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Optimize the Mutagenesis Strategy by Comparison to Develop High-Yield Alkaline Protease Strains

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Abstract:

Alkaline protease is a hydrolytic enzyme capable to degrade proteins into small peptides and amino acids, as a biocatalyst, it is applied in various fields such as detergent and leather industry. Here we report the different sources and pH values of alkaline protease and the bacterial species producing alkaline protease, discuss UV and ARTP and compare these two mutagenesis methods, and show that ARTP causes more DNA damnification than other mutagenesis methods and has the highest mutation rate caused by ARTP. In order to improve the protease production of Bacillus strains, the previous study has proposed some new and optimized mutagenesis methods. In addition, this paper demonstrates and highlights the considerable industrial potential of alkaline protease in detergents, leather processing, silver recycling, medical use, food processing, feed, chemical industry, and waste treatment, in which detergent and leather industry are more used.

Keywords: UV; ARTP; alkaline protease.

1. Introduction

Proteolytic enzymes can be seen everywhere in various organisms. Proteases are essential in physiological processes such as cell growth, metabolism, and differentiation. Alkaline proteases are one of the hydrolases capable of grading proteins into small peptides and amino acids produced by microorganisms, especially Bacillus sp. Alkaline protease is widespread availability in food, detergent, leather ,medical and so on. In the detergent industry, the demand for enzymes is still increasing [1,2]. Due to the rapid growth in demand of these enzymes in different industries, manufacturers have been looking for some improved techniques to enhance the yield, vitality and stability of proteases. Mutagenesis is usually induced by physical and chemical factors. Physical and chemical mutagens are the main means to obtain high-yielding and excellent bacteria strains. Compared with DNA recombinant technology, physical mutagens such as UV and chemical mutagens have overwhelming advantages because of their low procedure cost. (ARTP) are fresh and promising physical mutagenesis techniques that use plasma jets to cause mutations in a variety of microorganisms. Plasma jets contain a variety of reactive substances

that can cause DNA damage and induce microbial mutation [3,4]. ARTP can operate at common atmosphere without requiring extreme environments, thus reducing the cost of the equipment. ARTP induced mutations are of high frequency, which increases the possibility of obtaining ideal mutants. Overall, ARTP is a potentially valuable tool for microbiological technology study. The main intent of this research is to discuss how to obtain Bacillus strains with high yield proteases, compare different mutagenesis methods and propose the optimization of mutagenesis strategies, demonstrating its application in the detergent and leather industry. The main contents of this article include: (i) selection of strains; (ii) comparison of UV and ARTP; (iii) optimization of mutagenesis strategies; (iv) evaluation of industrial applications.

2. Selection of Strains

Proteases can decompose macromolecular proteins into some amino acids and small polypeptides. Different microbiomes will produce proteases of different structure and function and can be classified as serine proteases, half-serine proteases, acid proteases, and metalloproteases. In general, serine proteases are alkaline and have optimal activity at pH 7.0 – 11.0. Because serine proteases are in high demand in industry, the focus in this review is on serine proteases. Although several microorganisms are known to produce proteases, Bacillus strains are often the primary preferred source of commercial alkaline proteases [5,6] due to their specialized ability to secrete a large number of highly active enzymes. Bacillus licheniformis, Bacillus subtilis, and B. pupais are the most commonly used species in the industrial production of alkaline protease. To date, The active producers of filament amino acid proteases are bacteria and fungi, and many of them are well known. The Bacillus licheniformis NMS from soil produced by hot springs, Bacillus alcalophilus LW8 from high salt lake water. Sspore ogenic mutant strains of Bacillus are also used in industry, where extracellular proteases are produced for a longer time because the final product does not switch to sporulation. By using the alkaline protease-positive sporogenic mutant [7], a five-fold increase in enzyme production was observed. Other microorganisms that produce serine proteases were found to be wastewater from man-made alkaline environments, such as food, textiles, tanneries, potato processing units, paper-making units, calcium carbonate kilns, detergents, and other industrial processes with pH values usually around 10 and above. Additional studies reported a new Bacillus isolate CEMB 10370 . CEMB 10370 Can produce serine protease, which exhibits optimal activity at 50°C, and the soil samples are derived from diverse habitats, including garden soil, tannery, soap factory, etc [8]. This study aimed to investigate the comparison of alkaline proteases produced by Bacillus under different mutagenesis.

3. Comparison of UV and ARTP

Therefore, so far, people use some chemical substances (such as alkylating agent, azide, etc.) and physical γ -ray, x-ray, ultraviolet light, particle radiation and other substances as a mutagen to improve the mutation rate and screening beneficial varieties, the physical mutagen has long been playing an important role in the modification of industrial strains. UV radiation is the most commonly used physical mutagenic agent because this method is easier to operate and safer. Mutants that produce more proteases than their parent strain can be obtained by UV irradiation. Mutagenesis using different exposure times, 10 to 30 cm away from UV lamps (usually 30w bactericidal lamps, 2540-2550A), and portions of the appropriately diluted bacterial suspension strains were dispersed on lb plates and incubated at 37 C for the appropriate time. Colonies were formed after culture and further studied, the usual flow of UV mutagenesis. As a new method of physical mutagenesis, ARTP has some unique, ARTP is a kind of new developed based on whole-cell mutagenesis tools, physical plasma is composed of neutral particles and charged particles completely or partially ionized gas, they are usually divided into high temperature and low temperature plasma, working gas (such as helium, argon, nitrogen, oxygen), etc. Based on mutagenesis breeding, this part compares UV mutagenesis and ARTP mutagenesis by the degree of DNA damnification,rate of mutation and enzyme activity.

A previous study by Yuting Huang used a rapid and simple umu-microplate test to analyze and the condition of DNA damage was compared between the two mutagenesis methods , and the Yuting Huang study selected strain Typhimurium TA1535 / pSK1002 for the experiment [9]. Among the obtained data (Table 1), the maximum IR value in the group of UV was 8.4, and the maximum IR value can reach 11.42. The higher IR value indicates the higher degree of DNA damage to living bacterial cells. Table 1 shows that mutations ARTP result in more DNA damnification for detecting microorganisms compared to other conventional mutagenesis methods. The study also determined the net increment of biomass per cell (D (E / Biomass)) after the processing of mutation with diverse mutagenesis ways, which is a symbol of the DNA damnification condition and repair ability of diverse mutagenesis ways.

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Table 1. To Compare the genotoxicity of strain S . typhimurium treated with diverse mutagenesis methods[9]

	Dose	G	IR
4-NQO/mg•L^{-1}	0.05	0.87	1.80
	0.10	0.78	2.05
	0.15	0.66	2.45
	0.20	0.48	2.62
DES/mg•L^{-1}	1	0.14	1.47
	5	0.06	-
	10	-	-
	20	-	-
UV/s	1	0.88	1.68
	2	0.78	1.61
	4	0.66	2.67
	6	0.57	3.83
	8	0.53	5.20

ARTP treatment produced the highest D value, far beyond the treatment of the UV . It is suggests that ARTP causes more DNA damnification than other ways. In addition, the mutation rate treated with diverse mutagenesis (ARTP, UV mutation) was researched in another Yuting Huang study [9] (Figure 1). It has been shown that the highest mutation rate is caused by ARTP among all the mutagenesis ways researched. The mutagenesis of ARTP is far greater than DNA damage by other mutagenic methods in single living cells.

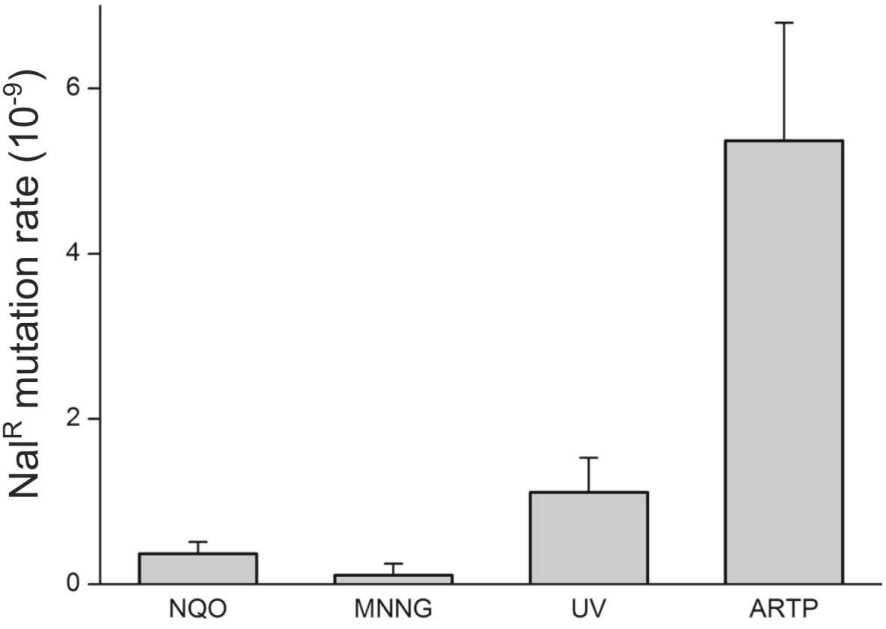

Fig. 1 Mutation rates of bacterial cells treated with the different methods of mutagenesis [10].

It is clear that ARTP has its own unique dna-damage mechanism, and the ARTP discharge spectrum when using helium as the initial gas reveals that plasma jets are composed of some active particles, which are characterized by high concentration and high uniformity, this may be important for mutagenesis. When ARTP mutagenesis was performed, either DNA or nucleotide breaks were found. ARTP can evoke some several complex DNA damages and lead to mutations in diverse ways, clarifying that it has a higher mutation rate relative to other mutational approaches. The comparison between UV and ARTP found that ARTP has the advantages of higher mutation rate, better genetic stability, and no pollution of natural [10]. ARTP mutagenesis can cause sublethal damage to living

organisms, including DNA damnification and protein denaturation and so on [11], which may be the essential reason for the significant increase of alkaline amylase expression level in B. subtilis under ARTP mutagenesis.

4. Optimization of the Mutagenesis Strategy

By improving the mutagenesis method, the mutagenesis efficiency can be improved to obtain the mutant strains with higher protease production than the original mutant strain. Several improved mutagenesis methods will be described below.

4.1 Optimization of UV Mutagenesis

In UV mutagenesis, dishes with viability between 0.1 and 10% were generally selected for isolation, Chibani Hiba Rahman performed mutagenesis by exposing the strain to UV radiation (from 0 to 21min, at 3min intervals,10cm) [12]. After 3 min of UV treatment, the survival rate decreased sharply to 15.07%, respectively. After 20 minutes of UV treatment, Bacillus survival rate reached 0.001%, which may be due to the distance from the ultraviolet lamp, adjusting the distance in UV mutagenesis for different purposes. Bacillus underwent mutagenesis and selected mutant strains using different exposure time and radiation intensity when using UV mutagenesis. Elif Demirkan The study exposed of Bacillus E6-5 at different distances (5 to 15 cm at 5 cm intervals) at 120 min(from the 1 min). A 254 nm 30w bactericidal lamp provided UV irradiation [13].

By treatment with UV irradiation , a small number of mutants were obtained after 5 min at a distance of 15cm. One mutant had the largest diameter of the protease region in the mutant obtained that showed a 1.2-fold increase in protease production in B. subtilis compared to the parental type. There is potential value to investigate whether subdividing the distance and time interval of strain exposure according to the study will yield mutant strains with higher yield.

4.2 Combined Mutagenesis

Neha Thakur performed combined mutagenesis with UV as a physical mutagenic, EMS and MMS as a chemical mutagenesis [14]. Under UV irradiation alone, the protease activity of the mutant was increased to 2599±2.6U/ml. The optimized mutants were further treated with different concentrations of chemical mutation to further increase the yield to 3209 U / ml. The enzyme activity of the mutant strain was 2.24 times higher than that of the wild strain.

4.3 „Segmented“ UV Mutagenesis

In conventional UV mutagenesis, weak UV penetration, and insufficient duration of UV irradiation and incorrect dosage. Mutated strains were obtained by ‘segmental’ UV mutagenesis. L. R. Valiullin’s team used a segmented UV mutating the strain, irradiated the culture suspension repeatedly with UV and incubated for in the dark. In that study, Bacillus was alternately irradiated for 30 seconds, the microorganisms were incubated in the dark for 30 min and the new mutant strains of B. subtilis were obtained [15]. These bacteria produced better amounts of hydrolysing enzymes than B. subtilis produced by common UV mutagenesis.

4.4 Optimization of ARTP Mutagenesis

ILiu’s study set up six different time groups at 10s intervals and treated at 100W and a specific gas volume [16]. Surviability significantly decreased with increasing mutagenesis time. Positive mutagenesis rate was highest at 50s and higher than negative mutation rate.The study selected 50s as the best mutation parameter. By screening, a high-yielding strain A59 was obtained. The enzymatic activity of strain A59 was up to 8433 U / mL, an increase of 23.38%. According to this experiment, considering the gradient of treatment time and selecting the better mutation parameters in ARTP mutagenesis obtained the higher yielding strains of alkaline protease mutant.

5. Conclusion

In this study, comparing UV and ARTP, after discussing the sources of alkaline proteases, showed that ARTP causes stronger DNA damage and causes the highest mutation rate in ARTP in UV and ARTP. In mutagenesis, selecting the appropriate exposure time and mutagenic distance can obtain mutant strains with high yield, so it is of some research value to subdivide the distance and time interval of strain exposure according to studies to obtain higher yield. In ARTP, the gradient of treatment time was reduced and the better mutation parameters were selected, resulting in more productive alkaline protease mutant strains. This study lists some applications of alkaline protease in industry, showing the important role of alkaline protease. In the field of medical science, alkaline protease medical materials, drug formulation, anti-cancer drugs have broad application prospects.

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