Research Paper

Volume : 5 | Issue : 1 | JANUARY 2016 • ISSN No 2277 - 8179

Studies on bacteriorhodopsin synthesis in UV induced Mutant Halobacterium salinarium with different time of UV exposure

Microbiology

KEYWORDS : H. Salinarium, Bacteriorhodopsin, Halorhodopsin (HR), Sensory Rhodopsins (SR I& II) Halorhodopsin transducer protein (Htr I & II), UV iduced mutant strain.

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ABSTRACT

Experiments on UV irradiation on Halobacterium salinarium was performed under 270 wave length UV source at 6.5 cm distance with different time of exposure from 10 seconds to 8 minutes for bacteriorhodopsin pigment synthesis and phototatic response. BR pigmented phototactic resistant H. salinarium colonies were isolated from 10 seconds to 50 seconds UV exposed culture. White colour, non phototactic thread like mutant colonies were isolated from 1 minute to 6 minutes UV exposed colonies. The results show that the UV radiation directly affects the flagellar movement and BR expression, also quantitative analysis of BR synthesis was done in the cultures. The obtained results which is strongly stated that the UV light affects the BR expression depends on time of exposure and time of intervals.

INTRODUCTION

Halobacteria are the major group of archea and it is absolute dependence on a high concentration of $NaCl_2$ and usually have a growth optimum about 2- 4 molar $NaCl_2$ (17 – 23%). This prokaryote traps light energy photosynthetically without presence of chlorophyll and exposed to low concentration it synthesises a modified cell membrane called purple membrane, which contains Bacteriorhodopsin (BR)[1]. The BR is a hydrophobic integral membrane protein is a two dimensional crystalline lattice and 25 kilo Dalton molecular weight and contains 7 closely packed α helices crossing the cytoplasmic membrane. The BR is directly convert sunlight into chemical energy, a retinal based protein plays key role function of this protein[2]. The BR has photoelectric properties and high thermal stability which is useful for optical memory and artificial retina photon switch[3]. Light induced sensory response are among the oldest scientific observations on the bacterial behaviour, the mechanism is involved in the response to light are probably the best understood in *H. Salinarium* which combines the mechanisms used for proton translocation of bacteriorhodopsin and aerotactic response. The aerotactic response of *H. Salinarium* is methylation dependent and inhibition of methylating donors resulted defective aerotoxis[4].

Giovanai *et al* explained the proton transfer mechanisms of bacteriorhodopsin with single mutation or double mutation processes like UV and Gamma irradiation[5] and genome of *Halobacteria* NRC-1 were sequenced by Baliga *et al*, proved the gene regulatory control of energy transduction under aerobic conditions and mechanisms for a stress response to extraordinary level of UV radiation[6]. A tremendous advantage of bacteriorhodopsin's organic nature is useful to genetic engineering[7]. The aim of the present study is the role of bacteriorhodopsin in the UV induced mutant of *H. Salinarium* and study the level of the pigment production at different time intervals and check the plasmid DNA presence in the UV induced strains.

MATERIALS AND METHODS

Collection of *H. salinarium* strain

H. salinarium (MTCC 1598) was obtained from Institute of Microbial Technology (IMTECH) at Chandigarh, India. The viability of *H. salinarium* was maintained by making regular subculture in *Hallobacterium* defined medium. The medium is composed of Sodium chloride- 250gm, Magnesium sulphate- 20gm, Potassium chloride- 0.2gm, Peptone- 3gm, Citrate- 5gm, Casein acid hydrolysate- 10gm in 1.0 litre of distilled water and also the same medium was used for isolation of the mutant *H. salinarium* colonies.

UV irradiation

5.0 ml of *H. salinarium* culture was taken in different sterilized petriplates and irradiated under Ultraviolet (UV) light source (270 wavelengths) at 6.5 cm distance from the light source with different time intervals from 10 seconds to 8.0 minutes. 10 seconds time interval was given up to 60 seconds time of exposure and 1.0 minute time interval was given from 1.0 minute to 8.0 minutes time of exposure. 0.1ml of was taken from all UV irradiated *H. salinarium* culture and inoculated on the appropriately labelled defined agar medium plates and 50ml broths. All the broths and plates were incubated at 37˚C for 1 week. After incubation, resistant *H. salinarium* bacterial colonies were observed with and without rhodopsin pigment production both in agar and broths medium. The amount of rhodopsin pigment production was measured as Optical Density (OD) values under UV-Spectrophotometer at 590nm and the optical density values are noted and given in the table.

Genomic DNA isolation

Total genomic DNA was extracted by standard method Smoker and Barnum[8]. *H. salinarium* cell pellet was collected from 5.0ml of UV exposed culture broth by centrifugation at 6000 rpm for 10 minutes. The cell pellet was suspended with 500µl of lysis solution (50mM Tris HCl pH 8.0, 5mM EDTA pH 8.0 and 50mM NaCl). Lysozyme was added to obtain a final concentration of 1.0mg/ml and the suspensions was incubated at 55˚C for 30 minutes. 10µl of proteinase K (1mg/ml) was added in the incubated solution and 20µl of 10%SDS were added and again incubated at 55ºC for 10 minutes or until the solution cleared. The lyzed bacterial solutions was chilled on ice and extracted with equal volume of Phenol: Chloroform:Isoamyalcohol (25:24:1), thoroughly mixed this solution and supernatant was collected. Equal volume of 4M ammonium acetate (pH-5.5) was added with the supernatant and double volume of isopropanol was added with this mixer. Total genomic DNA was precipitated by centrifugation at 12, 000 rpm for 10 minutes at -4ºC. The DNA pellet was allowed to air dry and the dried pellet was dissolved in 30µl of TE buffer (10mM Tris-HCl pH8.0 and 0.1mM EDTA pH8.0) and stored the pellet at -20ºC. Like this the remaining different times of UV exposed and non-exposed *H. salinarium* culture's genomic DNA were extracted and stored. 3.0 µl of gel loading buffer was added in that DNA solution and mixed thoroughly. 15µl of DNA solution was taken and added in appropriately labelled well of 1.2% agarose gel, 100 voltages electrical

charge were given to the gel and run the gel for 2 hours. After that, the gel was stained with Methylene Blue stain solution for 30 minutes, the excess stain were removed from the gel by using distilled water and then, the presence of plasmid DNA were checked in UV exposed *H. salinarium* as a deep blue colour band in the agarose gel.

RESULT

Isolation of UV resistant *H. salinarium* colonies

Phototactic pink coloured UV resistant *H. Salinarium* colonies are appeared on defined agar plate medium those are UV irradiated from 10 seconds to 30 seconds with 10 seconds intervals. Very mild phototactic pale pink coloured resistant bacteria colonies are appeared; those were UV irradiated from 40 – 50 seconds with 10 seconds intervals. White colour, non aerotactic resistant *H. Salinarium* colonies are appeared in the agar medium those were irradiated from 1 minute to 6 minutes with 1minute intervals. No resistant colonies were observed at 7 and 8 minutes UV exposed bacterial cultures. UV resistant pink coloured and white coloured *H. salinarium* bacteria pictures are given in the Table 1.

Table 1

OPTICAL DENSITY VALUE OF BACTERIORHODOPSIN PRODUCTION IN UV IRRADIATED *H. SALINARIUM* WITH DIFFERENT TIME OF EXPOSURE

S.No.	UV Exposure time	OD value at 590nm
1.	Control	0.076
2.	10 seconds	0.054
3.	20 seconds	0.050
4.	30 seconds	0. 046
5.	40 seconds	0.039
6.	50 seconds	0.026
7.	1 minutes	0.022
8.	2 minutes	0.017
9.	3 minutes	0.012
10.	4 minutes	0.008
11.	5 minutes	0.005
12.	6 minutes	0.004

Level of rhodopsin pigment productions

Non UV irradiated *H. salinarium* colonies are produced more pink colour and OD value of the pink colour production is 0.076 at 590nm wavelength light intensity. But in the UV irradiated *H. salinarium* colonies bacteriorhodopsin production levels are reduced depends on time of exposure. For example, 10 seconds exposed culture produced 0.054 OD value levels of bacteriorhodopsin and remaining exposed cultures bacteriorhodopsin production levels are reduced level when compared with balk culture and 10 seconds exposed culture (Table-1). No bacteriorhodopsin production was observed from 1 minute to 6 minutes exposed cultures and the culture's OD values are also reduced levels when compared with blank culture. The graph 1 shows the level of bacteriorhodopsin pigment in all the UV irradiated culture.

Graph 1

LEVEL OF BACTERIORHODOPSIN IN OD VALUES IN UV IRRADIATED *H. SALINARIUM* CULTURE

Isolation of Plasmid DNA

Bacterial chromosomal DNA were only observed from all the UV resistant bacterial cultures but no plasmid DNA even the colonies which is exposed under UV irradiation up to 6.0 minutes.

DISCUSSION

Biological systems have evolved mechanisms to appropriately respond to environmental stress that can damage proteins and DNA[9]. *Halobacterium* species produce a specialized region in the cell membrane, named purple membrane, which consists of a two dimensional crystalline lattice of a single chromoprotein, bacteriorhodopsin (BR). BR contains a 1:1 complex between a protein component bacterio-opsin & a chromophore retinal and carries out light driven proton pumping across the membrane[10,11]. The mechanism of proton translocation across the membrane by BR has been the subject of extensive study by biophysical and mutational approaches[12,13]. Bacteriorhodopsin is closely resembles to sensory pigment rhodopsin from the rods and cones of vertebrate eyes. Bacteriorhodopsin's chromophore is carotenoid derivative retinal that means aldehyde of vitamin A. Recently, retinal proteins similar BR have been discovered in some fungi and uncultivated marine planktonic bacteria, indicating a much wider distribution in nature than originally appreciated[14, 15].

H. salinarium normally depend respiration for their energy production. However, under low oxygen and high light intensity condition, the bacteria synthesize this BR pigment in late exponential to stationary phase. The pigment functions as an efficient light driven proton pump and absorb a photon by inside of the cell to outside and the pigment undergoes several conformational changes during photo cycle that can be used for the synthesis of ATP by chemo-osmatic mechanisms[16, 17].

In the presented study, UV resistant *H. salinarium* colonies were isolated with and without rhodopsin pigment expression at different time of UV exposure from 10 seconds to 6.0 minutes with 10 seconds and 1 minute intervals. Rhodopsin pigmented pink coloured UV tolerant bacterial colonies are isolated which is exposed up to 30 seconds and they have shown good phototactic function. Rhodopsin deficient and slow phototactic bacterial colonies were isolated from 40 seconds to 50 seconds UV light exposed cultures. Instead of pink colour colonies, white coloured colonies have appeared which is irradiated from 1 minute to 6 minutes under UV light. But no mutant colonies were isolated from 7.0 and 8.0 minutes UV exposed cultures. Like this, Spudich & Spudich has been characterized the rhodopsin deficient strain *Halobacterium* NRC1, pho81[18] and in 2004 Baliga *et.al.,* reported a systems-level study on the behaviour of *Halobacterium* NRC1 upon UV-C irradiation, two types of mutagenic lesions in DNA were observed in that bacterium and also white colourless mutant of *Halobacterium* NRC1 isolated through EMS mutagenesis[19].

The bacterium *H. salinarium* induces bacteriorhodopsin when grows in saturated salt concentration and oxygen level is low, as well as it induces three other retinal based light absorbing pigments; halorhodopsin (HR) , two Sensory Rhodopsin that is SR I and SR II. The SR-II is a constitution of retinal protein that absorbs blue light and undergoes a fast photo cycle and generating a signal to control the bacterial flagellar bundle rotation and SR I acts to produce positive signal in orange light and negative repellent signal in blue light[20, 21]. If blue (UV light) light is present the photopigment BR undergoes a fast transformation and is ready for go back to orange light absorbing form. But if blue light (UV light) is not present the photopigment undergoes slow transformation. In the present study, the *H. salinarium* exposed to prolonged UV irradiation from 1 minute to 6 minutes, the SR II proteins send signal to control the flagellar activity. Armitage J P stated that absorption of UV light in appropriate wavelength leads to an electron redistribution and a configurational chang-

Volume : 5 | Issue : 1 | JANUARY 2016 • ISSN No 2277 - 8179

es would occur in BR protein[4]. In this study, the UV light may change the configuration of BR protein so white colour mutant *H. salinarium* colonies were isolated even supply with appropriate saturated salt concentration and low oxygen levels in the culture medium and the current work have proved the Armitage J P statement.

Each SR protein (SR I & SR II) has an accompanying sensory protein, Htr I & Htr II, respectively. These proteins have cytoplasmic domains, homologous to those of the higly conserved domains of methylaccepting chemotaxis proteins (MCPs) BR, HR & SR I & II[22, 23] and if both the Htr I & II are deleted from *Halobacterium* the SR proteins able to pump protons just like the Schiff base driven bacteriorhodopsin (BR), but the presence of Htr prevents the proton pumping[24]. The UV light may redistribute the electrons via SR proteins; prevent the proton pumping in BR without the deletion of Htr proteins and also the SR II control the flagellar movement, so white colour thread like *H. salinarium* have isolated in the present study.

CONCLUSION

The results of present study indicates that the phototactic response of the bacteria have altered depends on the exposure of UV light intensity and also the OD values for quantitative analysis of BR have strongly proved that the UV light affects the BR expression depends on time of exposure and time of intervals. No plasmid was observed in all the UV mutant white colour cultures of *H. salinarium* cultures. The present investigation have suggested that further attention and research should be required to find out the clear studies about the bacteria tolerate in the UV light without damaging of cell activities and identify the full mechanisms of sensory rhodopsin, Htr pigments during electron transfer cycle in the cell membrane.

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