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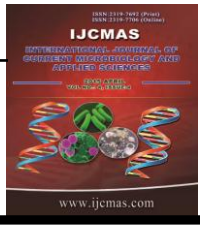

Original Research Article

Strain improvement studies on *Kurthia* species for the production of alkaline phosphatase

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A B S T R A C T

Strain development has been an essential prerequisite for efficient enzyme production process. Physical and chemical mutagens are prominent for the development of high yielding strains. In the present investigation, a strain of *Kurthia* species PN-1 producing alkaline phosphatase was subjected to ultraviolet irradiation. Mutant colonies were selected on the basis of macroscopic and morphological characteristics. The selected isolates were evaluated for their alkaline phosphatase producing capacities. The potent ultraviolet mutant strain was selected and further treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG). Mutation frequency was observed to be high when the survival rates were between 10% and 0.1%. The selected colonies were isolated and studied for increased alkaline phosphatase production. The alkaline phosphatase activity of the NTG mutant strain was 1.7-fold higher than the wild strain.

Introduction

Alkaline phosphatase (E.C.3.1.3.1) or orthophosphoric monoester phosphohydrolase is a hydrolase enzyme responsible for dephosphorylation at alkaline pH. The enzymes are wide spread in environment. Applications of alkaline phosphatases in molecular biology, diagnostics, dairy, biosensors, agriculture and aquatic ecosystem have been studied (Suresh and Das, 2014; Bel-Ochi *et al*., 2013; Tekaya *et al*., 2013; Rankin *et al*., 2010; Durrieu *et al*., 2003; Jordan *et al*., 1995). The constant increase of alkaline phosphatase applications in diverse areas demands the enhanced production of alkaline phosphatase. Highest possible yield of the fermented product can be majorly obtained with utilized microorganism where the potential productivity of the microorganism can be attained by strain improvement. Strain improvement has been essential in the development of process for fermentation products. Improved strains can be achieved with mutations in natural strain and subsequent screening.

Physical and chemical mutagens cause genetic variations by promoting errors in

replication or in repair of DNA (Trun and Trempy, 2004). Ultraviolet (UV) irradiation produces a variety of photoproducts of which the cyclobutyl dipyrimidines (dimers) and the pyrimidine-pyrimidone(6-4) adducts are predominant (Franklin *et al*., 1982; Patrick and Rahn, 1976). The photoproduct pyrimidine dimers constitute most of the lethal and premutagenic UV lesions (LeClerc *et al*., 1991; Kunz and Glickman, 1984). Photoreactivation of pyrimidine dimers with endogenous PyPy-specific photolyase avoids the high proportion of mutations (Lawrence *et al*., 1985). Chemical mutagens are stronger mutagenic agents that lead to transition mutations between GC and AT (Miller, 1972). *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) has been widely used as a mutagen in bacterial genetics since the discovery of its mutagenicity (Mandell and Greenberg, 1960). NTG is highly mutagenic which induces a high frequency of mutation (Rodríguez-Quiñones *et al*., 1984). The present investigation is to enhance the alkaline phosphatase production of *Kurthia* species PN-1 using UV irradiation and NTG as mutagens.

Materials and Methods

Microorganism: A strain of *Kurthia* sp. producing higher amount of alkaline phosphatase that was isolated from a crop field soil and designated as *Kurthia* sp. S-V-3 (Nalini *et al*., 2014) was selected. The isolate was redesignated as *Kurthia* sp. PN-1 (MTCC-9746). It was grown on nutrient agar slants at 37°C for 24 hr and maintained at 4°C.

Mutagenesis by UV: An 18 hr old slant culture of the isolate *Kurthia* sp. PN-1 was scrapped into 5 ml sterile distilled water and transferred into 45 ml sterile nutrient broth in a 250 ml Erlenmeyer flask. The flask was incubated at 37°C for 18 hr on a rotary shaker and aseptically centrifuged at 3000 rpm for 20 min. The supernatant was decanted and the cells were washed with sterile distilled water. Cells suspended in 5 ml sterile distilled water was diluted with 45 ml Tween 80 (1:4000) and thoroughly shaken for 30 min on rotary shaker. This suspension was used for UV irradiation.

A quantity of 4 ml of cell suspension was transferred aseptically into sterile Petri dishes and exposed to UV radiation at 2540-2550 A°. The exposure was carried out at a distance of 26.5 cm away from the center of the Germicidal lamp with occasional shaking. The UV light exposure times were 0, 5, 10, 15, 20, 25 and 30 min. Cell suspensions exposed to UV were stored in dark for overnight to avoid photoreactivation. Each irradiated suspension as well as the control sample were serially diluted using phosphate buffer of pH 7.0 and plated on nutrient agar medium. The plates were incubated for 24 hr at 37°C.

Survival curve was plotted and plates showing less than 1% survival rate were selected for the isolation of mutants. The isolates were selected on the basis of macroscopic differences and tested for alkaline phosphatase production.

Mutagenesis by chemical: An UV mutant strain producing maximum alkaline phosphatase was used for NTG treatment. Cerdá-Olmedo and Hanawalt (1968) reported the highest mutation frequency with NTG at pH 6.5. Cell suspension was prepared in the same manner as for UV treatment and diluted using phosphate buffer of pH 6.5. A stock solution of NTG of 10 mg/ml was prepared with phosphate buffer at 4°C and sterilized by passing through sterile bacterial proof filter. The cell suspension of 10 ml was added to 40 ml of

NTG solution to get a final concentration of 3 mg/ml of NTG. The mixture was agitated momentarily and immediately incubated at 30°C. Samples were withdrawn from the reaction mixture at appropriate time intervals and centrifuged immediately. The time intervals of exposure to NTG were ranged from 0 to 210 min. The cell pellets obtained were washed and suspended in 5 ml of sterile distilled water. A control was included without exposure to NTG. The treated samples were serially diluted with phosphate buffer of pH 7.0, plated and colony counts were made. Selected mutant strains from the plates having less than 1% survival rate were evaluated for their alkaline phosphatase activities.

Submerged fermentation: A 5 ml of 18 hr active inoculum was inoculated to the 45 ml of Pikovskaya medium (Pikovskaya, 1948) in 250 ml Erlenmeyer flask. The flasks were incubated at 37°C on rotary shaker for 48 hr. Uninoculated culture medium served as a control. Each experiment was conducted in triplicate and mean value was recorded.

Alkaline phosphatase assay: Culture broth was collected at the end of fermentation and centrifuged at 3000 rpm for 20 min. Supernatant was used to estimate the alkaline phosphatase activity by glycine assay method (Bernt, 1974).

Result and Discussion

Alkaline phosphatases play a vital role in the development of scientific applications and bioindustrial products. The activity of alkaline phosphatase was found to be used in the manufacture of anti-tumor compound for the conversion of etoposide phosphate to etoposide (Politino *et al*., 1996). Bacteria are effectively provided with significant alkaline phosphatase activities. The increased applications of alkaline phosphatases in various fields have gained importance in the production of bacterial alkaline phosphatases. The discipline encompasses with the strain improvement of *Kurthia* sp. PN-1 for the enhanced production of biomedically important enzyme alkaline phosphatase by mutations.

Mutations are used extensively in the strain improvement for enzyme production. The wild strain *Kurthia* sp. PN-1 produced 97.5 U/ml of alkaline phosphatase in the production medium (Nalini *et al*., 2014).

Selection of UV mutants and their alkaline phosphatase activity: The wild strain of *Kurthia* sp. PN-1 was subjected to UV irradiation. UV light owes mutations with genotoxic effect on bacterial chromosomes (Alcántara-Díaz *et al*., 2004; Rangarajan *et al*., 1999; Bridges and Mottershead, 1978; Doudney and Young, 1962). The number of survivals from each exposure time to UV radiation is represented in Fig. 1. Isolates were selected from 20 and 25 min of UV exposed plates. Selected mutants were provided with code numbers UV-1 to UV-15. The isolates were tested for their efficiency to produce alkaline phosphatase in culture medium.

Alkaline phosphatase production abilities varied in mutants. The results are shown in Table 1. Among all the mutants UV-9 produced maximum amount of 135.0 U/ml of alkaline phosphatase and it was 1.38-fold higher than the original strain, *Kurthia* sp. PN-1.

Isolation of NTG mutants and alkaline phosphatase activity: The selected UV-9 mutant was subsequently treated with NTG. NTG has been considered as a potent mutagen (Cerdá-Olmedo and Hanawalt, 1968; Adelberg *et al*., 1965). Its action is related to its decomposition products

(Cerdá-Olmedo and Hanawalt, 1968). Survival curve of NTG mutants is presented in Fig. 2. A total of 15 mutants were selected from the plates treated with NTG for 120, 150 and 180 min.

All the mutant strains, NUV-1 to NUV-15 exhibited increased alkaline phosphatase production than the wild strain. It is evident from Table 2 that NUV-7 was the predominant mutant involved in enhanced alkaline phosphatase production of 166.0 U/ml. It produced 1.7-fold higher alkaline phosphatase over the wild strain. The efficiency of mutants for alkaline phosphatase production is potential with NTG in comparison to UV irradiation.

Identifiable differences in the macroscopic characteristics indicating the mutations in isolates were observed when survival rates were between 10% and 0.1%. Isolates showed different responses to UV and NTG mutagens. The exhibited alkaline phosphatase activity of the mutants is probably due to the alteration of genotype in the isolates. Strain improvement is involved in the achievement of higher titres of the enzyme. Mutations of *Kurthia* sp. for increased alkaline phosphatase production have not been reported. It is concluded that the UV and NTG were effective mutagenic agents for strain improvement of *Kurthia* sp. PN-1 for enhanced alkaline phosphatase production.

Table.1 Alkaline phosphatase production of UV mutants

UV mutants	Alkaline phosphatase activity (U/ml)
UV-1	64.0
UV-2	93.0
UV-3	54.0
UV-4	97.0
UV-5	108.0
UV-6	50.0
UV-7	46.0
UV-8	70.0
UV-9	135.0
UV-10	124.0
UV-11	118.0
UV-12	68.0
UV-13	59.0
UV-14	90.0
UV-15	83.0
Kurthia sp. PN-1 (Wild strain)	97.5

Table.2 Alkaline phosphatase production of NTG mutants

NTG mutants	Alkaline phosphatase activity (U/ml)
NUV-1	110.0
NUV-2	98.0
NUV-3	117.0
NUV-4	157.0
NUV-5	144.0
NUV-6	126.0
NUV-7	166.0
NUV-8	139.0
NUV-9	154.0
NUV-10	130.0
NUV-11	148.0
NUV-12	127.0
NUV-13	120.0
NUV-14	134.0
NUV-15	105.0
UV-9 (UV mutant)	135.0

Fig.1 Effect of UV irradiation on survival of *Kurthia* sp. PN-1

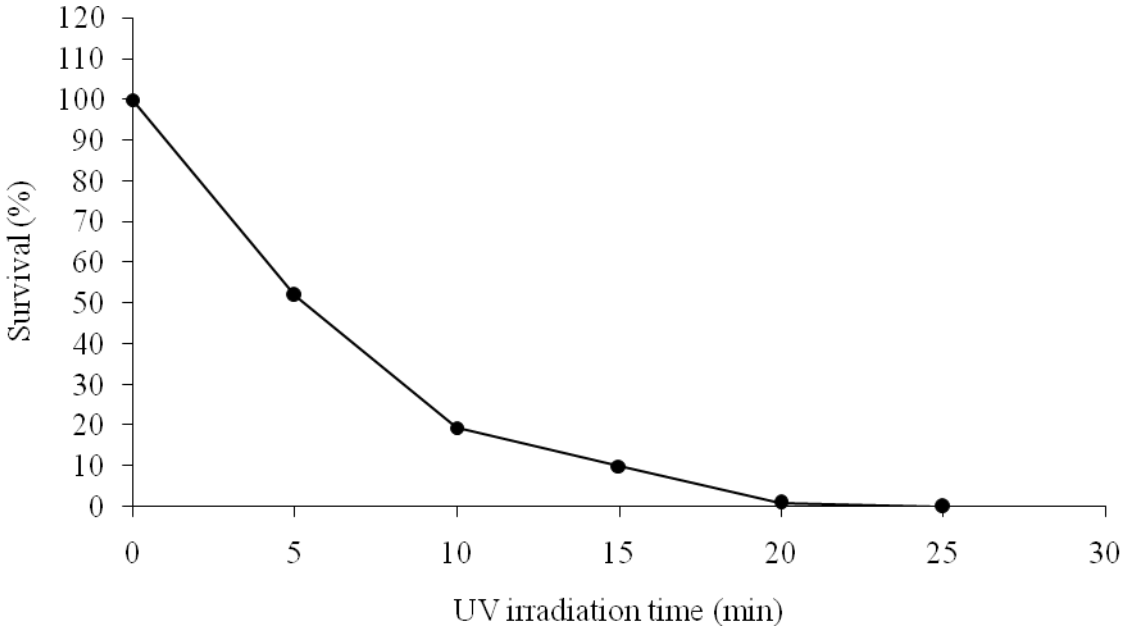
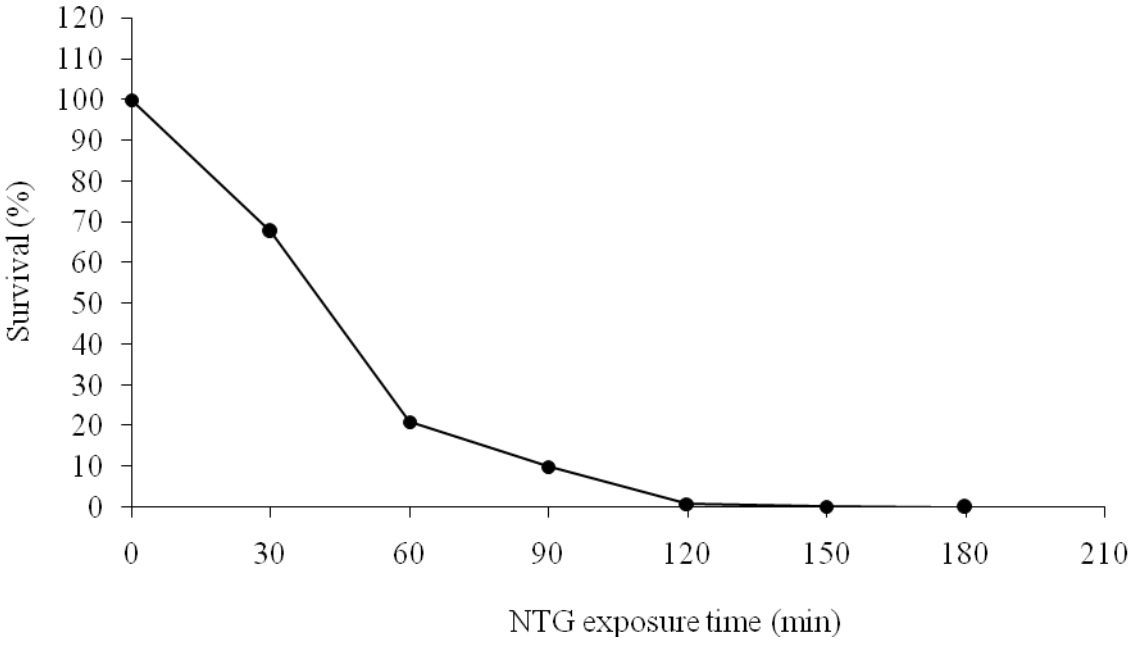


Fig.2 Effect of NTG treatment on survival of UV-9 mutant



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