



A Review: Extraction and evaluation of Gibberellic acid produced by bacteria under submerged fermentation

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Abstract: Gibberellic acid (GA3) is a plant hormone that stimulates the growth, cell expansion, division and development of a plant. They are an important natural phytohormone, a member of gibberellin family. It was discovered in 1930. It has a wide application in agriculture area and in horticulture. *Gibberellin* can be obtained from fungi, bacteria, and plants. Fungi especially *Gibberella Fuzikuroi* are preferred for gibberellic acid production. *Bacillus*, *pseudomonas* and *Rhizobacteria* isolates were isolated and characterized by Gram staining, motility test, plating on selective medium and performing biochemical test are done for the production of *Gibberellic Acid*. It effects stem elongation, elimination of dormancy, germination, flowering, sex expression, leaf and fruit senescence. Along with development there is an interaction of different environment stress situation viz., light, temperature, nitrogen and water etc. Its production by fermentation and new advances that are being carried out with special interest on SMF technique.

keywords: Gibberellic acid, Hormone, Submerged fermentation, *Rhizobacteria*, *Bacillus*, *Pseudomonas*

I. INTRODUCTION

The gibberellins defined as a group of naturally occurring plant hormone as they contain tetracyclic system are well known for phytohormone [1]. Phytohormone in particular gibberellic acid (GA3) 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. It is having intense effects and endogenous plants metabolites. This was confirmed by the isolation of runner bean *Phaseolus Coccineus*, in 1958 [2]. Microbial diversity in soil is considered as important for maintaining for the sustainability of agriculture production system. Region of contact between root and soil where the soil is affected by roots and they are designated as 'Rhizosphere'. The rhizosphere is the zone area of soil where they are influenced by roots through the release of substrate which directly affects microbial activity [3]. Bacteria are abundant in soil and seen in ubiquitous environment as they show symbiotic association with plants and help for their growth and development. Biological nitrogen fixation (BNF) is a physiological process that provides agriculture crop offering by ample N supply [4]. Being a leguminous plant, they initiate a symbiotic relation with rhizobia present in its root nodules and they support BNF [5].

It is one of the secondary metabolites produced by bacteria and is used by plant. Gibberellins have various roles in breaking seed dormancy, initiation of flowering, stem elongation and promotion of seed germination [6]. Plants and some microorganisms such as fungi and bacteria produce gibberellic acid. GA3 is applied to crops, orchards, and ornamental plants.

Where it plays a role in seed germination [7]. At present species belonging to fungal genera like *Fusarium*, *Gibberella*, *Sphaceloma*, *Neurospora* and *Phaeosphaeria* have been reported to produce gibberellins [8]. GA3 is largely being produced by submerged fermentation of the Fungus *Gibberella fujikuroi* at an industrial scale [9]. It is also synthesized by *Azobacter* [10], *Bacillus siamensis* BE76 [11], *Pseudomonas* spp [12]. It is highly valued industrially important biochemical selling at \$27-36/gm on the international market [13]. GAs act as a agent of environment signals allowing plants to respond, often speedily to changes in light, condition, temperature, water, nutritional status, other biotic and abiotic stresses. It is sensitive to alter in light quantity, quality or duration which aggregate increased or decreased GA content [26]. GAs present in endosperm, cotyledons and /or test seeds at behind time is unclear. Seeds impending maturity frequently contain high levels of GA-inactivating activity assuring against concentration of bioactive Gas in mature seed that could arouse premature germination and abnormal seedling growth [47]. Production of GA3 is considerably influenced by cultural condition. some are the important factor in obtaining high yield of GA3 which include Nitrogen, carbon source, pH, temperature, incubation time and optimization of the fermentation media [14]. GA levels affect the shielding against abiotic stress which is caused by reactive oxygen species [40], salt [41] or cold [42] and abiotic stress which is caused by pathogen [43]. The function is common to angiosperm, monocots and gymnosperms, some other are responsible for plant clades and have shown to control sex determination in ferns [44] or nodulation and arbuscular mycorrhizal association [45]. In this study, GA3 production by the plant growth promoting bacteria and optimum culture condition for a large-scale production of GA3 were investigated.

II. DISCOVERY OF GIBBERELIC ACID

Many studies on gibberellic acid isolated from plants and soil from fungi and bacteria [8]. The production biologically active substance in the culture of rapidly growing bacteria Rhizosphere was reported since 1960s Hussain and Vancura (1970) reported that this are an important factor for soil fertility they are biologically active substance [15]. The first report about gibberellic acid came from a group of Japanese scientists they focused on a disease called Bakanae, which particularly affected the rice. In 1998 it was observed in China, India and British Guiana [16]. Bakanae is a plant disease which mainly affect the seedling and this disease grow abnormally length, and have stem which are pale green than the normal plants [17]. In 1926 Kurosawa showed that the fungal extract applied to healthy plant produced the same symptoms as in Bakanae disease. In 1930 Yabuta and Hayashi purified the active compound from the fungal extract and called it as gibberellin. But this major discovery went unnoticed in western countries until after the end of World War II [18]. In 1950 researcher in England and United state perforated their own methodologies to isolate the active compound from *Gibberella* culture and named the compound gibberellic acid [19]. Because gibberellic acid application could induce elongation growth in genetic dwarf of pea and maize. It was surmised that gibberellic acid must also occur in higher plants by [2]. GAs usually give rise to 20 carbon atoms skeleton (C20-GAs) or 19 carbon atoms (C19-GAs), from this all are biologically active and GAs possess 19 carbon atoms [54].

III. CHEMISTRY AND STRUCTURE OF GIBBERELIC ACID (GA3)

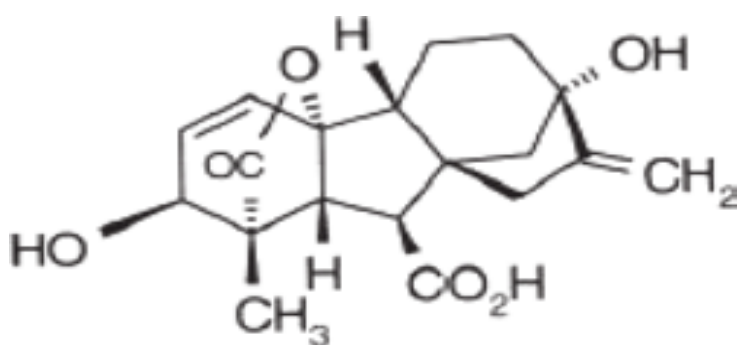


Fig 1: Chemical structure of gibberellic acid (GA3) [38]

Gibberellic acid (C₁₉H₂₂O₆) chemically characterized as a tetracyclic dihydroxy- γ in acid containing 2 ethylene bond and one free carboxylic acid group [20]. The first gibberellin (GA₃) was structurally elucidated in the mid-1950s [21]. GA₃ control many developments process and induction of hydrolytic enzyme activity [22]. Yabuta and Sumuki isolated gibberellins A and Gibberellins B in the form of crystals from the fungus *G. Fujukuroi* but soon after it was isolated from higher plants [34].

IV. BIOSYNTHESIS PATHWAY

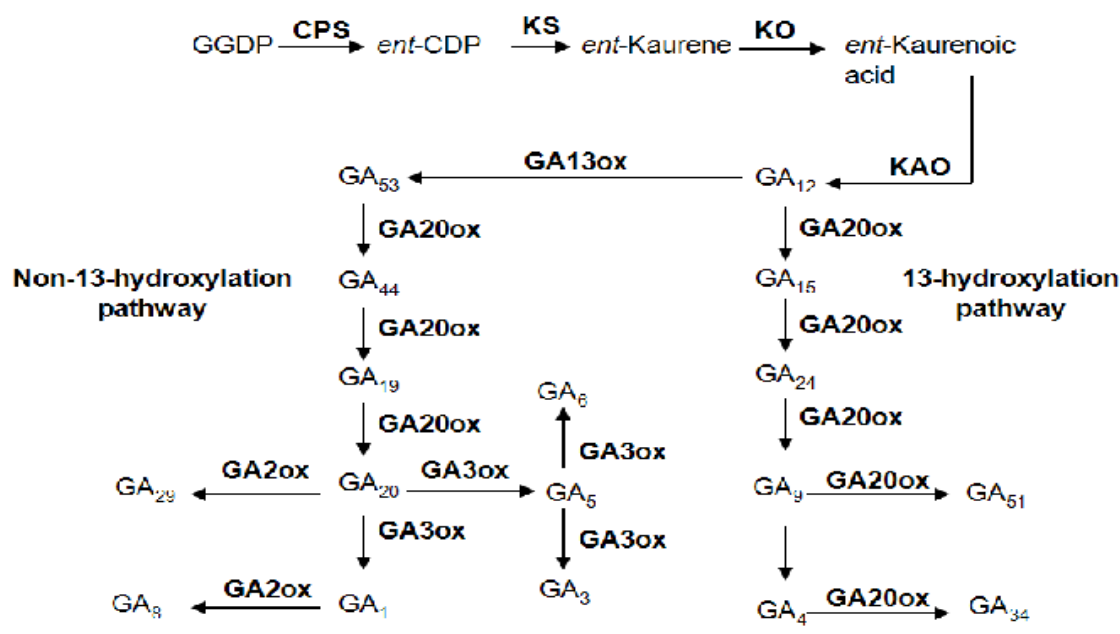


Fig 2: Biosynthesis of Gibberellic acid [39]

Gibberellin (GA) are endogenous plant growth regulators, having tetracyclic, di-terpenoid compounds. The developing seeds contain very high concentration of Gas a cytochrome comprehensive range of different structural forms [59]. After precious time and efforts to acknowledge the GA biosynthesis and movement, the specific site of bioactive GA in plants or tissue choose by bioactive GAs to commence their action has not yet been validated. The transport of active GAs and their intermediate was assisted by grafting experiment (6,7,8) and some Reports also stated that the appearance of GA in xylem and phloem exudates desired a long distance transport for GAs(4,5). Gibberellin being synthesized via terpenoid pathway. Gas existing in endosperm, cotyledons or testa of seeds at later enlightening stages is uncertain. Seeds approximately maturity contain huge amount of Gas in activating activity [39]. After valuable efforts and impressive progress has been made within the last decade in biosynthesis and signal transduction pathway. GID (GIBBERELLIC IN SENSITIVE DWARF) 1 inscribe a soluble GA receptor in rice and also a pathway has been emerged [52] in which growth repression is elevated by DELLA proteins through then regulations of transcription [57] also reviewed in [58]. Gibberellin confine within a pocket of GID1, causing a conformational transform that facilitates the formation of a GA -GID1-DELLA complex and demolition by 26s proteosome [60]. Some reports says that majority formed from methylerythritol G-Phosphate (MEP) pathway and there is specific plastid pathway for isoprenoid production [24,26] and other that GAs are formed from GGPP(*trans*-geranyl diphosphate) in proplastids(Aach)and this converted into *ent*-copalyl diphosphate synthase (CPS) which gradually converted to tetracyclic compound known as *ent*- kaurene synthase (KS).[25] via set of reacton catalyzed by different enzyme, counting two concecutive diTPSS, cytochrome P450 (CYP) and 2-oxoglutarate – dependent dioxygenases (2ODDS)in plants.(9).A pathway has come to light in which growth repression is bring into play by DELLA proteins through their regulation of transcription [57]. Ent- kaurene is reactively volatile and has been found to interchange with the outer environment and may function as a mediator of plant-plant communication [27]. Regulation start at later stages [61].In plants , GA₁₂-aldehyde is

converted to GA₁₂ which is oxidized at C-20 to form the 19-carbon gibberellins, GA₉, or is first 13-hydroxylated to GA₅₃, which is again oxidized at C-20 to yield GA₂₀. GA₉ and GA₂₀ are formed in parallel pathways, both involves oxidation of C-20 to alcohol and aldehyde, and the final formation of biological active 19- carbon Gas by loss of C-20^[23]. Require 3 enzyme viz, terpene synthase (TPSs), cytochrome P450 mono oxygenase (p450s) and 2-oxoglutarate dependent dehydrogenase (2 ODDs), for the biosynthesis of bioactive GA from GGDP in plants. The first GA intermediate product is generated in two cyclization steps from GGDP via *ent*-copalyl diphosphate (CPP). Sequential oxidation of *ent*-kaurene at position C-19 via *ent*-kaurenol and *ent*-kaurenal yields *ent*-kaurenoic acid, which is oxidized to generate the *ent*- 7 alpha - hydroxykaurenoic acid. A final oxidation at position C-6beta leads to the formation of GA₁₂ aldehyde^{[24],[25]}.

V. GA3 ON GROWTH AND DEVELOPMENT

GA₃ is a natural plant growth hormone called gibberellins .GA₃ promotes cell division and a number of plant development mechanism and encourages numerous effects on plant such as height, uniform flowering, reduced time to flowering and increased flower number and size^[28]. Gibberellic acid increases the fruit and its quality^[29].

VI. GA3 FORMATION PHYSIOLOGY IN FERMENTATION PROCESS

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 Industrially lag phase in nitrogen -limited media is undetectable as the strain requires little or no adaptation and growth in the fermenter start quickly due to the use of vigorous mycelial cells as inoculums^[30].

VII. PARAMETERS CONDITIONS FOR GIBBERELLIC ACID PRODUCTION

Thoughtful studies were carried out on optimization of nutritional and physical factors, such as supplementation of minerals and nitrogen to the substrate, initial pH and temperature, for upgrade yields of GA^[31].

VIII. NUTRITIONAL FACTORS INFLUENCING GA3 PRODUCTION

The kinetics of biomass and GA₃ production was firstly studied. During exponential growth phase glucose and phosphate are consumed .It start its production only after nitrogen is exhausted. The highest production of GA₃ is reached at an optimal point where the nitrogen concentration limits the formation of biomass and at the same time, inhibits the synthesis of the hormone^[30]

(a). NITROGEN

The presence of nitrogen in the medium is important for GA production due to regulatory process involving ammonium. However, synthesis of nitrogen only starts when the exhaustion of nitrogen occurs in the medium^[24].

(B). CARBON SOURCE

Suitable carbon source is synthesized in GA₃ that is glucose, sucrose, fructose, lactose and Maltose this effects the media. It was found that addition of fructose to media increased the rate of gibberellic acid production and by metabolizing fructose, pseudomonas sp. switched to stationary phase and began synthesizing gibberellic acid. Glucose and sucrose lower the production of gibberellic acid and they have been utilized for stimulating growth of microorganisms and suitable for carbon source^[32].

VIII. PHYSICAL CONDITION

(A). PH

Rice flour was used as *G. Fuzikuroi* easily infects and grow abundantly in rice plants. pH tested for where 2,3.5 and 5, because *G.Fuzikuroi* starts to produce gibberellins GA4 and GA, above pH 5.5 and we wanted to investigate production of gibberellic acid (GA3) ^[33].

(B). TEMPERATURE

The outcome of temperature on GA3 production is relative on the employed strain. Different condition of temperature was studied: 25°C ^[34], 27° ^[35], 28.5 ^[30], 28°C ^[36].

IX. Analysis and quantification of GA₃

The quantification and identification of GA3 which is present in medium are conducted by different method that is spectrophotometric, colorimetric, and fluorometric analysis ^[56].

X. Future prospects

Understanding the creation of DELLA mediated signaling and identifying the many partners of these proteins is presently a rapidly advancing area of research. Emerging verification that DELLA may act as a pointer of convergence for variety of hormones signaling pathways ^[62,63]. Development is likely to amplify the already substantial impact that GA-related research had on global agriculture.

CONCLUSION

Gibberellic Acid (GA3) is a plant growth regulator with a numerous highly valued application in agriculture. Industrially it is widely used for the production of (GA3) under submerged fermentation using *Bacillus*, *pseudomonas*, *Rhizobacteria*, *Azobacter*. Fortunately, symbiotic rhizobia are capable of triggering biological pathway which directly cause outcomes with direct and indirect effects on plants growth promotion and protection. Many bacterial isolates were reported earlier showing plant growth promoting activities and support our finding. As it has number of economics advantage in plant generation like horticulture, ornamental development of plants.

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