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Editorial

Strain Improvement and Microbial Biosynthesis

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Recent industrial biotechnology developments have revealed the enormous potential of microbial fermentation as an alternative to the chemical syntheses of many valuable compounds [1]. Global projections indicate that the 21st century will be the last for fossil fuel use, with coal resources completely depleted and oil production projected to cease by 2094. That is why a decade ago, the United States Department of Energy identified about 30 chemical compounds of which the microbial production has to be urgently developed. On the other hand, the new European strategy to make the continent climate-neutral by 2050 (the Green Deal) requires a circular economy and high levels of renewable feedstock valorization [2,3]. Therefore, profitable biotechnologies require new overproducers and need energy resources and substrates (such as plant biomass) to obtain chemical compounds in alternative ways, as the role of the microbial strains is of the greatest importance [4].

This Special Issue includes cutting-edge research on microbiology and bioprocesses aiming to improve the biosynthesis of target metabolites. It contains six research articles and three reviews, describing the isolation or improvement of new bacterial and fungal strains, process engineering, bioinformatics and alternative substrate use. Three of the articles are devoted to an industrial process development for obtaining valuable compounds. The potential of newly isolated strains to produce 2,3-butanediol (2,3-BD) was revealed in a study by Palaiogeorgou et al. [5]. The good qualities of new 2,3-BD overproducing *Klebsiella oxytoca* were coupled with the utilization of cheap substrates. After varying fermentation parameters such as temperature, carbon sources and aeration, the authors obtained a relatively high yield of 2,3-BD from the substrate sucrose and molasses: almost 115 g/L after 64 h fed-batch fermentation. Other advantages of the process are the relatively low temperature of 30 °C and the possibility of substrate color removal alongside the production of microbial metabolites. In this regard, representatives of the genus *Bacillus* and their diverse applications in the industrial production of valuable metabolites are emphasized in the narrative review of Arsov et al. about cloning systems in *Bacilli*. The article describes the latest developments in shuttle, integrative and CRISPR-Cas9 vectors applicable to different species such as *Bacillus subtilis*, *B. licheniformis*, *B. amyloliquefaciens* and *B. megaterium*. Genetic engineering approaches aim to increase the efficiency of bacilli in the production of α-amylase, β-amylase, pullulanase, lipase, xylanase, 2,3-BD, acetoin and poly-γ-glutamic acid, growth factors, vitamins, and amino acids, antimicrobials and peptides. The authors show both the broad perspective of metabolic engineering, as well as specific problems of gene cloning in Gram-positive hosts, among which are a small number of copies of autonomously replicating vectors (and the stringent copy number control), their structural and segregational instability, and a comparatively smaller number of transformants.

Attempting to obtain bacterial cellulose, Zheng et al. [6] used *Novacetimonas cocois* (*Komagataeibacter cocois*) and studied its genome for the first time. The carbon sources predicted as usable for bacterial cellulose production were glucose, sucrose, fructose, maltose and glycerol. The results of complete genome sequencing (3.5 Mbp chromosome and 6 plasmids) reveal the genetic basis for bacterial cellulose synthesis: the presence of two *bcs*

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operons (*bcs* I and *bcs* II). Classical symbiotic mechanisms of the co-synthesis of valuable metabolites are revealed by Chen et al. in a review of microbial vitamin C production. *Gluconobacter oxydans*, *Ketogulonicigenium vulgare* and associated bacteria mutually and sequentially stimulate the production of metabolites, which has been extensively engaged for a high final yield of the vitamin [7]. Koleva et al. reported the isolation and purification of the first known extracellular fungal catalase from the Antarctic isolate *Penicillium rubens*.

Two of the papers in the Special Issue consider the ability of yeast and fungi to overcome stressful environmental conditions and counteract toxic compounds. A physiogenomic approach to improve the isoamyl alcohol (IA) tolerance of *Saccharomyces cerevisiae* was applied by Song et al., since the toxicity of the final product to host cells limits the strain potential for IA industrial production. The elucidation of the molecular mechanisms of isoamyl alcohol action showed that it affects cell wall stability and cell membrane fluidity. The expression of genes related to ion homeostasis and energy production may play a protective role against IA stress. Gerginova et al. showed that *Gordonia* sp. and *Rhodococcus* strains grow well in media containing catechol, o-, m- and p-cresol. While the first species does not undergo any morphological changes in the presence of aromatic compounds, the cell size and shape of the second are altered, as shown via scanning electron microscopy.

Metabolic engineering strategies for the production of β-caryophyllene, a sesquiterpene with multi-pharmacological properties, employing *S. cerevisiae* and *E. coli* strains are observed in the third review in this Special Issue. Tsigoriyna et al. also see potential in three other microbial hosts: the photosynthetic cyanobacteria *Rhodobacter capsulatus*, *Synechocystis* sp. and *Synechococcus elongatus*.

Concerning new feedstocks for microbial fermentation, Alrdahe et al. develop a new promising approach to sustainably unlock plant biomass residues by optimizing the process through the combination of biodegradation with artificial intelligence. The authors performed a definitive screening design and artificial neural networks to optimize the degradation of common bean biomass utilizing the endophytic fungus *Trichoderma asperellum*. As a result, the fungal hydrolysate is rich in 12 essential and non-essential amino acids, phenolic, flavonoid and tannin compounds, and exhibits antioxidant, antibacterial and anticancer properties.

Summarizing the valuable research that has become available to the scientific community through this Special Issue, the complex development of biotechnology in the direction of recombinant DNA technologies, and metabolic and bioprocess engineering will play a leading role in the future. To date, most industrial processes are based on natural strains and their mutants. However, it was considered that the successful commercialization of a given microbial process requires the target product obtained in a minimum titer of 50 g/L achieved through the productivity of 3 g/L/h and a yield higher than 80% of the theoretical maximum [8–10]. Therefore, new methods and tools for the improvement of producing strains are needed for aiding the knowledge and technology transition from the laboratory to the industrial scale.

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References

1. Rusu, A.V.; Trif, M.; Rocha, J.M. Microbial Secondary Metabolites via Fermentation Approaches for Dietary Supplementation Formulations. *Molecules* **2023**, *28*, 6020. [CrossRef] [PubMed]
2. Head, I.M.; Gray, N.D. Microbial Biotechnology 2020; microbiology of fossil fuel resources. *Microb. Biotechnol.* **2016**, *9*, 626–634. [CrossRef] [PubMed]

3. Tsigoriyna, L.; Arsov, A.; Gergov, E.; Petrova, P.; Petrov, K. Influence of pH on Inulin Conversion to 2,3-Butanediol by *Bacillus licheniformis* 24: A Gene Expression Assay. *Int. J. Mol. Sci.* **2023**, *24*, 14065. [CrossRef] [PubMed]
4. Ramamurthy, P.C.; Singh, S.; Kapoor, D.; Parihar, P.; Samuel, J.; Prasad, R.; Kumar, A.; Singh, J. Microbial biotechnological approaches: Renewable bioprocessing for the future energy systems. *Microb. Cell Factories* **2021**, *20*, 55. [CrossRef] [PubMed]
5. Palaiogeorgou, A.M.; Delopoulos, E.I.M.; Koutinas, A.A.; Papanikolaou, S. Screening Bacterial Strains Capable of Producing 2,3-Butanediol: Process Optimization and High Diol Production by *Klebsiella oxytoca* FMCC-197. *Fermentation* **2023**, *9*, 1014. [CrossRef]
6. Zheng, Y.; Chen, M.; Li, J.; Fei, S.; Shang, S.; Liu, S.; Liu, L.; Li, C. Whole-Genome Analysis of *Novacetimonas cocois* and the Effects of Carbon Sources on Synthesis of Bacterial Cellulose. *Fermentation* **2023**, *9*, 972. [CrossRef]
7. Liu, Q.; Liu, M.; Chen, W.; Yuan, H.; Jiang, Y.; Huang, D.; Liu, H.; Wang, T. Recent Advances in 2-Keto-l-gulonic Acid Production Using Mixed-Culture Fermentation and Future Prospects. *J. Agric. Food Chem.* **2024**, *72*, 1419–1428. [CrossRef] [PubMed]
8. Jang, Y.S.; Malaviya, A.; Lee, S.Y. Acetone–butanol–ethanol production with high productivity using *Clostridium acetobutylicum* BKM19. *Biotechnol. Bioeng.* **2013**, *110*, 1646–1653. [CrossRef] [PubMed]
9. Vees, C.A.; Neuendorf, C.S.; Pflügl, S. Towards continuous industrial bioprocessing with solventogenic and acetogenic clostridia: Challenges, progress and perspectives. *J. Ind. Microbiol. Biotechnol.* **2020**, *47*, 753–787. [CrossRef] [PubMed]
10. Van Dien, S. From the first drop to the first truckload: Commercialization of microbial processes for renewable chemicals. *Curr. Opin. Biotechnol.* **2013**, *24*, 1061–1068. [CrossRef] [PubMed]