry have predictably been in the spotlight. Genetic improvement of bacilli for the production of α -amylase leads to a gradual increase in the yields obtained (**Table 1**). The goal of enhanced extracellular expression is achieved through signal peptide optimization and chaperone overexpression [9], the prevention of extracellular degradation by improving the folding environment [10], as well as by complex balancing of the entire secretion process [11]. Thus, the recombinant strain *B. subtilis* WHS9GSAB produced 35,779.5 U/mL α -amylase for 93 h, reaching a productivity of 384.7 U/mL/h [12].

Table 1. Application of recombinant *Bacillus* spp. with the highest production of industrially important compounds.

Strain	Vector	Compound	Genetic Source	Yield	Reference
<i>B. subtilis</i> WHS11YSA	pHYAmySA	α -amylase	<i>B. stearothermophilus</i>	9201.1 U/mL	[9]
<i>Brevibacillus choshinensis</i> (<i>B. brevis</i>) BCPPSQ	pNCamyS-prsQ	α -amylase	<i>B. stearothermophilus</i>	17,925.6 U/mL	[10]
<i>B. subtilis</i> WHS9GSAB	pHYGamySAsecYEG	α -amylase	<i>B. stearothermophilus</i>	35,779.5 U/mL	[11]
<i>Br. choshinensis</i> (<i>B. brevis</i>)	pNCMO2	β -amylase	<i>B. aryabhatai</i> CCTCC M2017320	5371.8 U/mL	[12]
<i>B. subtilis</i> WS9PUL	pHYcas9	pullulanase	<i>B. deramificans</i>	5951.8 U/mL	[13]
<i>B. subtilis</i> WB600	pMA5	lipase A	<i>B. subtilis</i>	1164.9 U/mL	[14]
<i>B. subtilis</i> DB10	pSKE194	xylanase	<i>B. subtilis</i>	1296 U/mg	[15]
<i>B. licheniformis</i> MW3	pKVM1	2,3-butanediol	<i>B. licheniformis</i>	123.7 g/L	[16]
<i>B. amyloliquefaciens</i> B 10-127	pMA5	2,3-butanediol	<i>B. amyloliquefaciens</i>	132.9 g/L	[17]
<i>B. subtilis</i> 168	pMA5	acetoin	<i>B. subtilis</i>	91.8 g/L	[18]
<i>B. subtilis</i> KH2	pKVM1 pMA5	poly- γ -glutamic acid	<i>B. subtilis</i> , <i>B. licheniformis</i>	23.28 g/L	[19]
<i>B. subtilis</i> G600	T7-BOOST *	GABA [†]	<i>B. subtilis</i>	109.8 g/L	[20]

References

* T7-BOOST, T7-Based Optimized Output Strategy for Transcription, a system based on the inducible promoters $P_{hy-spank}$ and $P_{xy/A}$; [†] GABA, γ -aminobutyric acid.

B. subtilis host strain 1A751 was engineered to express cellobiose 2-epimerase (CEase) from *Thermoanaerobacterium saccharolyticum* JW/SL-YS485. CEase is a curious enzyme able to convert lactose to epilactose (4-O-β-d-galactopyranosyl-d-mannose), an epimer of lactose difficult to obtain with purely chemical synthesis. While lactose is one of the main by-products in the cheese industry, epilactose reportedly has prebiotic properties [21].

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Platform chemicals like 2,3-butanediol (2,3-BD) and acetoin, previously derived from oil, can now be obtained in a more environment-friendly way. A number of microorganisms, including many *Bacillus* spp., have demonstrated strong producing abilities, in a few exceptional cases reaching about 15%, after the proper genetic modification [44].

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