STRAIN IMPROVEMENT STUDIES FOR THE ISOLATE *STREPTOMYCES MALAYSIENSIS* (TMS 1a)

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ABSTRACT

Strain improvement is an essential part of process development for products of fermentation. The developed strain can reduce the costs with increased productivity and can possess some specialized and desirable characteristics. The isolated *Streptomyces malaysiensis* (TMS 1a) is obtained from termite mound soil and was subjected to strain improvement for keratinase production. Then the isolate was subjected to UV and nitrous acid treatment. The wild isolate *Streptomyces malaysiensis* (TMS 1a) produces 20IU/mL of keratinase activity while UV mutant UV-7 yielded 32.0IU/mL and the nitrous acid treatment UV7N-8(TMS 1a) exhibited 46IU/mL Enzyme activity. The UV Mutant showed 60% higher activity than the wild strain, where as nitrous acid treated UV7 N8 mutant showed maximum activity which was 43.75% higher than the UV Mutant strain. The overall strain improvement programme increases keratinase activity 2.3 folds with respect to the wild strain. Thus these results show that UV and HNO_2 were effective mutagenic agents for strain improvement. Thus this effect is attributed to possible changes in the promoter zones of the gene coding for these enzymes due to UV exposure. The radiation might have deregulated the transcription of mRNA corresponding to these enzymes, which lead to an increased production.

Key words: strain improvement, keratinase activity, UV mutant, nitrous acid mutant, promoter genes, transcription.

INTRODUCTION

The science and technology of manipulating and improving microbial strains, in order to enhance the metabolic capabilities for biotechnological applications, are referred to as strain improvement. The success in making fermentation industry competitive depends greatly on continuous improvement of the production strains. Mutation is the primary source of all genetic variations and has been used extensively in industrial improvement for production [9][13].The use of mutation and selection to improve the productivity of cultures has been strongly established for over 50 years and is still recognized as a valuable tool for strain improvement of many enzyme producing microorganisms. Improvement of microbial strains for the over production of industrial products has been the hallmark for all commercial fermentation processes. Conventionally, strain improvement has been achieved through mutation, selection, or genetic recombination. Overproduction of primary or secondary metabolite is a complex process and successful development of improved strains requires knowledge of physiology, pathway regulation and control and the design of creative screening procedures. In addition, it requires mastery of the fermentation process for each new strain, as well as sound engineering know-how for media optimization and the fine tuning of process conditions [4]. Lower fermentation, manufacturing and capital cost can be gained from improvements in fermentor design engineering. However, the improvement of the microbial production strain offers the greatest opportunity for cost reduction without significant capital outlay. The potential productivity of the organism is controlled by its genome and, therefore, the genome must be modified to increase the potential yield of the product. Products yield can be increased by continual genetic modification of strain.

MATERIALS AND METHODS:

a) UV irradiation of the isolate TMS 1a and selection of mutants:

Strain improvement for the isolate TMS 1a was done by mutation and selection. The isolate was subjected to UV irradiation. The dose survival curve was plotted for selecting the mutants between 15% and 1% survivals. Mutation frequency was mentioned to be high when the survival rates were between 15 and 1%.The spore suspension of wild isolate was prepared in sterile distilled water and 4mL quantities were pipette out aseptically into sterile flat-bottom Petri dishes of 100mm diameter. The exposure to UV light was carried out in a Dispensing-Cabinet fitted with TUP 40 W Germicidal lamp that has about 90% of its radiation at 2540-2550 Å. The exposure was carried out at a distance of 26.5 cm away from the centre of the germicidal lamp. The exposure was carried out for 0, 30, 60, 90, 120, 180, 240 and 300 sec respectively. During the exposure, plates were gently rotated so as to get uniform exposure of the contents of the Petri dish. The treatment was done when all other sources of light were cut off and the exposure was carried out in

dark. The treated spore suspensions were transferred into sterile test tubes covered with a black paper and kept in refrigerator overnight, to avoid photo reactivation. On the next day pour plating technique was carried out for the serial dilution of the samples up to 10^7.

After that, 0.1mL of each sample was inoculated into YEME medium plate and incubated for 7 days at 28 °C. The colonies number in each plate was counted. Assumption was made that each colony was formed from a single spore. Plates having survival rate between 15 and 1% were selected for the isolation of mutants. The stable mutants were selected based on the consistent expression of the phenotypic character and maintained on starch casein agar medium for experimental purposes.

b) Nitrous acid treatment

The cell suspension of the selected UV mutant was prepared by using acetate buffer pH 5. To 9 mL of the cell suspension in buffer, 1mL of sterile stock solution of 0.01 M sodium nitrite was added. Samples of 4 mL are withdrawn at 0, 30, 60, 90, 150, 180, 240 and 300 sec and each of 1mL samples was neutralized with 0.5 mL of 0.1 M NaOH and serially diluted and plated on YEME medium. The dose-survival curve was constructed after nitrous acid treatment. Plates having survival rate between 15 and 1% were selected for the isolation of mutants. The stable mutants were selected based on consistent expression of the phenotypic character up to six generations and maintained on YEME slants. Incubation of plates was done at 28 °C for 5 days.

RESULTS AND DISCUSSION

Table 1.1: Effect of UV irradiation on the isolate TMS 1a

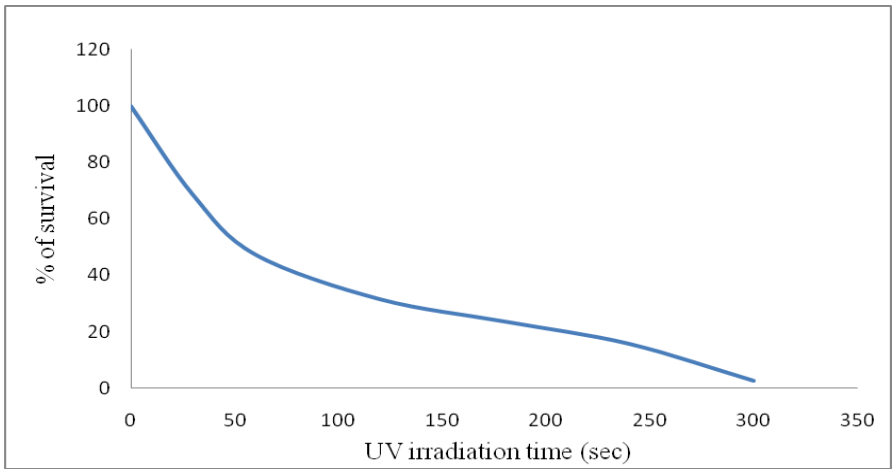

Fig 1.1: Survival curve of the isolate TMS 1a after UV irradiation

Table 1.2: Keratinase activity of UV mutants of the isolate TMS 1a

Irradiation time (sec)	Number of colonies/mL after Irradiation ($x10^6$)	Percentage kill (%)	Survival percent (%)
0	38	0	100
30	26	31.5	68.5
60	18	52.6	47.4
120	12	68.4	31.6
180	9	76.3	23.7
240	6	84.2	15.8
300	1	97.3	2.7

UV mutants	Keratinase activity (IU/mL)
UV-1	15
UV-2	24
UV-3	05
UV-4	16
UV-5	21
UV-6	13
UV-7	32
UV-8	15
UV-9	28
UV-10	14
Wild strain	20

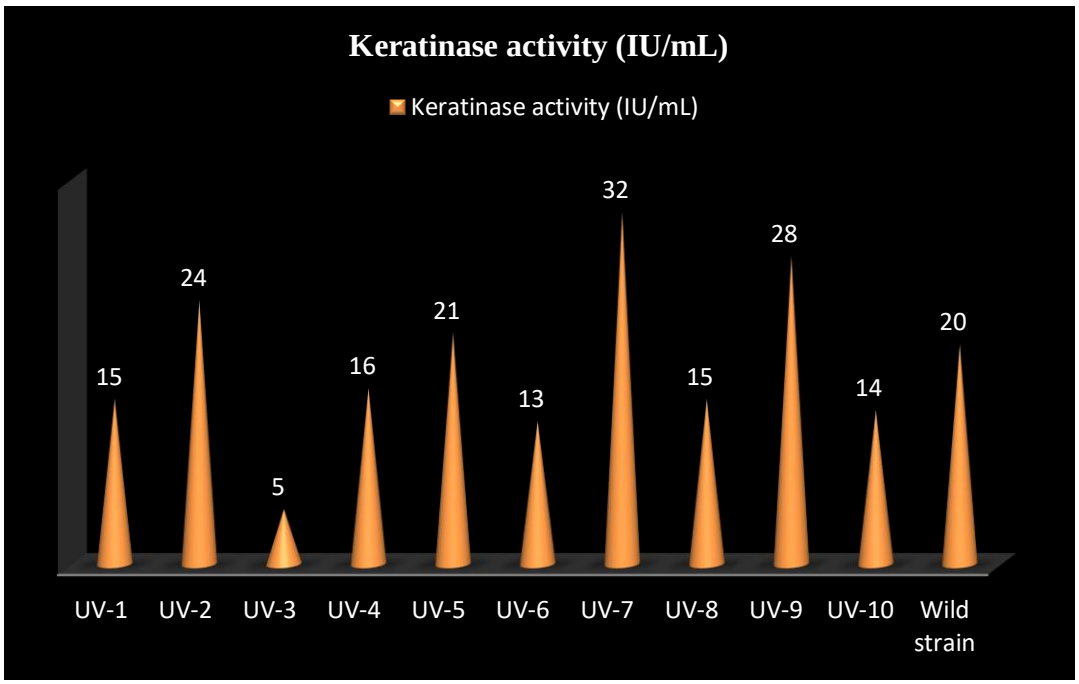

Fig 1.2: Keratinase activity of UV mutants of the isolate TMS 1a

Table 1.3: Effect of nitrous acid on UV-7 mutant strain of the TMS 1a isolate

Exposure time (sec)	Number of colonies/mL after exposure ($x10^6$)	Percentage kill (%)	Survival percent (%)
0	51	0	100
30	34	33.3	66.7
60	26	49	51
90	19	62.7	37.3
120	14	72.5	27.5

180	11	78.4	21.6
240	8	84.3	15.7
300	3	94.1	5.9

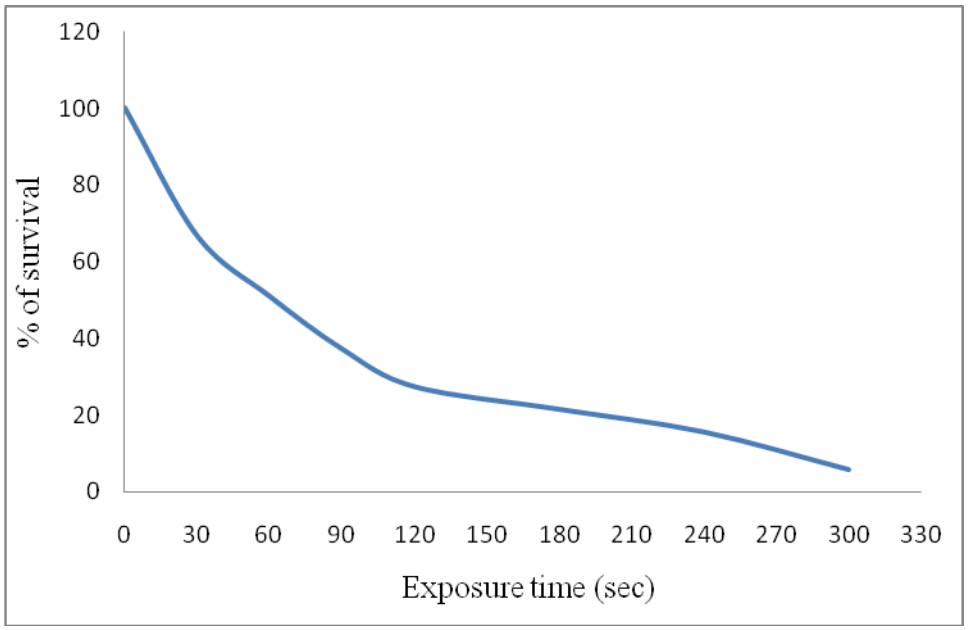

Fig 1.3: Survival curve of UV-7 mutant after nitrous acid treatment of the TMS 1a isolate

Table 1.4: Keratinase activity of UV-7 mutant after nitrous acid treatment of the TMS 1a isolate

UVF-7 mutants	**Keratinase activity (IU/mL)**
UV7N-1	18
UV7N-2	23
UV7N-3	16
UV7N-4	08

UV7N-5	13
UV7N-6	28
UV7N-7	24
UV7N-8	**46**
UV7N-9	12
UV7N-10	09
Wild strain	20

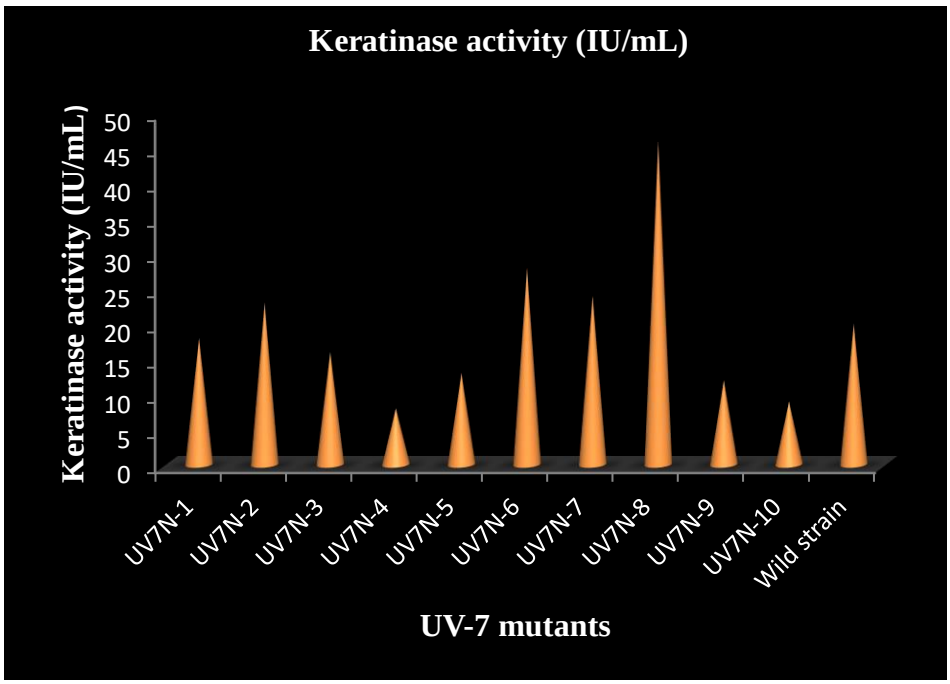

Fig 1.4: Production of keratinase activity of various nitrous acid treated UV-7 mutants from the isolate TMS 1a

Genetic improvement is one of the promised approaches for increased production of enzymes by industrially important microorganisms. In the present investigation, physical and chemical mutagens used were UV and nitrous acid consecutively.

- **Isolation of UV mutants and determination of keratinase activity:**

The wild isolate TMS 1a was subjected to UV irradiation at varying intervals of 0,30, 60, 120, 180, 240, 300 sec respectively. The number of survivals from each exposure time was represented in Fig 1.1 Plates having survival rate between 15 and 1% (300 sec) were selected for the isolation of mutants as shown in Table 1.1. A total of 10 UV mutants were isolated and determined for their keratinase production capacities by submerged fermentation and the activity was determined according to the method [5]. The results indicated that among the ten UV mutants, UV-7 showed highest keratinase activity (32.0 IU/mL) when compared to wild strain (20.0 IU/mL) and the results were shown in Table 1.2 and Fig 1.2. Production of keratinase enzyme by mutant isolate UV-7 mutant has improved by 60% improvement over the wild strain. Hence the best mutant UV-7 was selected for nitrous acid treatment for further improvement in keratinase production.

- **Isolation of nitrous acid mutants of UV7 strain and determination of keratinase activity:**

The selected mutant UV7 was subjected to nitrous acid treatment. The number of survivals from each exposure time for UV7 was represented in Fig 1.3. Plates having survival rate between 15 and 1% (240 and 300 secs) were selected for the isolation of mutants as shown in Table 1.3. A total of 10 mutants were selected and their keratinase activities were determined. The results indicated that the mutant UV7N8 (TMS 1a) yielded 46.0 IU/mL, which was 43.75% improvement over the UV mutant strain UV7 (32.0 IU/mL) and the results were shown in Table 1.4 and Fig 1.4.

The wild isolate TMS 1a produced 20.0 IU/mL of keratinase activity while the UV mutant UV7 yielded 32.0 IU/mL and the nitrous acid mutant UV7N-8 (TMS1a) exhibited 46.0 IU/mL enzyme activity. The UV-7 mutant exhibited 60% higher activity than the wild strain, where as nitrous acid treated UV7N8 mutant showed maximum activity which was 43.75% higher than UV mutant strain. The overall strain improvement programme increased keratinase activity 2.3 fold with respect to wild strain. Thus these results indicated that UV and HNO_2 were effective mutagenic agents for strain improvement.

Enhancement of keratinase activity was observed in all the strains after exposure of UV irradiation. This effect is attributed to possible changes in the promoter zones of the gene coding for these enzymes due to the ultraviolet exposure. The radiation might have deregulated the transcription of mRNA corresponding to these enzymes, leading to an increased production. Since ultraviolet radiation affects mainly the hydrogen bonds of pyramidine bases (cytosine + thymine) the most vulnerable regulatory sequences must have been those containing the highest concentration of C+T [14]. It can be assumed that keratinase might be under the control of such regulation.

Parekh *et al.*, (2000) [11] investigated that in many cases, mutations by UV are harmful, but occasionally it may lead to a better adapted organism to its environment with improved biocatalytic performance. The potential of any microorganism to mutate is an important property conferred by DNA, since it creates new variations in the gene pool. The main challenge is to isolate those strains which are true mutants that carry beneficial mutations. Varalakshmi *et al.,* (2009) [15] investigated that long period of exposure to UV light (>5 minutes) proved to be detrimental to the enzyme production. With increase in exposure time, the production of all the enzymes was reduced with studies on *A. niger* JGI 24 in which increased time of UV exposure resulted in decreased alpha amylase production. On 20 min exposure to UV, amylase activity was found to be zero.

Cai *et al.*, (2008)[3] reported keratinase production and keratin degradation by a mutant strain of *Bacillus subtilis* KD-N2 producing keratinolytic activity about 2.5 times that of the wild-type strain with 1 mg/ml MNNG solution. The mutant strain has produced inducible keratinase in different substrates of feathers, hair, wool and silk under submerged cultivation. Ashis *et al*., (2011) [1] described the improvement of alkaline β-keratinase production by ethyl methyl sulphonate (EMS)-induced mutant *Brevibacillus* sp. strain AS-S10-II and biodegradation of the waste chicken-feather was purified by alkaline β-keratinase from this mutant strain. When compared with wild strain, the mutant strain (EMS-05) exhibited more growth rate, less generation time and significantly higher rate ($p < 0.010$) of alkaline β-keratinase production.

Ellaiah *et al*., (2002) [7] reported a 200% increase in lipase yield by *Aspergillus niger* mutant of UV and NTG. Bapiraju *et al*., 2004 [2]has reported, a 232% increase in lipase production was achieved by strain improvement of indigenous isolate *Rhizopus sp.* BTS-24 by natural selection and induced mutations employing UV and NTG. Ganesh Kumar and Takagi, (1999)[8] have reported strain improvement using mutagenesis and recombinant DNA technology can be applied to augment the efficiency of the producer strain to a commercial status. A fivefold increase in the yield of enzyme was observed by the use of enzymes and tailor-made proteins from alkalophilic microorganisms with enhanced production yield.

Mutagenesis with only UV irradiation seemed less efficient in strain improvement for protease production [10]. The mutant strain selected after UV irradiation only showed a 44% increase in protease activity with an alkalophilic bacterial strain of *B. pantotheneticus* [12]. However, the protease activity in Pseudomonas sp RAJR044 was enhanced 2.5-fold by UV mutagenesis [6]. This deviation can also be due to the various bacterial strains used. In general, chemical mutagenesis is more efficient for improving protease production. However, combination of multiple mutagenic treatments may give better results was reported by Xia et al., 2012 [16].

Conclusion

The new isolate and keratinase produced by it, offer immense potential for further studies. Sequencing of the purified enzyme, comparison of the sequences to find out peculiarities which make enzyme unique and in depth studies in ungula and cosmetics will be highly useful. Assessing the performance of the enzyme in clinical conditions in patients and in industrial processes is another useful area with lot of potential applications.

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Conflict of interests

The authors declared no conflict of interest.

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