**Fronteiras: Journal of Social, Technological and Environmental Science**
http://periodicos.unievangelica.edu.br/fronteiras/
ISSN 2238-8869

*Article*

# The Production of Metabolites by Saccharomyces Cerevisiae and its Application in Biotechnological Processes

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**ABSTRACT**

*Saccharomyces cerevisiae* yeasts are widely known and used in biotechnological processes, as they have an excellent metabolic capacity that results in the formation of natural products with high added value. Thus, this study aims to present a view on the production of metabolites by *Saccharomyces cerevisiae* and their application in biotechnological processes. For this, a bibliometric analysis was carried out on the scientific production regarding the use of yeasts in biotechnological tests for the production of substances by activating their metabolic pathways. The articles found in the range between the years 2014 to 2019 are mostly research articles 57% and the rest 43% review. The analysis of the production of articles per year showed an oscillation for both research and review articles, and the countries with the highest publication rate are the United States and China. The data demonstrate a growing interest in secondary metabolic pathways of *S. cerevisiae*. These microorganisms can be used for the production of different metabolites that are of industrial interest, as they have a purity content that results in high commercial value.

**Keywords:** yeasts; biotechnology; metabolic routes.

**RESUMO**

As leveduras *Saccharomyces cerevisiae* são amplamente conhecidas e utilizadas em processos biotecnológicos, pois apresentam uma excelente capacidade metabólica que resulta na formação de produtos naturais de alto valor agregado. Assim, este estudo tem como objetivo apresentar uma visão sobre a produção de metabólitos por *Saccharomyces cerevisiae* e sua aplicação em processos biotecnológicos. Para tanto foi realizado uma análise bibliométrica, sobre a produção científica a respeito da utilização de leveduras em ensaios biotecnológicos para a produção de substâncias pela ativação das suas vias metabólicas. Os artigos encontrados no intervalo entre os anos de 2014 a 2019 são na maior parte artigos de pesquisa 57% e os demais de revisão 43%. Já a análise da produção de artigos por ano apresentou uma oscilação tanto para os artigos de pesquisa como para os de revisão e os países com maior taxa de publicação são os Estados Unidos e a China. Os dados demonstram um crescente interesse pelas rotas metabólicas secundárias da *S. cerevisiae*. Estes microrganismos podem ser

Submissão: 21/06/2021 Aceite: 03/08/2021 Publicação: 30/12/2021

v.10, n.3, set.-dez.. 2021 • p. 174-184. • DOI http://dx.doi.org/10.21664/2238-8869.2021v10i3.p174-184.

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utilizados para a produção de diferentes metabólitos que são de interesse industrial, pois possuem um teor de pureza que resulta em alto valor comercial.
**Palavras-chave:** leveduras; biotecnologia; rotas metabólicas.

## 1. Introduction

In nature, there is a diversity of microorganisms that have specific and important functions within the ecosystem. They are responsible for the decomposition and cycling of organic and mineral residues and pollutants present in the environment, supporting soil fertility and promoting balance between systems (Mendes et al. 2013). Due to their numerous metabolic pathways, they are mainly explored for use in bioprocesses producing several metabolites of industrial interest, among the microorganisms we can mention the *Saccharomyces cerevisiae* yeasts, which are used for the production of ethanol, an important biotechnological product (Nandy & Srivastava 2018). This product is obtained sustainably and is ecologically correct, since it has helped to minimize environmental impacts, being an important biofuel today.

Yeasts are heterotrophic unicellular microorganisms from the kingdom Fungi and have characteristics typical of the group, such as the presence of cell wall and organized nucleus with nuclear membrane with varied shapes, such as: spherical, oval, elliptical, apicular, triangular, among others, and reproduction usually occurs through budding (Kurtzman et al. 2011), and may also present sexual dimorphism and produce pseudo-hyphae and true hyphae during its growth (Koch et al. 2018; Demuyser & Van Dijck 2019). They are generally classified as Ascomycota and Basidiomycota due to the formation of ascospores (ascomycetes) and basidiospores (basidiomycetes) (Kurtzman et al. 2011).

The best-known species of yeast is *Saccharomyces cerevisiae* is also the most used in different industrial bioprocesses. These microorganisms are used in various processes, such as food fermentations, wine, beer and bakery production, thus, yeasts are being studied, especially the *S. cerevisiae* species (Borneman & Pretorius 2015; Nandy & Srivastava 2018), as they can be excellent producers of compounds and have the ability to convert various substrates, in addition to their high multiplication capacity and easy conservation (Moysés et al. 2017). These microorganisms are considered as independent living entities that perform the bioconversion of sugars (anaerobic glycolysis), with the purely objective of obtaining the chemical energy necessary for their survival (Tortora et al. 2012; Ginovart et al. 2018).

In ethanol production, the yeasts used are *Saccharomyces cerevisiae*, which has a high tolerance to stress factors, supporting high concentrations of ethanol in the medium (Caspeta et al. 2015). However, these microorganisms can succumb to extreme conditions of osmolarity and temperature influencing their fermentation performance (Yamakawa et al. 2019; Rivera et al. 2017). During fermentation, yeasts assimilate sucrose, which is present, mainly in sugarcane, and glucose from an enzymatic process present in corn starch (Mattanovich et al. 2014), that is, yeasts use these substrates for their metabolism and naturally produce, in their metabolic pathways, ethanol in larger quantities and other metabolites in smaller quantities.

The production of many of these compounds is directly related not only to the availability of the carbon source but also the nitrogen source and other essential nutrients present in the substrate, however, the variation in the concentration of these sources can alter the yeast metabolic pathways and perhaps produce other compounds at the expense of ethanol (Dzialo et al. 2017). A good example is the activation of the Ehrlich pathway and consequently the formation of higher alcohols (Gamero et al. 2015; Stribny et al. 2015). However, this route is little explored during the fermentation process, that is, underutilized, however, the production of fusel alcohols can be applied in various segments of the industry.

Higher alcohols are medium-chain aliphatic compounds of more than three carbon atoms with branched or aromatic secondary residues, also known as fusel alcohols, are volatile alcohols derived from fermentation with more than two carbons and therefore a molecular weight and point of higher than ethanol (Cordente et al. 2019). Due to their useful chemical properties, higher alcohols have numerous applications, such as the production of alternative biofuels, in addition to being applied as a raw material for flavouring products in various industrial segments (Nozzi et al. 2014).

These microorganisms are considered a bioindustry of compounds, as they have numerous metabolic pathways that promote the assimilation of different substrates, resulting in the formation of natural products with high added value. Such products are formed by the activation of certain metabolic pathways, in which essential nutrients for the maintenance of cells and their physiological functions are shared. However, if there are disturbances in the environment that affect yeasts, they tend to activate other pathways such as the Ehrlich pathway that starts with the transamination of the amino acid to α-ketoacid which is then decarboxylated to the corresponding aldehyde and then either reduced to ethanol or oxidized to fusel acid. α-ketoacids are intercessors in the biosynthesis of certain amino acids, being considered as a link between the anabolic and catabolic processes of this microorganism (Amaya-Delgado et al. 2018). In this sense, this study aims to present an overview of the production of metabolites by *Saccharomyces cerevisiae* and its application in biotechnological processes from 2014 to 2019.

## 2. Material and Methods

### *2.1. Nature of research*

For this study, bibliometric analysis was used, in which a survey of scientific production regarding the use of yeasts in biotechnological tests for the production of substances by activating their metabolic pathways was carried out.

The methods used were:

a) descriptive research, which according to Kauark et al. (2010), presents the predetermined characteristics of the population or phenomenon, or the creation of relationships between variables, making use of standardized techniques for data collection;

b) quantitative research to enumerate and measure data. According to Prodanov and Freitas (2013), the quantitative approach believes that everything can be quantifiable, which suggests pouring opinions and information into numbers to categorize and interpret them. With the idea of understanding how the knowledge or field of science is structured.

According to Prodanov and Freitas (2013), for research using this method, documents must undergo a critical evaluation regarding their structure and the knowledge presented. Furthermore, Pereira et al. (2018). point out that understanding the context of scientific publications in a particular research area helps to understand the researched phenomena.

### *2.2. Data Collection Procedures*

Data collection was performed through searches in the ScienceDirect database, with a time frame in the period from 2014 to 2019. For the search, the terms between quotation marks and the AND operator ("yeasts" AND "*Saccharomyces cerevisiae*" AND "metabolic pathways"), to search for articles containing both terms. Only open access articles were evaluated and variables delimited to assist in the compilation of data, so the articles were selected and evaluated according to the following specifications:

1. Types of articles (research or review article)
2. Year of publication;
3. Country of publication
4. Metabolites produced;

### *2.3. Data analysis*

Data analysis and treatment were performed using Excel 2019 software.

## 3. Results and Discussion

The articles found in the time frame between the years 2014 to 2019 with open access are mostly composed of research articles with 57% and the remaining 43% are review articles (Figure 1). Research on the use of *Saccharomyces cerevisiae* yeasts for the production of metabolites is in evidence, given the conditions of use of this microorganism and its metabolic pathways that provide the obtainment of numerous natural substances that have high economic value, as they can be used in various industrial processes and easy to handle.

Yeasts, given their characteristic of great adaptive capacity and flexible metabolism that responds quickly to the conditions of the nutritional environment and the intrinsic factors of the bioconversion processes (Félix et al. 2020), are being applied and studied in several areas of biotechnology in a multidisciplinary way, since they are considered good model organisms.

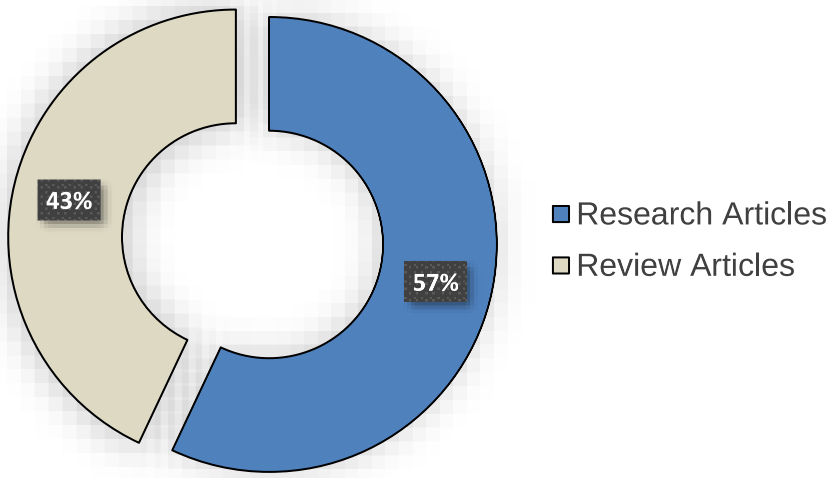


Figure 1- Scientific publications with open access in indexed journals from 2014 to 2019. Source: Prepared by the authors.

The analysis of the production of articles per year showed an oscillation for both research and review articles. The highest percentages of publication of review articles were in 2015, 2018 and 2019, while research articles presented 22% of publications in 2016, falling by 6% in the following year and returning to an increase going to 21%. However, in the years 2016 to 2018, research articles showed a significant increase in publications using these microorganisms in the most varied areas of study (Figure 2). Probably, the growing use of *Saccharomyces cerevisiae* yeasts is related to their characteristics, as they are easy to handle, fast and have a high capacity to convert and activate their metabolic pathways and their relevance in the production of biotechnologically applicable metabolites in the most varied industrial segments.

In recent decades, research on the *Saccharomyces* genus has been highlighted, mainly regarding the use of these yeasts for the efficient and viable production of ethanol (Zhang et al. 2015), in addition to several other processes such as the production of vitamins, proteins, enzymes, heterologous proteins and in products of pharmaceutical interest through manipulation with new metabolic pathways that are possible through genetic improvements and in secondary compounds with a focus on biorefineries.

Furthermore, with the possibility of genetic manipulation of *S. cerevisiae*, it can be used to produce compounds of industrial interest (Naghshbandi et al. 2019; Kawai et al. 2019). As an example, the possibility of producing resveratrol that occurs naturally in plants and has a defence function against infections by pathogens (Li et al. 2015), given its chemical properties and the possibility of use in drugs (Mei et al. 2015). Another promising possibility is the use of modified yeasts for the production, on a commercial scale, of flavonoids (Trantas et al. 2015), in a process considered cheap, since renewable carbon sources containing glucose can be used and the process of fermentation for the production of chemical products aimed at the composition of medicines. This type of process has high economic feasibility, especially concerning the amount of product that can be obtained (Gold et al. 2015).

In the analysis of articles published by origin, within the period, it was observed that the main countries with the highest publication rate for both review articles and applied research, with emphasis on the United States with 18% and 21% respectively, followed by China with 10% review articles and 12% research-related publications and after Germany with 12% review and 10% research (Figure 3). However, although Brazil has a low publication rate for scientific articles, it still conducts studies and uses selected and customized yeasts in processes such as the production of ethanol that uses sugarcane juice as a substrate, generating a percentage ranging from 80 to 90% of the national production of this biofuel. It should be noted that this production process is highly profitable and sustainable and that it has a great economic and environmental appeal, making the country the second world producer in the production of bioethanol.

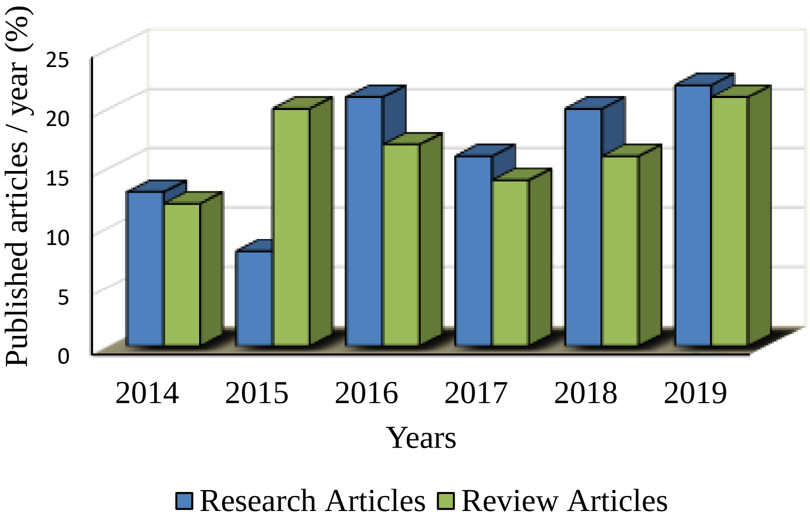


Figure 2 - Evolution of the number of articles published per year for the time frame between 2014 and 2019 of open access. Source: Prepared by the authors.

Furthermore, the development of the areas of metabolic engineering and synthetic biology is increasingly in evidence and brings as an economic alternative for the sustainable production of chemical products using microbial cells (Liu & Nielsen 2019), and it is also notorious that investment in genetic engineering and its applications it drives studies on the use of systems biology to control cell behaviour and direct it to a specific metabolic pathway, with a view to a specific biochemical product or new products through biocatalysis (Nandy & Srivastava 2018).

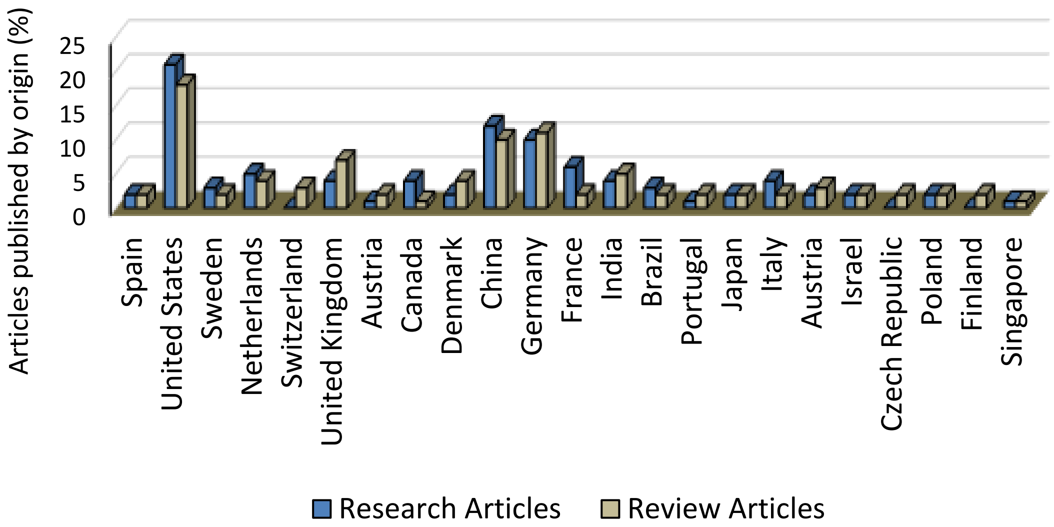


Figure 3 - Geographical distribution of publications with open access from 2014 to 2019.Source: Prepared by the authors.

In the analysis of the studies that report on the metabolic routes with the production of certain compounds, it was observed that the relevant researches are about the production of secondary metabolites 25%, the volatile compounds 25% and the production of enzymes 18% (Figure 4). The data demonstrate a growing interest in secondary metabolic pathways of S. cerevisiae with a view to products other than ethanol.

This is because the same yeast that is traditionally used in the fermentation of wine, beer and bread, naturally produces, alongside ethanol and $CO_2$, a range of higher alcohols and esters with a strong impact on sensory properties and product quality (Chain et al. 2019; Gonçalves et al. 2018).

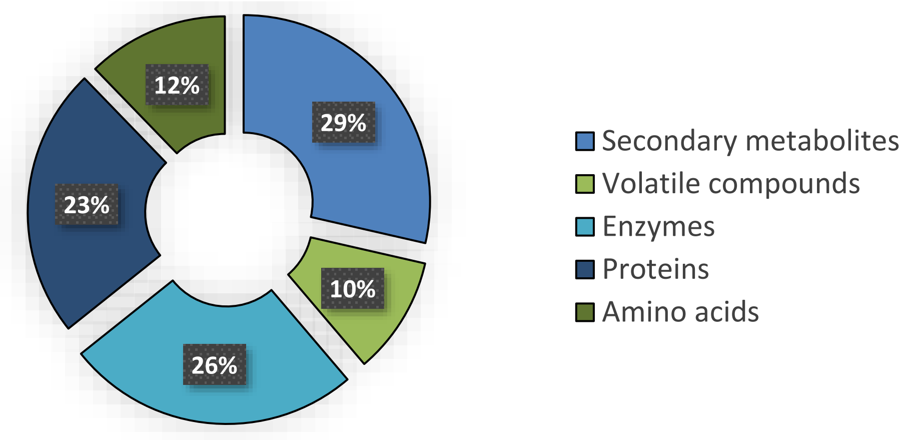


Figure 4 - Studies related to the production of metabolites by yeasts. Source: Prepared by the authors.

Yeasts can be used to obtain products ranging from therapeutic proteins and industrial enzymes and different metabolites, it is enough to give the yeast conditions so that biosynthesis occurs, as according to Knudsen et al. (2015), in the use of *S. cerevisiae*, nutrients and an assimilable carbon source are needed to obtain a specific product such as in the case of ethanol or in a spectrum of products and by-products such as glycerol and other alcohols, such as higher alcohols that have commercial value (Nandy & Srivastava 2018).

Higher alcohols are medium-chain aliphatic compounds of more than three carbon atoms with branched or aromatic secondary residues, also known as fusel alcohols, are volatile alcohols derived from fermentation with more than two carbons and therefore a molecular weight and point of higher than ethanol (Cordente et al. 2019). Due to their useful chemical properties, higher alcohols have numerous applications, such as the production of alternative biofuels, in addition to being applied as a raw material for flavouring products in various industrial segments (Nozzi et al. 2014).

These microorganisms can also be genetically manipulated and start to produce chemical compounds aimed at different industrial segments such as the production of oils (Zhou et al. 2016), organic acids widely used in the health area, being used in the composition of drugs and nutraceuticals (Nandy & Srivastava 2018). However, one of the great challenges for these bioprocesses is to balance resources and optimize the kinetics of biosynthesis, especially concerning the accumulation of cell biomass and product formation, as the concentration of biomass is essential to ensure the productivity needed for the processes commercials (Williams et al. 2016).

Notably, the interest in researching secondary metabolites may lie in the possibility of using these compounds in different segments, such as being applied in processes that use volatile compounds such as ethanol. In addition to the possibilities of producing enzymes that can be used in different processes, such as the production and composition of medicines and supplies for the health area. These compounds are of natural origin and have high added value. Metabolic engineering can also be used to regulate the biosynthesis pathways of this microorganism and thereby control the production of other substances such as the production of sesquiterpene, monoterpene and muconic acid (Peng et al. 2017), through activation of target proteins, induction or even expression of another protein (Natsume & Kanemaki 2017).

In this context, it can be analyzed that several studies report the numerous metabolic routes present in *S. cerevisiae*, which give rise to the production of various compounds. Some are activated through substrate supplementation since some routes can be activated by the presence of some compounds or even through modifications and incorporation of genes that can increase the production of organic or other products. In Table 1 are some examples of compounds that can be obtained from the metabolism of *S. cerevisiae*.

Some yeast strains have specific characteristics that allow obtaining certain compounds from the biocatalysis of different carbon sources as a result of this process, several metabolites such as ethanol, glycerol are produced (Jullesson et al. 2015; Tortora et al. 2012), trehalose (Da Silva Santos et al. 2018), in addition to the production of enzymes that have wide application in biotechnological processes (Barbosa et al. 2020), in other products of industrial interest such as aromatic compounds, including higher alcohols, fatty acids, acetates, ethyl esters and carbonyls, among others (Cordente et al. 2012; Mouret et al. 2014; Nedocvic' et al. 2015).

**Table 1** – Metabolites produced by *Saccharomyces cerevisiae* and their use in biotechnological processes.

| Metabolites | Application | Reference |
|---|---|---|
| Triacetic acid lactone | - Chemicals industry | Cardenas & Da Silva (2014) |
| Benzylisoquinoline alkaloids | - Drug composition | Trenchard & Smolke (2015) |
| Carotenoids | - Food industry<br>- Feed manufacturing<br>- Nutritional supplements<br>- Cosmetic and pharmaceutical products | Xie et al. (2015) |
| Fatty acids | - Production of biofuels and derivatives | Tang Lee & Chen (2015) |
| Isobutanol | - Chemicals industry | Generoso et al. (2015) |
| Artemisin | - Drug composition | Krivoruchko & Nielsen (2015) |
| Glycerol | - Food industry<br>- Chemical industry<br>- Cosmetic and pharmaceutical products | Klein et al. (2016) |
| p-coumaric acid | - Flavoring chemical industry | Rodriguez et al. (2017) |
| Terpenes | - Biofuel production<br>- Perfume ingredients<br>- Drug production | Lian et al. (2018) |
| Dihydrochalcone | - Chemical industry<br>- Drug production | Eichenberger et al. (2017) |
| Proteins | - Food industry<br>- Drug production | Wang et al. (2017) |
| Cannabidiol | - Chemical industry<br>- Drug production | Zirpel et al. (2017) |
| Enzymes | - Industrial processes | Rabeharindranto et al. (2019) |
| Ethanol | - Chemical industry<br>- Biofuel<br>- Drug production | Ferreira et al. (2019) |
| Monoethylene glycol | - Chemical industry<br>- Polyester polyethylene terephthalate (PET) production | Uranukul et al. (2019) |
| Lycopene | - Food industry | Ma et al. (2019) |

**Source:** Prepared by the authors.

Thus, it can be inferred that several biotechnological processes manipulate S. cerevisiae to make them more efficient in the production of several compounds, both quantitatively and qualitatively, with a view to their use in different areas of knowledge. Thus, it is currently the main target of synthetic biology, genetic and metabolic engineering. A good example is a cannabidiol, which can be obtained from the biosynthesis process, giving this product a high level of purity, low cost, does not use solvent for extraction and does not harm the environment. Artemisinin can also be produced by synthetic biology with the induction of metabolic pathways. This compound is an important drug and widely used in the cosmetic industry (Zirpel et al. 2017; MA et al. 2019). Such alteration or remodelling in *S. cerevisiae* has given these microorganisms the designation of small factories of sustainable bioactive molecules with high added value.

## 4. Final Considerations

The publications are mostly the result of applied research that has grown over the period and most articles published are from the United States. The profile of publications demonstrates that there is a growing interest in metabolites produced by *Saccharomyces cerevisiae* during the fermentation process.

Yeasts are a biological model that can be used for the production of different compounds of natural origin, in addition to being easily modified by genetic engineering and having their metabolic routes amplified and modified, resulting in higher production of compounds of industrial interest with the content of purity that provides a high commercial value. Biotechnological processes using *S. cerevisiae* enable the production of effective bioactive molecules in an environmentally sustainable way. However, further studies are needed concerning metabolic routes and substrates that provide not only the production of different compounds but in quantities that meet the demands.

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