Chapter 1

General Introduction

Shangkun Qiu

Institute of Applied Microbiology-iAMB, Aachen Biology and Biotechnology-ABBt, Worringer Weg 1, RWTH Aachen University, D-52074, Aachen, Germany

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1.1 Bioeconomy: A Sustainable Future

The bioeconomy represents a shift from a fossil-based economy to one that uses biological resources, processes, and principles to produce energy, chemicals, and materials sustainably. By harnessing the power of biotechnology, agriculture, and other bio-based sectors, the bioeconomy aims to create an integrated and sustainable approach to economic development.[1]

Central to the bioeconomy is the use of renewable biological resources. Plants, algae, and microorganisms are used to produce biofuels, bioplastics, pharmaceuticals, and other high-value products.[2] This approach reduces reliance on non-renewable resources and minimizes environmental impact by lowering greenhouse gas emissions and reducing waste.[1,2]

One significant aspect of the bioeconomy is the development of biorefineries. These facilities use biomass to produce a range of products, similar to how petroleum refineries produce fuels and chemicals.[3] For example, biorefineries can in future convert agricultural waste into bioethanol, biodegradable plastics, and other value-added products. This provides an additional revenue stream for farmers and promotes waste reduction and sustainable resource use.[3,4]

The bioeconomy also fosters innovation in agriculture through biotechnology to develop crops with improved yields, pest resistance, and nutritional content. These advancements can help meet the growing global food demand while reducing agriculture's environmental footprint. For instance, genetically modified crops that require fewer pesticides and fertilizers can significantly lower agricultural pollution.[3,5] However, such a future would rely on a strict agenda change in the EU.

Furthermore, the bioeconomy supports the transition to a circular economy, where waste products are reused and recycled, creating a closed-loop system. This includes developing biodegradable materials that replace conventional plastics, reducing plastic pollution, and promoting sustainable consumption.[6,7,8] At RWTH Aachen, an open loop recycling is suggested in the project catalaix.

In the context of synthetic biology, the bioeconomy leverages engineered organisms to produce complex molecules, such as pharmaceuticals and specialty chemicals, more efficiently and sustainably than traditional methods.[2] For example, engineered yeast strains can produce high-value natural products like ginsenosides, artemisinin, and other bioactive compounds, offering a renewable and scalable alternative to extraction from natural sources.[9,10]

The bioeconomy also addresses global challenges such as climate change, resource depletion, and environmental degradation.[6] By promoting sustainable practices and technologies, it aims to create a resilient economy that supports both human well-being and ecological health. Investments in bioeconomy-related research and development are crucial for driving innovation and unlocking new economic opportunities.[8]

Overall, the bioeconomy represents a transformative approach to achieving sustainable development, emphasizing the importance of biological resources and processes in creating a sustainable and prosperous future.[11] As we continue to advance in biotechnology and other bio-based sectors, the potential for the bioeconomy to contribute to economic growth, environmental sustainability, and societal well-being becomes increasingly significant.[1,8,11] The challenge is to compete with the petrochemical industry, an industry optimized for seven decades. Examples may be in the realm of natural products, where petrochemistry is less successful, e.g., in the production of terpenoids, like triterpenoids generally isolated from plants.

1.2 Introduction of producing recombinant ginsenosides by yeast

Ginseng (*Panax* spp.), a plant belonging to the *Araliaceae* family, has been a traditional ingredient in medicine throughout East Asia for several millennia.[12,13] The term "ginseng" is a translation of the Chinese words 人 參 (*Renshen*), "the essence of men".[14,13,15] The major pharmacologically relevant constituents of ginseng are ginsenosides, a class of triterpenoids that possess activities on different systems such as the cardiovascular, central nervous, endocrine, and immune systems (Table 1-1). Ginsenosides have shown potential against the coronavirus disease 2019 (COVID-19),[12,16,17,18] making them commercially valuable, with a global market exceeding one billion U.S. dollars annually.[2,17,18] Over 200 ginsenosides have been identified from 17 species in the *Panax* genus, which can be mainly categorized into two groups based on their aglycone number: oleanane-type pentacyclic and dammarane-type tetracyclic ginsenosides.[16,19] Dammarane-type tetracyclic ginsenosides can be further categorized into three groups based on the hydroxylation and carbohydrate moieties at C-3, C-6, and C-20 positions: protopanaxadiol (PPD)-type ginsenosides, protopanaxatriol (PPT)-type ginsenosides, and ocotillol.[20,21]

Table 1-1. Reported functions of PPD and its glycosylated derivatives.[22]

Compounds	Model	Suggested Function	Ref.
PPD	Male C57BL/6 J mice ICR mice Male *ob/ob* mice	Ameliorates metabolic syndrome by gut microbiota remodeling	23
PPD	HepG2 cells	Activates adenosine 50-monophosphate-activated protein kinase (AMPK) and regulates glucose and lipid metabolism	24
PPD	Human oral squamous cell carcinoma cell line KB	Inhibits the overexpression of P-glycoprotein in multidrug-resistant cancer cells	25
PPD	Human ALL cell lines Reh, RS4 Human Burkitt's lymphoma cell line Raji Peripheral blood mononuclear cells (PBMC)	Inhibits growth and cell cycle progression and stimulates differentiation of acute lymphoblastic leukemia	26,27
PPD	Human glioblastoma U251-MG U87-MG cells	Suppresses the viability of human glioblastoma cells by reducing the expression of N-cadherin, integrin β1, and cyclin D1	28
PPD	HCT-116 human colorectal cancer cells	Inhibits growth of HCT-116 colorectal cancer cells, inhibits apoptosis of cancer cells, and reduces the size of tumor cells	29
PPD	Male Kunming mice	Positive effect on the treatment of depressive psychiatric disorders	30
CK	HFD-fed OLETF rats	Enhances glucose metabolism and hepatosteatosis by regulating adenosine monophosphate-activated protein kinase (AMPK)	31
CK	—	Inhibits pancreatic lipase (PL) activity	32
CK	MIN6 pancreatic β-cells mice	Increases the insulin secretion in MIN6 pancreatic β-cells and suppresses ER stress-induced inflammation	33,34
CK	Type 2 diabetes mice HepG2 cells	Suppresses the hepatic gluconeogenesis by activating adenosine-5′monophosphate kinase	35
CK	Human umbilical vein endothelial cells (HUVECs)	Prevents oxidized low-density lipoprotein induced HUVECs inflammation and apoptosis	36
CK	Mouse model Mouse hippocampal HT22 cells	Restores the memory deficit by inducing Nrf2-mediated antioxidant enzymes and leads to cognitive improvement	36,37
CK	Brain disease models of mice	Controls the microglial activation by inhibiting mitogen-activated protein kinases and NF-κB/AP-1 activities, enhancing HO-1/ARE signaling and reactive oxygen species	38

CK	Sprague Dawley rats	Improves spontaneous GABA release by enhancing intradermal Ca^{2+} concentration	37, 39
CK	Human astroglial cells	Inhibits both NF-kB and JNK pathways to exert anti-inflammatory effects	40
CK	Male NIH mice	Exerts antidepressant-like effects by regulating monoamine neurotransmitters NA, adrenocorticotropic hormone, and corticosterone levels	41
CK	Human keratinocyte HaCaT cell line	Increases the production of hyaluronan by increasing the expression of hyaluronan synthase 2	42
CK	Human colon cancer cells	Improves human colon cancer cells convert to TRAIL-induced apoptosis by upregulating cell pro-apoptotic proteins and downregulating cell survival proteins	43
CK	A549 and H1975 cells	Suppresses the proliferation and apoptosis of non-small cell lung cancer by activating AMPK/mTOR and JNK signaling pathways	44
CK	Human breast cancer cell line MCF-7 cells	Inhibits the proliferation and apoptosis of MCF-7 cells	45
CK	SD rats Immortalized rat HSC line HSC-T6 cells	Ameliorates the liver function impairment by the hepatoprotective activity and anti-fibrotic	46
Rh2	Human promyelocytic leukemia cell line HL-60	Induces the differentiation of HL-60 cells by modulating PKC isoform levels	47
Rh2	Human astroglial cells	Inhibits both NF-kB and JNK pathways to exert anti-inflammatory effects	40
Rh2	Human hepatoma SK-HEP-1 cells	Induces apoptosis by Bcl-2-insensitive pathway activation of caspase-3 protease	48
Rh2	Prostate cancer cell line	Inhibition of the proliferation of prostate cancer cell lines	49
Rh2	Mice 3T3-L1 cells	Suppresses the metabolic disorders and anti-obesity by regulating the AMPK signaling pathway	50
Rh2	Male C57BL/6J mice	Exerts antihyperglycemic effect via inducing islet β-cell proliferation	51
Rh2	B16 melanoma cells HEK293 cells	Suppresses the proliferation of B16 melanoma cells and reduces the viability of HEK293 cells	52
Rh2	Mouse melanoma (B16) cell line	Inhibits the growth of the B16 cells line, leads to morphological alterations, and stimulates melanogenesis	53
Rg3	—	Inhibits the pancreatic lipase (PL) activity	32
Rg3	Male NIH mice	Exerts antidepressant-like effects by regulating Neurotransmitters NA, adrenocorticotropic hormone, and corticosterone levels	41
Rg3	Human endothelial cells (ECs)	Inhibits ECs apoptosis by inhibition of the mitochondrial caspase pathway	54
Rg3	Prostate cancer cell line	Inhibition of the proliferation of prostate cancer cells	49
Rg3	C57BL/6 and Balb/c mice Murine B16 melanoma cells	Inhibition of the lung metastasis of tumor cells	55
Rg3	Rat mesothelial cells	Inhibits the invasion of rat ascites hepatoma cells	56
Rg3	Hamster pancreatic b-cell line	Suppresses blood glucose level increase via enhancing insulin secretion	57
PPT	Human breast cancer cell line MCF-7 cells	Binds human estrogen receptor α (hERα) ligand-binding domain and acts as agonists of hERα	58
PPT	Human non-small cell lung cancer cell line A549	Directly binds the DNA-binding pocket of tumor suppressor P53 to involve in the regulation of the antitumor network	59
PPT	Murine RAW 264.7 macrophages	Inhibits the release of NO in RAW264.7 cells by suppressing the expression of the inflammatory enzyme iNOS	60
PPT	Murine RAW 264.7 macrophages Inflammatory model of mouse ear edema	Improves the anti-inflammatory activity by decreasing the production of pro-inflammatory cytokines TNF-α	61, 62
PPT	C57BL/6 male mice	Improves the abundance of gut microbiota, increases the concentration of short-chain fatty acids and their receptor proteins, and decreases colonic inflammation in antibiotic-treated mice	62
PPT	Male mice	Reverses the cognitive deficits induced by	63

		scopolamine, improves cholinergic system reactivity, and inhibits oxidative stress and acetylcholinesterase activity	
PPT	Male Sprague-Dawley rats	Promotes platelet aggregation induced by the P2Y12 receptor and decreases the impairment of cardio-cerebrovascular diseases	64
PPT	H9c2 cardiomyocytes	Inhibits H_2O_2-induced H9c2 cells cardiomyocytes injury by regulating PI3K/Akt pathway and decreases ROS generation and the apoptosis of H9c2 cells	65
PPT	Human EGFR-mutant non-small cell lung cancer cell lines	Inhibits lipid metabolism and cells proliferation by suppressing the expression of lipid metabolism key enzyme stearoyl-CoA desaturase 1	66
Rh1	Mouse melanoma (B16) cell line	Stimulates the melanogenesis of B16 cells line	53
Rh1	Mouse embryo fibroblasts 3T3-L1 cells	Suppresses adipogenesis and ameliorates obesity via inhibiting adipocyte inflammation and differentiation	47
Rh1	SD rats Immortalized rat HSC line HSC-T6 cells	Ameliorates liver function impairment by the hepatoprotective and anti-fibrotic activity	46
F1	Human dermal fibroblasts	Inhibition of collagen degradation and anti-skin aging	67
F1	Natural killer cells	Enhances the innate immune response	68
F1	Mouse 3T3-L1 embryo fibroblast cells	Suppresses lipid accumulation and reactive oxygen species (ROS) production via reducing expression of adipogenesis markers	69
F1	Male C57BL/6 mice ApoE-/- mice	Enhances the viability of ox-LDL-injured endothelial cells and exerts anti-atherosclerosis activities by suppressing the NF-κB signaling	70
F1	Human umbilical vein endothelial cells (HUVECs) Human brain microvascular endothelial cells (HBMECs)	Increases angiogenesis and ameliorate cerebrovascular function by activating the insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 1 receptor (IGF1R) pathway	71
F1	Human HaCaT keratinocytes	Reduces the apoptosis of human HaCaT keratinocytes induced by ultraviolet-B radiation	72

Ginsenosides are synthesized from squalene or 2,3-oxidosqualene via the mevalonate pathway, a pathway present in most eukaryotes and well-studied in yeast. [14,20,73] Acetyl-CoA is first converted to isopentenyl diphosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), the precursors of squalene.[74] From 1.5 moles of glucose, one mol of IPP and three moles of NADPH can be formed in yeast.[20] An improved stoichiometry from glucose using a synthetic pathway was reported, too.[75] Isopentenyl diphosphate isomerase (Idip) converts IPP to DMAPP,[20] followed by condensation of two IPP and one DMAPP by farnesyl diphosphate synthase (Fps/Erg20p) to farnesyl diphosphate (FPP).[20,76] Two FPP are subsequently converted by squalene synthase (Sqs/Erg9p) and squalene epoxidase (Sqe/Erg1p) to one 2,3-oxidosqualene,[20,76] which is the common precursor for the yeast sterol ergosterol and many plant triterpenoids, requiring alternative enzymes (Figure 1-1).[20,76]

Plant natural products are widely used in the food, cosmetic, chemical, and pharmaceutical industries,[77,12,14,78,79] primarily in the form of plant materials or extracts. Two primary methods are used to obtain plant natural products: direct extraction from plant biomass and chemical synthesis.[16] However, plants often contain low amounts of the desired molecule, thus employing a considerable amount of biomass, which in turn requires the intense use of organic solvents during extraction.[80,17,20] Additionally, the supply of derived natural products might easily affect plant protection status, as some plants grow exclusively in a particular environment or at a very slow rate.[77,16] For instance, ginseng roots require approximately six years of growth until harvest, experiencing varying influences related to weather, soil, and the presence of pathogens, while hemp grows quickly and is easily farmed for natural compounds[16,81]. Nonetheless, the number of

natural products obtained directly from plants is still modest when compared to the sheer number of plant natural products reported in the academic literature.[82,83,84] Chemical synthesis is often economically unattractive due to the complex structures of natural products, making microbial synthesis a promising alternative.[85,86,87,88]

With the advent of synthetic biology, designing living systems for the tailored production of bioplastics, functional foods, and natural products has become feasible.[89] In recent years, several natural products with application potential have been synthesized using engineered microbes.[12,90] Three prominent examples are farnesene, artemisinic acid, and amorpha-4,11-diene production by yeast. For example, α-farnesene was produced by engineered *Yarrowia lipolytica*, and its production was improved during fed-batch fermentation via the expression of the HMG-CoA reductase (Hmgp) from *Bordetella petrii* and acetyl-CoA acetyltransferase from *Escherichia coli*, reaching a final titer of 26 g/L.[77,91] Artemisinic acid production in *S. cerevisiae* was increased by overexpressing essential genes involved in the mevalonate pathway as well as optimizing the electron transport chain and yeast cultivation, reaching titers of 25 g/L.[92,9] Amorpha-4,11-diene production by *S. cerevisiae* expressing the amorpha-4,11-diene synthase (ADS) from *Artemisia annua* harboring a fully engineered mevalonate pathway, including three copies of the truncated gene *tHMG* encoding the *Sc*HMG-CoA reductase, and using unrestricted ethanol as carbon source, reached a titer of 40 g/L.[93]

Yeasts have long been domesticated and were used for the production of bread, beer, and wine.[94,95,96] Yeasts are simple eukaryotic cells with complete organelle structures, which allow posttranslational modifications of proteins, thus ensuring the activity of many enzymes found in other eukaryotes, including plants.[80,89] Yeasts also possess redox systems that provide similar biological and physical environments as encountered by cytochrome P450s in mammals and plants.[80,20] Due to these reasons, yeasts are considered excellent cell factories for producing plant natural products, with biosynthetic pathways of varying complexity.[94,97]