

Production of Gibberellic Acid by *Aspergillus Niger*: A Review

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ABSTARCT

Gibberellic acid (GA₃) is a plant hormone found naturally in plants and fungi. It is a white to pale-yellow solid with the chemical formula C₁₉H₂₂O₆. Although plants naturally produce GA₃, it is often produced commercially from fungi for agricultural use. The production of GA₃ through fermentation methods, including submerged fermentation (SmF) and semi-solid-state fermentation (SSSF), is explored, with a focus on optimizing production and reducing costs. Additionally, the review delves into the factors influencing GA₃ production, both physical and chemical parameters. It emphasizes the use of agro-industrial residues as cost-effective sources for GA₃ production, promoting environmentally friendly recycling and reducing the environmental impact. Quantification methods for GA₃, are discussed, highlighting the challenges and benefits associated with each method. Furthermore, the applications of GA₃ in horticulture, agriculture, and the food industry are outlined, demonstrating its diverse range of effects on various plants and crops.

Keywords: *Aspergillus niger*; Gibberellic acid(GA₃); Fermentation; Quantification; Applications.

1. INTRODUCTION

Plant hormones are involved in several stages of plant growth and development, it is essential for plant defence mechanism. Gibberellins (GAs) are a large family of structurally related diterpenoid acids that occur in green plants and some microorganisms [1]. Among more than 100 gibberellic acids, the major bioactive gibberellic acids, including GA₁, GA₃, GA₄, and GA₇, commonly have a hydroxyl group on C-3β, a carboxyl group on C-6, and a lactone between C-4 and C-10. GA₁ has been identified frequently in a variety of plant species [2]. The main product of gibberellin biosynthesis is gibberellic acid (GA₃), which is formed from GA₄ via GA₇ GA₃ [3]. GA₃ is an important member of the gibberellin family and acts as a natural plant growth hormone, controlling many developmental processes, and is gaining great attention all over the world due to its effective use in agriculture, nurseries, tissue culture, tea [4]. The gibberellins defined as a group of naturally occurring plant hormone as they contain tetracyclic system are well known for phytohormone. Phytohormone in particular gibberellic acid are key growth hormone for controlling different physiological mechanism including plant growth and composition, flowering, leaf, expansion, stimulation elongation and osmoregulation stimulation in internodes, germination [5]. This review provides an overview of gibberellic acid (GA₃), covering its history, biosynthesis pathway, physical and

chemical properties, production methods, factors affecting production, quantification methods, and applications.

2. HISTORY/DISCOVERY OF GA₃

In the 1930s, a group of scientists and farmers in Japan stumbled upon a puzzling disease affecting rice fields, characterized by excessive stem growth, yellowing of plants, and poor seed production [4][6]. This disease, known as bakanae, spread to other regions like China, India, and British Guiana [7]. Although identified over a century ago, the specific *Fusarium* species responsible for bakanae's varied symptoms remain unclear. Research in Japan led to the recognition of a pathogen broadly termed "*Fusarium moniliforme*." [9]. In 1926, Kurosawa demonstrated that applying fungal extract to healthy plants induced bakanae symptoms [8]. This extract, later purified by Yabuta and Hayashi in 1930, was named Gibberellin [8]. However, this breakthrough remained largely unnoticed in the West until after World War II. In the 1950s, Imperial Chemical Industry (ICI) in Britain initiated a program to enhance gibberellin production by selecting high-yield strains of *F. moniliforme*. Through fermentation studies and purification steps, they isolated and termed the resulting compound "Gibberellic acid (GA₃)" [10].

3. BIOSYNTHESIS PATHWAY

GA synthesis occurs via the terpenes route from Geranylgeranyl diphosphate-GGPP by plants and by fungi [11]. Different researchers have extensively discussed this route. The biosynthesis of gibberellic acid GA₃ as a secondary metabolite was first reported by Hanson, 1983[12]. Thus, the biosynthesis pathway can be summarised in 3 phases,

3.1 Phase 1: Conversion of GGPP to ent-kaurene

GGPP is a linear molecule of 20 carbon atoms that is formed when four isoprenoid molecules are joined together. This molecule is changed by an ent-copalyl diphosphate synthase (CPS) into ent-copalyl-diphosphate, which is then turned by an ent-kaurene synthase (KS) into ent-kaurene, a tetracyclic chemical.

3.2 Phase 2: Conversion of ent-kaurene to GA₁₂

The sequential oxidation in C-19 of ent-kaurene to form ent-kaurenoic acid is catalyzed by ent-kaurene oxidase (KO) in plants and P450-4 in fungi. This ent-kaurenoic acid oxidase (KAO) in plants and P450-1 in fungi then converts the ent-kaurenoic acid to GA₁₂-aldehyde.

3.3 Phase 3: Converting GA₁₂ to other GAs

In plants, GA₁₂-aldehyde is first transformed to GA₁₂ and subsequently to GA₉ by the action of GA₂₀-oxidase, which is in charge of producing C₁₉-GAs. A parallel route results in the 13-hydroxylation of GA₁₂ to produce GA₅₃, which is then transformed into GA₂₀ by C₂₀-oxidase.

Next, by adding a 3β-hydroxyl group, GA₃-oxidase changes GA₂₀ and GA₉ into GA₁ and GA₄, respectively. GAC 3-oxidase transforms GA₂₀ into GAC, which is how GA₃ is created. This stage varies throughout species and is contingent upon the surrounding circumstances. GA₁₂-aldehyde is 3β-hydroxylated in fungi to generate 14-aldehyde, which is then oxidized to form GA₁₄. Afterwards, the oxidation of C₂₀ transforms this final one into GA₄. The first bioactive molecule to develop is GA₄, which undergoes desaturation to become GA₇, which is subsequently 13-hydroxylated to become GA₃. The 13-

hydroxylation of GA₄ yields GA₁. Similar biosynthetic mechanisms are used by fungi and plants to convert GGPP to ent-kaurene and then to GA₁₂-aldehyde. The order in which the stages of 3β-hydroxylation and 13-hydroxylation occur in plants and fungi causes the pathways to differ from the stage at which GA₁₂-aldehyde is converted to other GAs [13].

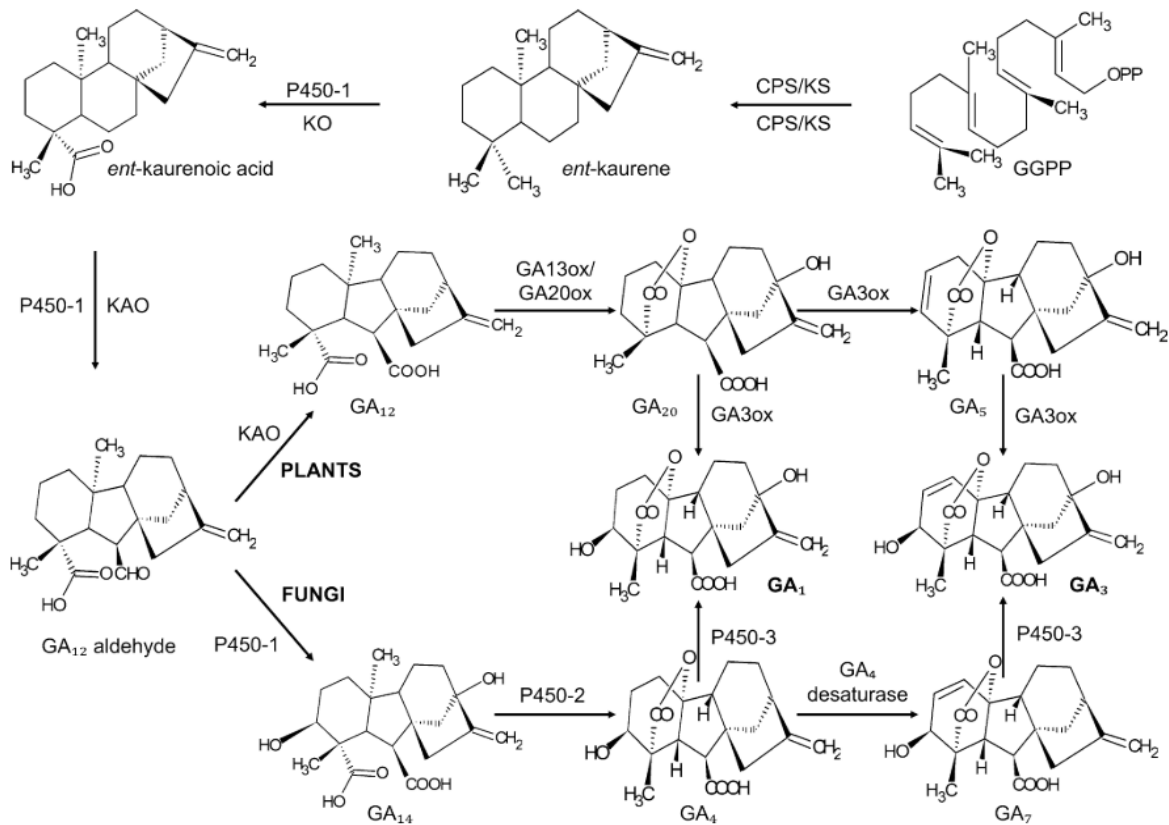


Figure 1: Describes the biosynthesis pathway for GAs in plants and fungi [13].

4. PHYSICAL AND CHEMICAL PROPERTIES OF GA₃

Gibberellic acid (C₁₉H₂₂O₆) chemically characterized as a tetracyclic dihydroxy-γ-lactonic acid containing 2 ethylene bond and one free carboxylic acid group [14]. GAs can possess a skeleton with 20 carbon atoms (C₂₀-GAs) or 19 carbon atoms (C₁₉-GAs), in which all biologically active GAs possess 19 carbon atoms [15]. Currently, around 140 known different GA molecules have been isolated from plants and microorganisms, but only a part of them are biologically active, whereas most of them are precursors of minor importance. Those with the highest biological properties and that are commercially available are GA₃, GA₄, and GA₇ [16][17].

Chemically, GA₃ is a tetracyclic dihydroxy γ-lactonic acid containing two ethylene bonds and one free carboxylic acid group. Its chemical structure (-CHO) is presented in Figure 2.

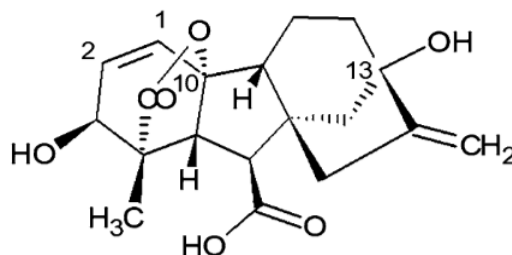


Figure 2: Structure of Gibberellic acid [13].

Melting point: It has a melting point of around 233–235 °C [4].

Solubility: It is soluble in alcohol, acetone, ethyl acetate, and butyl acetate, and it is sparingly soluble in petroleum ether, benzene, and chloroform. GA₃'s ability to dissolve in water is low, reaching only 5 g /L [4].

Stability: It is a white crystalline powder which is stable in dry conditions and readily decomposes at high temperatures, at alkaline pH values, and in aqueous solutions. Its half-life in aqueous solutions is approximately 14 days at 20 °C and 2 days at 50 °C. Therefore, the lack of stability can be associated with a C1–C2 double bond in its chemical structure, making the molecule more reactive. The same occurs with GA₇ whereas GA₄ is the most stable of the three GAs. It has also been suggested that the loss of the γ -lactone ring can lead to GA₃ biological inactivation, and therefore, the presence of γ -lactone ring and C1–C2 double bond in GA₃ structure are crucial for maintaining its biological activity [4].

5. PRODUCTION OF GA₃

Low amounts of GA₃ obtained from plants stimulated the production of this hormone through fermentation. Since then, efforts have been made to optimize its production, reduce costs, increase productivity, and discover new ways of production [18]. Fermentative production of gibberellic is a classic example of secondary metabolite production as the phase of growth can be clearly distinguished and related to nutritional and environmental states operating in the fermenter [19].

There are 3 types of fermentation which can be performed in order to obtain GA₃:

- Solid-state fermentation (SSF)
- Submerged fermentation (SmF)
- Semi-solid-state fermentation (SSSF)

5.1 SSF is a process in which the microorganism grows on a solid matrix in the absence of free water that serves as a support/ substrate for microorganism development. Water is present only in a small amount that is sufficient for microorganism growth.

Advantages of SSF: High product yields, low bacterial contamination, less wastewater generation, easier product recovery, and low-cost agro-industrial sub-products that can be used as substrates.

Limitations of SSF: SSF scale-up is difficult due to problems with the control of fermentation parameters [20][21].

5.2 SmF is the conventional and preferred technique. The microorganism is cultivated in a liquid medium, usually a synthetic medium.

Limitations of SmF: It requires higher amounts of water, energy, and space, as well as higher costs of product recovery [21].

5.3 Semi-solid-state fermentation (SSSF) is an unusual technique and little studied for GA₃ production. This technique overcomes some drawbacks presented by SSF and SmF, especially related to heat and mass transfer, nutrient availability, and production cost. In this case, the microorganism is cultivated in a liquid medium with solids in suspension [21].

Gibberellin can be obtained from fungi, bacteria, and plants. At present species belonging to fungal genera like *Fusarium* (*Fusarium oxysporum*), *Penicillium* (*P. corylophilum*, *P. cyclopium*, *P. funiculosum*), *Gibberella*, *Sphaceloma*, *Neurospora*, *Aspergillus* (*Aspergillus flavus*, *A. niger*), *Phaeosphaeria* and

Rhizopus stolonifera have been reported to produce gibberellins., It is also synthesized by Azobacter, Bacillus siamensis, Pseudomonas spp, Rhizobium phaseoli, Azospirillum brasilense, and Phaeosphaeria sp., [22][23]. Industrially, GA₃ is produced by submerged fermentation (SmF) using the ascomycetous fungus Gibberella fujikuroi, renamed Fusarium fujikuroi. It is also possible to produce GA₃ by chemical synthesis or by extraction from plants, but these methods are not economically viable [4]. Microbial production of GA in a cost-effective manner is an important factor which is considered in industrial processes, and this may be achieved by utilization of agro waste materials. Generally, agro-residues and forest products are considered the best sources of cheap substrates [23]. This practice provides environmentally friendly recycling, reducing the environmental impact, and enables a possible reduction of the final product cost. Soybean, sugar beet, sweet potato, potato, and sorghum residues; wheat and rice straw; corn, rice, and soybean husks; and sugarcane and cassava bagasse can be used as substrates for GA₃ production. Processing waste from the coffee industry, fruit industries, and oil mills is also employed [13].

The table 1 given below describes the various ways which can be employed in the production of gibberellic acid by fermentation from A.niger.

Table 1: Production of GA₃ in different conditions

Organism	Substrate	Fermentation	pH	Temperature (c)	Duration (days)	GA ₃ (g/L)	References
A. niger	Corn corb	Solid State	5.1	30	11	6.1	[27]
A. niger	mevalonic acid (precursor)	Submerged	5.5	30	9	0.164	[16]
A. niger	Synthetic Czapek-Dox broth	Solid State	5	30	12	0.23	[22]
A. niger, from melochia rhizosphere	Glucose Czapek-Dox medium	Solid State	-	28	7	0.24	[23]
A. niger	Banana peel	Submerged	5.5	25	7	13.5	[24]
A. niger	Rice flour	Solid State	5	29	4	0.20	[25]
A. niger (Fursan)	Mollases	Solid State	-	30	12	155	[26]

6. FACTORS AFFECTING GA₃ PRODUCTION

The microorganism's development depends critically on the chemical and physical environments. Among the nutrients that most affect GA₃ production, carbon and nitrogen sources are the most important.

6.1 Carbon Source:

Glucose and sucrose are commonly used as carbon sources for GA₃ production, though mannitol, maltose, starch, plant meal, or mixtures of quickly and slowly utilized carbon sources, e.g., glycerol, glucose and galactose [28][4][29]. Even use of vegetable oils such as sunflower oil is also seen [30]. Glucose

concentrations above 20% at the start of fermentation should be avoided, as they cause catabolic repression [19].

6.2 Nitrogen Source:

As ammonium regulates the process, both the amount and quality of nitrogen in the medium are critical to gibberellin fermentation. High yields of GA₃ are reached when the nitrogen concentration in the media is low. In fact, GA₃ production begins when nitrogen is exhausted [19]. Nitrogen sources such as ammonium sulphate, ammonium chloride, glycine, ammonium tartrate, corn steep liquor, and plant oil are preferred [30]. The influence of the C to N ratio is directly related to GA₃ production. Thus, the amount of C and N must allow for the initial active mycelia growth in a nitrogen-limited medium [19]. Thus, the C: N ratio normally used to get a better yield of GA₃ is between 6: 1 and 45: 1, but C: N can be different, from 10:1 to 25: 1 in the early stages, and from 25: 1 to 200: 1 in the final stages [19]. The use of agro-industrial residues as carbon or nitrogen sources was extensively reported for GA₃ production with different fermentation systems. This approach undoubtedly offers recycling that is friendly to the environment, lessens its impact, and opens the door to potential cost savings on the finished product. Generally speaking, these residues contain all the nutritional supplies needed for the growth of microorganisms. (Soybean, sugar beet, sweet potato, potato, and sorghum residues; wheat and rice straw; corn, rice, and soybean husks; and sugarcane and cassava bagasse can be used as substrates for GA₃ production. Processing waste from the coffee industry, fruit industries, and oil mills is also employed) [13].

6.3 Physical factors:

Physical factors affecting GA₃ production are temperature, pH, agitation, aeration and water activity.

6.3.1 pH:

For GA₃ production, pHs of 3.5-5.8 are generally used. Therefore, there are few studies describing the pH values used for GA₃ production. [31] used an amberlite inert support in SSF with an initial pH of 4.5. [32] used an amberlite inert support in SSF with an initial pH of 4.5. [32] used cassava flour with an initial pH of 6.0. [28] used coffee husk and cassava bagasse as a substrate/support for GA₃ production with an initial pH between 5.0-5.4. [33] used rice flour with an initial pH of 5.0. [34] used milk permeate with an initial pH of 6.0. [35] used glucose and corn steep liquor with an initial pH of 5.0.

6.3.2 Temperature:

The optimum temperature varies from 25 to 32 °C depending on the used strain [19].

6.3.3 Aeration:

Because the biosynthesis of gibberellins involves many oxidative steps, which are catalyzed by cytochrome P450 monooxygenases, dioxygenases and dehydrogenases, a high aeration condition is critical for an optimal production process. In fact, the oxygen demand increases during exponential phase of the mycelium growing. After the exponential phase, it occurs the stationary phase, where the biosynthesis of gibberellins begins. proposed an aeration rate of 0.25 vvm (volume per volume per minute), independent of the reactor volume. Holme and Zacharias (1965) used different values of 0.6 vvm for 5 L reactors and 0.25 vvm for 20 L reactors [36].

6.3.4 Agitation:

Agitation should allow efficient homogenization as well as promote the mass transfer inside the fermenter [13].

6.3.5 Water activity:

The mass transfer of water and other solutes across the cell membrane is thought to be facilitated by the water activity of the medium. In actuality, altering and regulating this parameter may help manage the microorganism's metabolism. Several papers describe moisture content and water activity (aw), for both SSF and SmF, as critical variables that limit microbial growth, metabolite production and product efficacy [37].

6.3.6 Mineral salts and trace elements:

In addition to carbon and nitrogen, the production of other metabolites requires magnesium, potassium, phosphate, and sulphate. In spite of this, very little information is known on how trace elements affect the microbial synthesis of GA₃. Typically, the component combinations of Czapek-Dox and Raulin-Thom media effectively meet the criteria. Impurities in commercial media typically satisfy the requirements for trace elements [4].

7. GA₃ QUANTIFICATION METHODS

Initially, the assessment of GA₃ promoted growth modifications of various plant organs and components served as the basis for the methods for quantifying GA₃. Current applications for bioassays include high specificity and sensitivity requirements; however, they are not appropriate for monitoring GA₃ during fermentation when fast findings are needed. Simpler and quicker techniques utilizing colorimeters, spectrophotometers, and fluorimeters are recommended for this purpose [38]. The spectrophotometric approach, first presented by Holbrook, Edge, and Bailey in 1961, is among the most straightforward and often utilized techniques for figuring out GA₃ concentrations in fermentation research. Though it isn't unique to GA₃, this approach is rapid and easy to use, making it perfect for measuring larger concentrations and quantities of materials. Gibberellic acid is produced by hydrogen chloride-induced broth acidification of GA₃[13]. The drawback of these techniques is that the sample needs to be pre-treated in order to get rid of any contaminants that may have been present in the fermentation broth. Several gibberellins may also be produced during the fermentation process that produces GA₃. Since GAs have no fluorescence, low ultraviolet (UV) absorption, and no distinguishing chemical properties that might serve as the foundation for a particular chemical assay, quantifying GAs has always been very challenging [39]. When it comes to analysing GAs, mass spectrometry (MS) is very useful when compared to traditional detection techniques like ultraviolet light. Moreover, MS has the benefit of positive analyte identification and has a greater sensitivity and universal specificity than any other current detector. The most effective option for the quantitative examination of endogenous Ga is gas chromatography coupled with mass spectrometry (GC-MS), an extremely selective and sensitive analytical technique that is becoming more widely available [40]. Derivatization processes are a necessary step for GC separation even though the GC-MS technique performs admirably. With regard to these polar and thermally labile phytohormones, high-performance liquid chromatography (HPLC) presents a potentially appealing strategy. In HPLC, traditional UV or photodiode array detection (PDA) was widely utilized to separate phytohormones from plant samples [41]. With the advent of liquid chromatography coupled with mass spectrometry (LC-MS), it is now possible to directly analyse GA molecules in the LC eluent without having to undergo the chemical modification needed for GC analysis. In recent times, capillary electrophoresis coupled with mass spectrometry (CE-MS) has become a potent instrument for the identification of charged species. This technology has proven effective in the examination of phytohormones and various other naturally occurring substances, including proteins, oligonucleotides, and

amino acids [42]. CE offers improved separation efficiency and lower sample and reagent consumption, making it a popular choice as an HPLC substitute [43]. In addition to these techniques, the amount of GA₃ in the sample can also be measured using fluorometric analysis by reacting it with 85% sulfuric acid at 4 °C. This technique's drawback is that gibberellic acid and GA₃ cannot be distinguished from one another, which could result in quantification errors. Consequently, careful consideration must be given to this procedure while preparing the sample, and the amount of gibberellic acid in the sample must be taken into account when measuring total fluorogen, or GA₃ [44].

8. APPLICATIONS OF GA₃:

Various applications of GA₃ can be majorly seen in 3 industries i.e. Horticulture, Food industry and Agriculture which are described in table 2,3 and 4.

8.1 Horticulture

Table 2: Applications in horticulture

Target	Action	References
Peaches and nectarines	Inhibits flowering	[45]
Fruit “Yu Her Pau” litchi	Increases fruit weight	[46]
Grape fruits	Can increase the mass and number of berries, reduces the number of seeds	[47]
“Tahiti” lemon acid	Has an effect on flowering and fruit fixation	[48]
Influenced all the vegetative parameters of ‘Chandler’ strawberry	Crown height, crown spread, petiole length, leaf number and leaf area	[49]

8.2 Food Industry:

Table 3: Applications in food industry

Target	Action	References
Malt	Can improve the malting process and reduce the amount of time required for the process	[55]

8.3 Agriculture:

Table 4: Applications in agriculture

Target	Action	References
Effects of GA ₃ and Calcium Chloride in restoring the metabolic alterations resulting from salt stress in linseed	Increased plant height, number of branches, number of leaves, leaf area, fresh and dry weights	[50]

Maize plants	Reverses the effects of salt stress	[51]
Dwarf pea seeds	Shoots growth stimulation	[52]
Trichocereus terscheckii seeds	Promotes seed germination in white light and darkness	[53]
Potato tubers	Promotes multiplication and cell elongation, dormancy breakage	[54]
Infection of potato leaf by P. infestans	Acts on parasite esporangiosporos inhibition	[55]
Sugarcane	Can improve productivity	[55]

9. CONCLUSION

Gibberellic acid (GA₃) is a plant hormone that stimulate the growth, cell expansion, division and development of a plant. It is highly valued for its applications in agriculture, horticulture and food industry. Different GA₃ commercial products are reported and available in the world market, enabling its application in large diversity of cultivars. The current industrial method for producing GA₃ is based on SmF and employs either *Fusarium moniliforme* or *Gibberella fujikuroi*. Nonetheless, novel approaches to the manufacturing of GA₃ are being generated by the ongoing testing of various production methods. In this regard, the SSF technique has demonstrated some financial benefits over the SmF procedure in terms of metabolite production and the value-adding of agro-industrial leftovers that can serve as substrate supports. The chemical and physical circumstances play a critical role in the development of the microbe and, subsequently, in the production of its metabolites, regardless of the fermentation method employed.

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