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Enhanced FK-506 Production by a New Strain *Streptomyces Glaucescens* SS-13 through Ultraviolet Irradiation-Induced Mutagenesis

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Abstract: ***Tacrolimus (FK-506) is a significant polyketide produced by Streptomyces sp. It is considered the most important immunosuppressant molecule, and its estimated market size in 2030 for tacrolimus is around US$10 billion. The lower productivity of the product during the fermentation causes major challenges from an industrial perspective. In this study, we performed classical UV mutagenesis to enhance the production of Tacrolimus (FK-506) by a newly reported strain, Streptomyces glaucescens MTCC 276. The wild-type strain was a lower producer of FK-506 and lacked industrial importance. We have screened several mutant strains to identify a potent isolate with higher productivity. The results indicated that the screening of the mutant strain S. glaucescens, SS-13, led to a maximum tacrolimus production of approximately 125 mg/L. We also investigated the effect of the surfactant Tween-20 to improve tacrolimus production. Additionally, our findings on the effect of Tween-20 revealed that an optimal concentration of 10 g/L resulted in a higher yield of around 96 mg/L. Therefore, further chemical mutagenesis and fermentation studies can be conducted with this potent mutant strain to maximize its titer value. The SS-13 mutant strain shows promise for efficient and significant industrial application in the future. The growth parameters, such as pH, temperature, and nutrient feeding, were also studied to enhance the production of FK-506.***

Keywords: Tacrolimus; *Streptomyces glaucescens;* UV mutagenesis; Fermentation; Tween-20

1. Introduction

Tacrolimus (FK506) is a 23-membered polyketide produced by different *Streptomyces sp.* It is the most used medication for the treatment of graft rejection and is preferred as a first-line immunosuppressant regimen(Sarumathy et al., 2016). FK506 suppresses the immune system by inhibiting signal transduction cascades required for T-lymphocyte activation during tissue rejection (Motamedi and Shafiee, 1998). In mammals, the activities of FK506 are relatively specific for lymphocytes, with only limited effects observed in other cells and tissues(Pires et al., 2020). It has been approved by the FDA since 1994, and widely used for heart, kidney, and bone marrow transplantation, as well as various autoimmune diseases, particularly inflammatory and hyperproliferative skin disease, and cutaneous manifestations of immunologically induced illness(Kino et al., 1987). The global tacrolimus market value in 2022 is estimated to be US$6.485 billion. A CAGR of 4.7% is expected during the forecast period (2022-2030). The estimated market size in 2030 for tacrolimus is around US$9.38 billion(Patel et al., 2020). During the last few years, a great deal of work has been put into elucidating several crucial genes of the biosynthetic cluster to meet the growing demand for FK506. So, numerous experiments have been carried out, which include exogenous feeding, metabolic pathway changes, and conventional breeding techniques, including mutagenesis(Ye et al., 2021).

Research universities and pharmaceutical companies have shown more focus on the FK506 production because of its clinical and pharmacological significance; in addition, it has broader applicability. These advantages made a greater demand for tacrolimus, and many industries started focusing on the production and developing the product more effectively and economically in previous years(Xia et al., 2013). The enhancement of streptomyces strain production is crucial upon the isolation of high-yielding strains, the cultivation parameters, mutagenesis, and metabolic engineering. For the efficient and cost-effective manufacturing of tacrolimus, researchers have utilized many techniques, including metabolic engineering to create novel strains and parameter optimization of fermentation processes(Singh et al., 2017). The labor-intensive method of numerous rounds of mutagenesis and screening is usually used to obtain the industrial strains(Cen et al., 2024). Strain Improvement or optimization of physicochemical and nutritional conditions might lead to an enormous increase in the fermentative production of microbial metabolites(Yan et al., 2021). In addition to optimization, a highly effective strategy for enhancing the secondary metabolites production involves supplementing the fermentation medium with appropriate exogenous nutrients, inducers, and growth factors, including precursors, surfactant, and metal ions(Ordóñez-Robles et al., 2018). Earlier, extensive research activities were done to investigate how different amino acids affect the Tacrolimus production. The results indicated that lysine, valine, proline, leucine, and threonine will significantly enhance tacrolimus productivity(Barreiro et al., 2024).

The development of a reliable, defined medium was essential in order to properly investigate the regulatory mechanisms of tacrolimus production. The physiology and biochemistry of a microorganism's generation of secondary metabolites may usually be investigated more easily when a

straightforward, chemically defined medium is used(Sambyal and Singh, 2020). The relatively low yield of tacrolimus through the fermentation process represents a significant obstacle to its industrial-scale production. Previous reports showed that random mutagenesis was utilized to evaluate the overproducing mutants of tacrolimus(Barreiro and Martínez-Castro, 2014). Hence, it is important to identify new microbial strains that have the potential to produce tacrolimus. The list of strains producing Tacrolimus FK-506 is given in Table 1.

Table 1: List of Streptomyces species reported for Tacrolimus production.

Strain	Details	Reference
Streptomyces tsukubaensis	First reported and key source of tacrolimus, 0.1-3g/L titer.	Zhang et. al. (2025)
Streptomyces hygroscopicus	This strain can produce about 10-12 mg/L	Kawai, S., et al. (2001).
Streptomyces clavuligerus	Reported titers are about 50-495mg/L	Kim and Park et.al. (2007)
Streptomyces friuliensis	Reported titers are around 8 mg/L	Zhan, J., et al. (2008).
Streptomyces venezuelae	Reported titers are about 5-60mg/L	Kim et.al .(2015)
Streptomyces glaucescens MTCC 5115	Reported titers are about 5-10µg/L	WO2005038009A2

This is the first report on the mutagenesis of *Streptomyces glaucescens MTCC 276. In* this current study, we have carried out multiple UV mutations on the *Streptomyces glaucescens* MTCC 276. The UV mutants were screened for the production of tacrolimus. The best mutant was studied for the effect of surfactants such as Tween-20 & Triton X-100 on enhancing the titer of Tacrolimus.

2. Materials and Methods

Lab Chemicals and Dehydrated Media

All the chemicals and reagents were of analytical grade (A.R.). Glucose, Potassium phosphate, Magnesium sulfate, Sodium nitrate, Copper sulfate, Calcium carbonate, 2M NaOH, HCl, Antifoam, and Tween 20 were obtained from Sisco Research Laboratories (India). The dehydrated media, such as Yeast extract, Malt Extract, Corn starch, Soya bean meal, Agar, and Soya peptone, were purchased from Himedia Laboratories (India).

Selection and cultivation methods

Streptomyces glaucescens (MTCC 276) was obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India.

Streptomyces sp. MTCC 276 was maintained on slants and GYMA medium in Petri plates consisting of glucose 4, malt extract 10, yeast extract 4, and agar 15, Calcium carbonate 2 (g/L), pH 7.3, and incubated at 28°C. The glycerol stocks were used for further studies.

Morphological appearance in various media

The strain *Streptomyces glaucescens* MTCC 276 was selected for the study. The culture was streaked to ISP Medium 1, ISP Medium 2, and ISP Medium 4, which are employed for the characterization of Streptomyces species under the International Streptomyces Project (ISP)(Singh et al., 2017) and GYMA Plate for revival. The slants and plates were kept in an incubator oven for 5 days at 25°C.

Macroscopic and Microscopic characterization

Bacterial morphology was studied macroscopically by observing the growth characteristics on an ISP-2 media plate. The colony features, such as shape, size, texture, colony growth (length and width), aerial mycelium presence or absence, spore chain morphology, the color of the colony, and diffusible pigment production, were observed and identified with the help of referring to the taxonomic guides(Martínez-Castro et al., 2013). The bacterial isolates were visualized under a light microscope by using the Gram-staining procedure. Moreover, further morphological and biochemical characterization was performed for the isolates to verify the identity of *Streptomyces sp.*

Seed medium composition

The seed media consists of Corn starch 25, Glucose 5, Soya peptone 3, Yeast extract 5, Malt extract 7, and Calcium carbonate 1(g/L). The media was prepared using RO water, and the pH was adjusted to 6.8 using 10% sodium hydroxide solution. The prepared media was aliquoted into four flasks (50ml media in 250ml conical flasks) labeled as Control, 5 min, 10 min, and 15 min, and then sterilized by autoclaving at 121°C for 20min. A loopful of inoculum was taken from the UV-mutated plates and inoculated into seed media, which was incubated at 28˚C for 3-5 days.

Production medium composition

The production flask was prepared using Corn starch 40, Glucose 20, Soya peptone 20, Soya bean meal 20, Magnesium sulphate 0.25, Sodium chloride 1, Copper sulphate 0.025, calcium carbonate 3 (g/L), and 1% Strucktol was added as an antifoaming agent. The media components were dissolved in RO water, and the pH was adjusted to 6.8 using 10% sodium hydroxide solution. The media was aliquoted into four flasks, labeled as Control, 5 min, 10 min, and 15 min, and sterilized.

UV Mutation procedure

A loopful of the previously grown colonies from a slant was taken and dissolved in an Eppendorf tube containing sterile saline (1ml). The microbial sample (0.5 µL) was spread on each Petri plate using an L-shaped rod, and the plates were labeled as negative control, 5 min, 10 min, and 15 min. The inoculated plates were kept under UV radiation at different time intervals of 5, 10, and 15 minutes, except for the negative control, and the plates were incubated at 28°C for 3-5 days for germination. The Philips TFP 20 M UV screen produces ultraviolet light at a wavelength of 213 nm, which is within the germicidal UVC range known for effectively damaging microbial DNA. This wavelength is commonly used to induce mutations and sterilize cultures. When bacterial cultures are exposed to this UV source, a killing rate of 40% to 80% is typically achieved, which balances lethality with enough surviving cells for effective mutagenesis(Korbekandi et al., 2010).

Following UV exposure, the treated bacterial cells are incubated at 28°C for around 5 days. This incubation period

allows sufficient time for the surviving cells to grow and form colonies, facilitating the identification and isolation of mutants generated by the UV irradiation process(Sun et al., 2023).

The UV exposure at 213 nm causes DNA damage that leads to mutations, the partial killing rate ensures viable cells survive for mutation screening, and the incubation at 28°C for 5 days allows visible mutant colonies to develop for further study. This method is a standard and effective protocol for UV-induced mutagenesis in bacteria such as Streptomyces(Kim et al., 2023).

Mutant colonies were randomly picked for screening. About 15 different colonies from each UV mutation plate were inoculated into 5 mL of modified ISP-2 broth media in a 10ml tube. The modified ISP-2 medium consists of glucose (4g/L), yeast extract (4g/L), malt extract (10g/L), and pH 7.2. The tubes were incubated at 28°C for 5 days in shaking conditions. The product titer was analyzed by HPLC to identify the best one for each category, which was taken for a production trial to confirm the productivity of the UV mutant using the seed and production methods as mentioned in previous sections.

Fermentation Conditions

a) **Seed generation conditions:** A loopful of the colony was taken from the different intervals of UV-mutated plates and was inoculated into the seed flasks. The incubation of the seed flask was carried out at 28˚C for 5 days on an orbital incubatory shaker.
b) **Production fermentation conditions:** The production flasks were inoculated with 10% v/v well-grown culture from the seed flask and incubated at 28°C, 200 rpm for 9 days on an orbital incubatory shaker.
c) **Effect of surfactant on tacrolimus production:** Different concentrations of Tween-20 (0, 10, 20, and 30 g/L (w/v)) were added to each 500 mL Erlenmeyer flask containing 50 mL of media, respectively. The inoculum 10% was added into the production media and incubated at 28°C, 200 rpm for 168h. The optimal concentration of Tween-20 was determined by HPLC analysis of culture broth for tacrolimus yields. The pH, PMV were also calculated for all the flasks.
d) **pH, packed mycelium volume (PMV), and HPLC analysis:** The pH and packed mycelium volume were evaluated for all the production media. For PMV evaluation, broth (10mL) was centrifuged at 3000rpm for 10 minutes. The supernatant was measured and calculated by using the formulae:

PMV (%) = (Volume of broth−Volume of Supernatant)/ (Volume of broth) X 100

The HPLC-based method was used in the analysis of reaction samples. The product formation was analyzed using the Reverse Phase–HPLC method. A reverse phase C-18 column with a dimension of 250mm X 4.6mm, 5 micron size was used for the analysis of the percentage conversion of product for all the molecules under study(Patel et al., 2020).

The mobile phase is 0.1% Trifluoroacetic acid in water and acetonitrile as the eluent in a gradient mode. The gradient was set at 40% acetonitrile initially and increased up to 60% in a 45-minute linear gradient. The flow rate was 1.6mL/min, and the compounds were identified by a UV-Vis detector at UV 210 nm. The column temperature was set at 60°C, and the sample cabinet was set at 15°C. The product concentration was measured using the following formula.

$$Product\ titer\left(\frac{mg}{L}\right) = \frac{Area\ of\ the\ sample}{Area\ of\ the\ Standard}\ x\ Standard\ concentration\ x\ dilution\ of\ sample$$

3. Results and Discussions

The present study aims to identify a new strain of *Streptomyces glaucescens* MTCC 276 to produce Tacrolimus using the best reported media and to evaluate UV mutagenesis for the increased production of Tacrolimus (FK 506) for commercial feasibility.

Morphological appearance of the strain on different media

The morphological appearance of the strain *S.glaucescens* was evaluated using the earlier reported literature(Martínez-Castro et al., 2013). *Streptomyces glaucescen*s shows distinct morphological features on various media, typical of Streptomyces species, with some specific characteristics depending on the medium used. The cultivation in rich media such as ISP 2 (yeast extract–malt extract agar), S. *glaucescens* forms colonies with well-developed substrate mycelium and aerial mycelium; the aerial mycelium often appears grayish to whitish with a powdery or hairy texture due to spore formation(Vishwanatha et al., 2017). On starch-containing or inorganic salt media (like ISP 4), colonies may show production of colored pigments, such as yellow or brownish hues in substrate mycelium or the reverse side of plates. Growth on minimal or other selective media can be variable, but generally slower, with less aerial mycelium and sporulation(Wildermuth, 1972).

The microscopic features of the spores of S. *glaucescens* tend to be somewhat unique, with a "hairy" or coarse spore surface, appearing globose to oval under electron microscopy. Spore chains are often short and spiral-shaped. The mycelium does not typically fragment into smaller elements and is composed of branching hyphae(El-Naggar and El-Ewasy, 2017).

The results showed that the color of the aerial mycelia varied depending on the type of media used for the growth. The morphology was observed as a slight ash-brownish color, thick colonies on ISP-2 agar medium, a grey color on ISP-4 medium, and dark, orange-colored colonies in GYMA media, details provided in Table 2. The spores were greyish in ISP-2 medium and off-white to green in ISP-4, and a whitish color was noticed in GYMA media. Diffused pigments were sometimes observed in the revival slant prepared using ISP-2 medium, as shown in Figure 1.

Table 2: Culture characteristics of *S. glaucescens*

S. No.	Medium	Growth pattern observed	Remarks
1	Yeast extract malt agar (ISP-2)	Gray, powdery colonies were observed	Good Growth and no colour pigment observed.
2	Inorganic salts starch agar medium (ISP-4)	Slight brown-creamy color, thick colonies	Good Growth and a slight pink colour pigment were observed in the back of the colony.
3	Glucose, Yeast extract, malt Agar (GYMA)	Orange to brown colored thin colonies with aerial mycelium were observed	Good Growth and orange colour pigment observed in the back of the colony

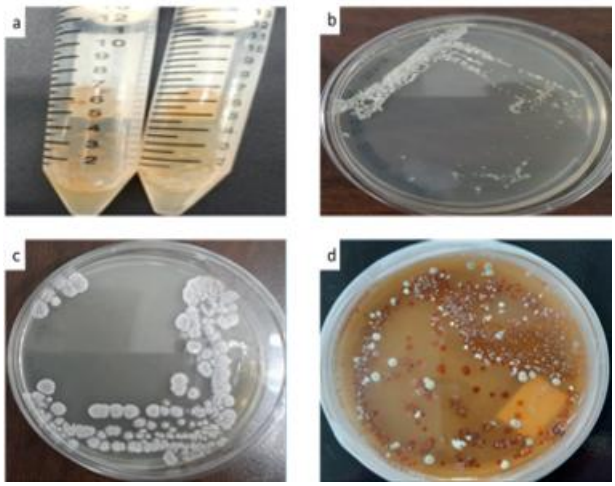

Figure 1: Microbial culture pictures showing (a)Revival slants, (b) Cultures on ISP-2, (c) ISP-4, (d) GYMA plates

Identification of cultural characteristics of Strain *Streptomyces glaucescens* MTCC 276:

The Morphological and biochemical characterization of the isolate was conducted to determine its taxonomic identity as per standard methods(Shirling and Gottlieb, 1966). Microscopic observation after Gram staining analysis revealed characteristics consistent with those of *Streptomyces* species with thread-like mycelial growth. The methodology used for *Streptomyces glaucescens* MTCC 276 served as a reference and was compared with previously reported data(Vishwanatha et al., 2017). As noted by Waksman (1961), colony color and morphology alone are not sufficient for genus-level identification; thus, morphological characteristics remain the primary criteria for classification, as detailed in Table 3. The biochemical test results, presented in Table 4, further support the identification of the isolates as belonging to the *Streptomyces* genus.

Table 3: Morphological characteristics for potential strain

Characteristic	Result
Gram's staining character	+
Morphology	Long branching filaments
Substrate mycelia	Off-White to light brown
Spore/colour	+/ Gray
Spore Chain morphology	Rectiflexibiles
Spore morphology	Rough
Spore-bearing hyphae	Monoverticillus/pale brown
Metabolism	Aerobe

Table 4: Biochemical features of potential strain

Tests	Result
Catalase	Positive (+)
Oxidase	Negative (-)
Citrate	Positive (+)
Methyl red	Negative (-)
VP	Positive (+)
Starch hydrolysis	Positive (+)
Indole	Negative (-)
Nitrate reduction	Positive (+)
Glycerol Utilization	Negative (-)
Glucose	Positive (+)
Lactose	Positive (+)
H_2S formation	Negative (-)

UV Mutagenesis and screening for tacrolimus high-producing strains

Extensive scientific evidence supports the enhanced production of metabolites in *Streptomyces* sp. through mutagenesis(Singh et al., 2017). The UV mutagenesis has been reported to enhance the production of tacrolimus in *Streptomyces* species. Similarly, in this study, *S. glaucescens* MTCC276 went through UV treatments with varying times of 5 min, 10 min, and 15 min. Figure 2 represents the culture growth on the GYMA Plate and microscopic observations of the mycelial growth in the flask medium. Approximately 50 mutant colonies obtained through the UV mutagenesis were selected for the evaluation of the tacrolimus production via shake flask fermentation.

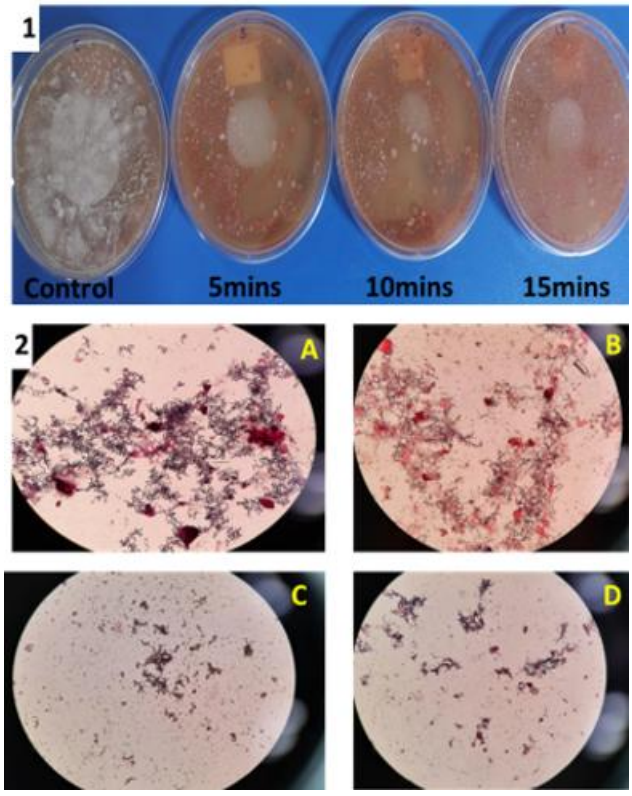

Figure 2: (1) UV-mutated strains on GYMA plate (2) Microscopic images of mutant strains from flask medium (A) 5mins (B) 10mins (C) 15mins

Morphological Comparison of Control and Mutant Cells

The changes in colony morphology for the mutant after exposure may be due to the UV radiation effect being examined. The developed mutants were compared for probable changes in their morphological characters. The bacterial colonies were rounded, smooth, and substrate mycelium was off-white, and aerial mycelium was gray or orange in control cells. Significant morphological differences were noticed in the bacterial colonies exposed to UV radiation(Du et al., 2014). The UV-derived mutants exhibited smaller, flatter colony sizes, and some mutants produced abundant spores, while others secreted orange-colored pigments. The data indicated that mutants with higher spore production and reduced, orange-colored pigment secretion demonstrated comparatively greater productivity of tacrolimus.

Screening of UV Mutants

Mutant colonies were randomly picked for testing. About 50 different colonies from each UV mutation plate were inoculated into 5 mL of modified ISP-2 broth media in a 10ml tube. The modified ISP-2 medium consists of glucose (4g/L), yeast extract (4g/L), malt extract (10g/L), and pH 7.2. The tubes were incubated at 28°C for 5 days under shaking conditions. The product titer was analyzed by HPLC to identify the best one for each category, which was taken for a production trial to confirm the productivity of the UV mutant using the seed and production methods mentioned in the sections. Most of the mutants did not provide significant product formation. The best 3 mutants of each category were presented in Table 5. The mutant strains SS-1, SS-9, and SS-13 gave maximum titer values of about 173, 294, and 405 mg/L.

After 5 days, the broth sample was taken from each flask to check pH and PMV (Packed mycelial volume). PMV analysis: About 10 ml of the mutated sample was taken from each flask in a labeled Falcon tube and centrifuged at 10000 RPM for 10 min and calculated for PMV%.

Table 5: HPLC results for the mutant screening

Category	Name	Titer (mg/L)
UV_5min	**Mutant_1**	**173**
	Mutant_2	154
	Mutant_3	125
	Mutant_4	102
	Mutant_5	162
UV_10min	Mutant_6	129
	Mutant_7	179
	Mutant_8	136
	Mutant_9	**294**
	Mutant_10	194
UV_15min	Mutant_11	205
	Mutant_12	259
	Mutant_13	**405**
	Mutant_14	332
	Mutant_15	264

In-process analysis of the production flasks

The potent mutant strains were reevaluated to check the productivity. After 5 days, the broth sample was taken from each flask for checking pH and PMV (Packed mycelial volume). PMV analysis: About 10 ml of the mutated sample was taken from each flask in a labeled Falcon tube and centrifuged at 10000 RPM for 10 min and calculated for PMV%. The results were tabulated in Table 6.

Table 6: In-process analysis results for the seed flasks

S. No.	Flask name	pH	PMV
1	Control	7.16	8.50%
2	UV_ Mutant_1	7.42	12%
3	UV_ Mutant_9	7.34	13.20%
4	UV_ Mutant_13	7.43	13.50%

Production titer analysis results

HPLC Sample Preparation Procedure

The best three mutants were re-examined, and a duplicate production flask was fermented using the production medium for five days. The production flasks were harvested on 5th day and taken for the HPLC analysis against Tacrolimus internal standard. About 5 ml of sample was taken in a Falcon tube, added to an equal volume of 100% acetonitrile, and vortexed for 5 minutes. The tubes were centrifuged at 10000 RPM for 10 min, and the supernatant was collected and analyzed. Table 7 & Figure 3 show the results of in-process pH, PMV, and HPLC quantification of Tacrolimus titer analysis results.

Table 7: In-process analysis results for the production flasks

Flask name	pH	PMV%	Tacrolimus Titer (mg/L)
Control_1	8.4	11.2	52
Control_2	8.33	11.4	48
UV_ Mutant_1	8.4	10.3	163
UV_ Mutant_1	8.44	10.5	158
UV_ Mutant_9	8.31	11.2	254
UV_ Mutant_9	8.35	11.7	211
UV_ Mutant_13	**8.23**	**12.5**	**415**
UV_ Mutant_13	**8.17**	**11.5**	**398**

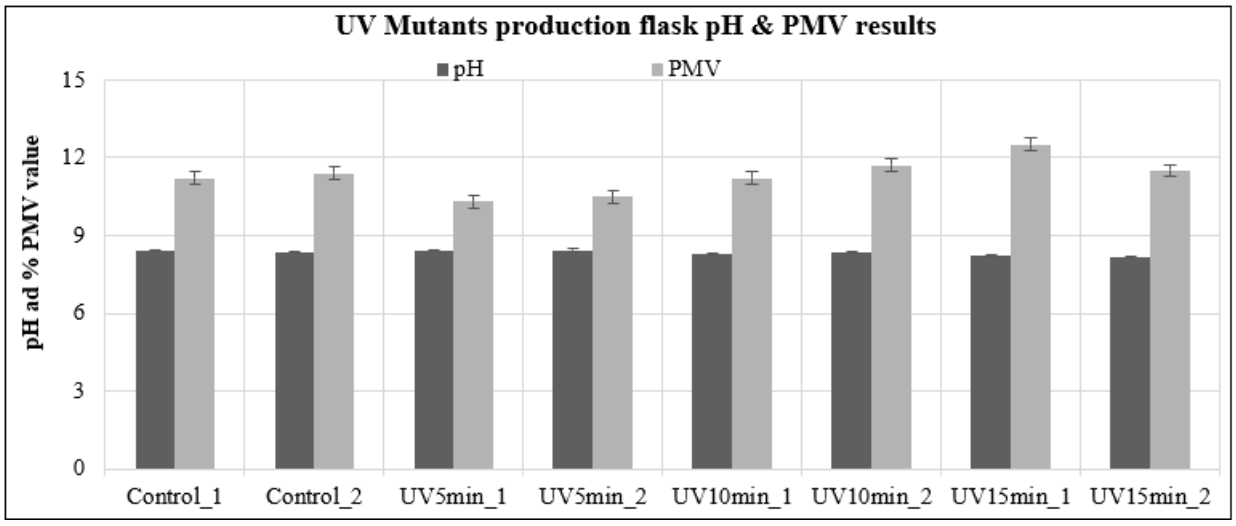

Figure 3: Bar graph showing the in-process analysis trend for the UV potent mutants

Effect of surfactants (Tween-20) on tacrolimus production

The best UV mutant strain 13, was subjected to Tween-20 treatment(Biswas and Paul, 2016). The procedure followed was as mentioned in the materials section. The optimum Tween-20 concentration was determined by analyzing the tacrolimus yields in the culture broth using reversed-phase HPLC. It is observed that as the concentration of Tween-20 increases, there is a decline in the trends of PMV and pH, due to the toxicity effect. The activity was slightly higher on the T-1 flask compared to the control flask, T-2 showed lower activity than T-1, and T-3 had very poor activity. The study indicates that the surfactant Tween 20 significantly affects the growth and activity of the culture. More experimentation is needed to determine the optimal concentrations; currently, the T-1 flask with 10 g/L showed the highest activity (Figure 6). As the concentration of Tween-20 increased, a decrease in the trend of PMV and pH was observed (Figure 7). The activity was slightly higher on the T-1 flask than on the control flask; T-2 was lower than T-1, and T-3 showed very poor activity. The study indicates that the surfactant Tween 20 significantly influenced the growth and activity of the culture. More experiments are needed to determine the optimal concentrations; currently, the T-1 flask with 10 g/L showed the highest activity, as tabulated in Table 8.

Table 8: Effect of Tween-20 on tacrolimus production by mutant SS-13

Details	Tween 20	pH	% PMV	Activity (mg/L)
Control	0	7.78	19.1	381
T-1	10g/L	7.8	25.6	360
T-2	20g/L	7.7	20.5	511
T-3	30g/L	7.7	15.7	231

4. Conclusion

The present study highlights the significance of mutagenesis and strain improvement strategies to enhance the biosynthetic potential of Streptomyces species. The ultraviolet mutagenesis provided a high-yielding mutant strain, *Streptomyces glaucescens* SS 13, which was successfully obtained, demonstrating a substantial increase in tacrolimus production as 415mg/L, compared to its parental counterpart. Morphological variations among the mutants, such as altered colony size, spore abundance, and pigment secretion, provided useful indicators for selecting strains with superior productivity.

Further optimization of the fermentation process revealed that supplementation with Tween 20 at an optimal concentration of 10 g/L significantly enhanced the FK-506 tacrolimus yields to 511 mg/L, underscoring the importance of exogenous nutrient and surfactant addition in secondary metabolite biosynthesis. These findings not only validate mutagenesis as a reliable approach for strain improvement but also emphasize the role of medium optimization in maximizing metabolite output.

The SS 13 mutant strain, with its enhanced productivity profile, represents a promising candidate for industrial exploitation. Future work involving chemical mutagenesis, advanced fermentation strategies, and possibly genetic engineering could further elevate production levels, making large-scale manufacturing of tacrolimus more economically viable. Collectively, this study demonstrates that systematic strain improvement coupled with process optimization can pave the way for sustainable and efficient production of pharmaceutically important biomolecules, thereby strengthening the industrial applicability of Streptomyces mutants.

Conflict of Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Contribution of Authors:
We certify that the authors identified in this article contributed to this work well, and they will bear full liability for claims relating to the content of this article.

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