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Gibberellic acid production by *Fusarium fujikuroi* SG2

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Present study isolates efficient strains of gibberellins producing fungal strains from 'bakanae' diseased root system of rice plants for their GA_3 production potentials in Czapek-Dox liquid medium/improved medium. One of the isolates SG2 (GA_3, 1175 mg/l) produced higher GA_3 than standards strains of *Gibberella fujikuroi*, which was identified as *Fusarium fujikuroi* SG2 (MTCC4649). While studying GA_3 production pattern by this strain, gibberellin synthesis initiated on 3rd day and reached maximum by 9th day of fermentation.

Keywords: *Fusarium fujikuroi*, Gibberellic acid, Isolation, Submerged fermentation

Introduction

Gibberellins are biologically active, endogenous hormones in higher plants and products of secondary metabolism in certain fungi[1]. The latter fact offers possibility of producing gibberellins on a large scale by microbial fermentation. *Fusarium fujikuroi* (syn *F. moniliforme*, *Gibberella fujikuroi*) is used for commercial production of gibberellins at high cost. In this study, *F. fujikuroi* was isolated from bakane diseased rice plants and screened for gibberellic acid (GA_3) production under submerged fermentation (SmF).

Experimental

Microorganisms

Soil and plant samples including infected ear heads, culm and roots of 'bakanae' diseased rice plants, collected from Modakurichy area of Erode district, Tamil Nadu, were used as source of inoculum for isolation of GA_3 producing fungi. Soil samples were serially diluted in sterile distilled water and plated on Potato-Dextrose Agar (PDA) medium. Plant samples were placed on PDA plates after surface sterilization. Plates were incubated at 30±1°C for 3-4 days and discrete colonies, resembling colony characteristics of *F. fujikuroi*, were subcultured and maintained on PDA slopes. Isolated cultures were purified by hyphal tip method[2]. Isolates (4) each of *F. fujikuroi* and *Fusarium* spp. along with two strains of *G. fujikuroi* and one *F. moniliforme* were used as reference cultures. Cultures were maintained in PDA under refrigerated condition.

Identification of Isolates

Isolated fungal cultures were identified as per reported[3,4] procedures. All isolates were tested to produce 'bakanae' disease in healthy rice seedlings by adding spore suspension of isolated cultures to sterile soil, in which plants were grown under microbiologically controlled conditions. Plants were irrigated with sterile distilled water over a period of 21 days. Colony morphology including pigmentation was observed in Petri plates after 5 days of growth on PDA. To study mycelial and conidial characteristics, few isolates were grown on agar block placed on a microslide. At one corner of the block, spores were inoculated and covered by a cover slip[5]. After 5 days, growth was observed under microscope for mycelial and conidial characterization.

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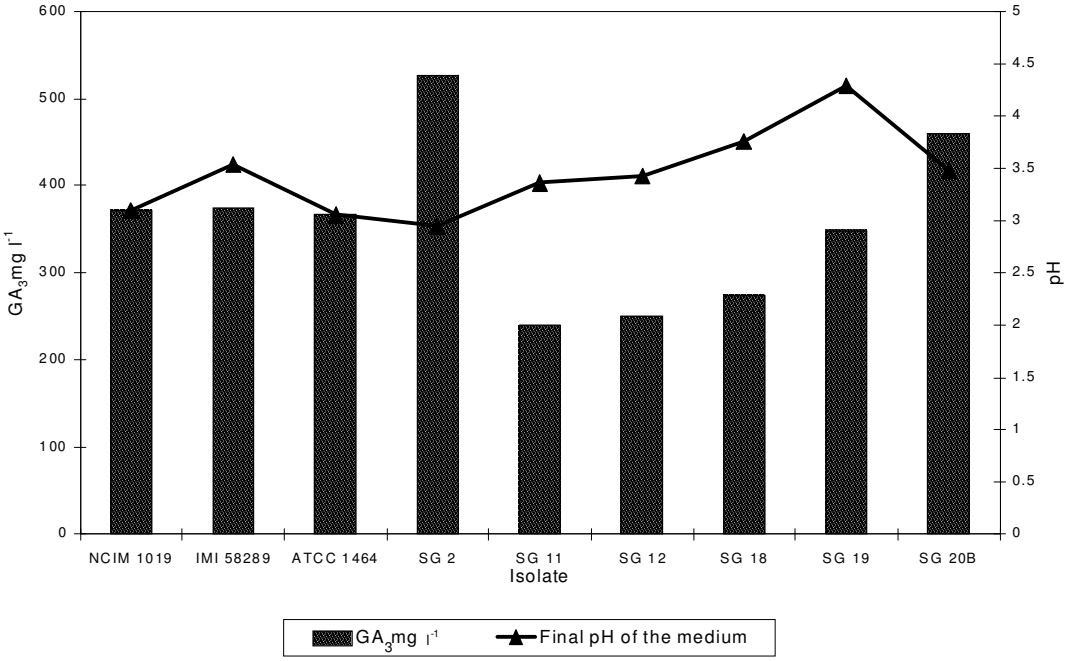

Fig. 1—GA_3 production by *Fusarium* isolates under submerged fermentation

Production, Extraction and Estimation of GA_3 and Determination of Dry Matter

Cultures (Fig. 1) were tested for their ability to produce GA_3 after growing in Czapek-Dox broth at 29±1°C for 7 days on rotary shaker (150 rpm). Samples were removed after 7 days of fermentation for estimation of dry mycelial biomass, final pH and GA_3. After 7 days of fermentation, culture broth was filtered through pre-weighed filter paper. Filter paper along with mycelial matter retained on filter paper was dried to constant weight at 60°C, weighed and mycelial weight calculated. pH of filtrate was determined on pH meter and set to pH 2.5 with 10% hydrochloric acid (HCl). Acidified broth was extracted three times with ethyl acetate (1:3 medium to solvent ratio). Aqueous phase of all three stages was discarded and solvent portion was pooled, dried and residue collected for estimation of GA_3[6]. In this method, gibberellic acid is converted to gibberllenic acid, which was detected at A_{254} nm (Absorbance at 254nm) in a double beam UV spectrophotometer (Varian Cary 50 Scan, Australia).

To scale up GA_3 yield, all test cultures were screened in modified medium[7] (glucose, 100 g/l; ammonium chloride, 1 g/l; and rice flour, 2 g/l; besides magnesium sulphate and potassium hydrogen phosphate; pH 5). *F. fujikuroi* strain SG2 was selected based on its maximum GA_3 production. Its ability to produce GA_3 in Czapek-Dox broth medium was further studied up to 9 days and gibberellin synthesis and sugar utilization by the fungus were estimated[8].

Results and Discussion

Isolates (21) as tentatively *F. fujikuroi* were made from different parts of rice plants having 'bakanae'. Based on morphological and microscopic observations, 8 isolates were identified as *Fusarium*. Further characterization including plant infection revealed that only 4 cultures (SG2, SG18, SG19, SG20B) were to be *F. fujikuroi*. Colonies of isolated cultures (SG2, SG18, SG19, and SG20B) produced cottony growth on PDA plates, which had violet, bright red, orange and bright orange pigmentations, respectively. Microscopic observations revealed the presence of branched, septate and hyaline mycelium, numerous microconidia and less numbers of macroconidia and sometimes chlamydospores. Identity of isolate SG2 as *F. fujikuroi* was confirmed by IMTECH, Chandigarh, and has been deposited in Microbial Type Culture Collection and gene bank (MTCC) as MTCC 4649.

In this study, results on GA_3 production and biomass formation under shake flask fermentation varied from strain to strain. Isolate SG2 produced highest GA_3

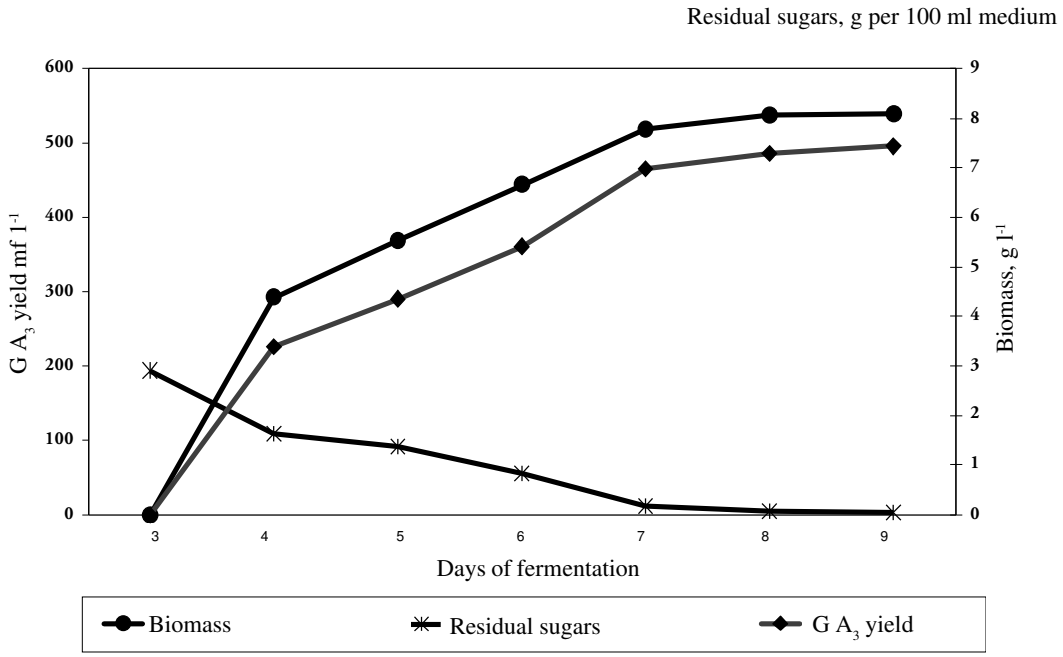

Fig. 2—GA_3 production pattern by SG2 in submerged fermentation

(526 mg/l) followed by SG20B (460 mg/l) and IMI 58289 (375 mg/l), with a lowest GA_3 yield (86 mg/l) with *Fusarium* spp. FC1. In general, final pH of medium and fungal biomass were 2.95-5.10 and 3.37-6.85 g/l respectively. In cultures of NCIM1019, ATCC1464, SG2 and SG20B, gibberellin yield (had direct proportion to) coincides with final pH, whereas all other cultures had higher pH and lower gibberellin yield (relationship) (Fig. 1).

While comparing GA_3 levels in modified medium, all isolates had a significantly higher level than in Czapek-Dox medium. Isolate SG2 produced 1175 mg/l followed by isolate SG20B (1085 mg/l) and ATCC1464 (985 mg/l). These results are according to earlier findings that GA_3 yield could be increased up to 1g/l by using an optimized medium having low concentration of nitrogen and carbon[9]. Also, initial pH of media (5.5), which was under control in these experiments, resulted in higher gibberellin yields than earlier reports[10,11].

F. fujikuroi SG2 was selected as superior strain for GA_3 production based on its gibberellin yield [gibberellin synthesis initiated on 3rd day and reached maximum by 9th day (495 mg/l) of fermentation], both in conventional and improved medium under SmF. GA_3 yield obtained on 9th day was at par with yield obtained on 7th and 8th day of fermentation, indicating optimum period of fermentation may be fixed as 7 days and further extension of incubation beyond 7th day did not influence much on gibberellin yield. Similarly, residual sugars available in medium after fermentation were also on decreasing trend. Complete utilization of sugar (98%) in medium was observed on 9th day of fermentation (Fig. 2). Based on initiation and completion of gibberellin synthesis and sugar utilization, gibberellin production is divided into five different phases as: I) Phase of about >96 h during which growth, nutrient utilization began and almost 50% of sugar utilized for synthesis of 46% of GA_3; II) Phase of steady growth of about 48 h duration characterized by increased growth and nutrient utilization (sugar and nitrogen); III) In 24 h duration of this phase, dry weight of mycelium reached maximum, ultimately leading to maximum production of GA_3; and IV) & V) In this phase (48 h), trend in increase of GA_3 and biomass was lesser and almost all sugar has been consumed.

Biomass formation and GA_3 production followed same trend. However, cell lyses was observed on 9th day in some cultures. Though depletion of nitrogen was

observed before 4th day of fermentation, presence of traces of nitrogen from 4th to 9th day of fermentation might be due to fungal cell protein (data not shown). This study also proved that GA_3 synthesis starts upon depletion of nitrogen, which is in agreement with earlier reports[10,12]. This investigation paves way for commercial exploitation of GA_3 producing strain (SG2) to meet local needs of gibberellic acid requirement.

Conclusions

Commercial production of GA_3 and related gibberellins not only helps in boosting agriculture but also various industrial processes. *F. fujikuroi* SG2 produced GA_3 (1175 mg/l), which was higher than the standards strains of *G. fujikuroi.*

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