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Influence of UV and Gamma Rays Irradiation on Luminescent Bacteria and Exploring Their Efficacy as Biosensor

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Abstract: Toxicity monitoring of harmful radiations is an indispensable issue of modern radioecology. As bioluminescent bacteria have the simplest structure to epitomize the biosphere also their bioluminescence can act as an indicator of the condition, hence assay systems based on luminous bacteria can be used as sensors for monitoring the environmental radiotoxicity. The present investigation explores the measurement of bacterial luminescence which can be easily computed. Further, the bioluminescent bacterial strains were used to evaluate the impact of UV and Gamma irradiations. Random Amplified Polymorphic DNA(RAPD) was carried out to observe alterations underexposure. Further by using a phylogenetic tree, a comparative study of the effect of UV and Gamma rays has been carried out. It has been revealed that the isolated strains have shown remarkable sensitivity towards radiation exposure present in the environment and therefore they could be used as the potential biosensing elements for developing an on-site pollution monitoring biosensor.

Keywords: Bioluminescence; UV irradiation; Gamma irradiation; RAPD; Biosensor.

1. Introduction:

In recent times, radiation is a phenomenon prevailing in our daily lives, deriving from natural and artificial sources. Living organisms are profoundly affected by using radiation-provoked cell damage, threatening healthy and diseased tissues alike. The excessive rise in radiation pollution is also an imperative concern of current ecology. Assessing the toxicity of radiation to marine organisms under low dose irradiation conditions and understanding toxic impact mechanisms is a challenge for scientists working in related fields [1, 2]. The constant increase in the number and range of manmade sources of electromagnetic radiation creates a high level of interest in studying their effects on living organisms [3]. Also in today's world, wireless technologies are universal. Next-generation services are proceeding to drive the work out of both new substances as well as amendment of previous locales. Yet anthropogenic radiofrequency, intense radiations from mobile towers and microwave electromagnetic fields are one of the most widely proliferating environmental issues of concern. Hence their effects on biological processes particularly on human health are now an alarming issue for the whole world [4]. This is also of key significance that such radiation pollutants should be detected and controlled in natural atmospheres because control and preservation of quality and wellbeing from environmental emissions have also been a significant element in maintaining public health and well-being [5, 6]. The identification and tracking of such organic pollutants in environmental matrices is also of considerable importance and a requirement for remediation

and safety risk assessment.

Microorganisms are the easiest and most important component of aquatic habitats, and their physiological indices may act as an indicator of the overall state of habitats. Bacterial illumination is not only an appealing natural phenomenon but also it is a bacterial physiological feature that is significant in ecological investigations. Considering the present investigation, marine luminous bacteria are found to be the most potential candidates. Bioluminescence is the process in which natural chemical reaction emits visible light. In recent years, luminous marine bacteria have been used as bioassays to control water toxicity [7]. Advances in molecular biology have improved the rapid speed and high detectability of bioluminescence spectroscopy. Bioluminescent bioreporters in particular have been well recognized in the laboratory and can be commonly used in environmental monitoring due to their ability to produce an easily measurable light signal [6]. The principal physiological/key parameter is the luminescence intensity could be easily measured through the interventions of the luminometer. Such properties allow quantitative monitoring of biological processes in applications of medical, diagnostic and drug discovery. Due to these unique properties, bioluminescence can be used as an ultrasensitive and selective bioanalytical device. [8, 3]. The Bioluminescent assays in particular, due to their ability to generate an easily measurable light signal, have been well validated in the laboratory and extensively applied in environmental monitoring. Their advantages imply are speed, sensitivity, simplicity, and availability of the devices [4].

For the molecular typing of bacterial species, there are some methods of polymerase chain reaction (PCR)-based DNA fingerprinting. Randomly amplified polymorphic DNA (RAPD) is a PCR-based technique where changes in the DNA sequence at sites in the genome are detected with the help of arbitrary primers [8,9]. Against this background, the present study aims to enumerate the effects of UV and Gamma exposure on isolated strains of bioluminescent bacteria using the RAPD-PCR technique and its phylogenetic relationship tree studies, which could help to develop a biosensor for perceiving the intensity of these radiations existing in the environment.

2. Material and Methods:

2.1. Sampling and isolation of Luminescent Bacterial Strains

To achieve the objectives of this investigation, samples were collected from marine water sources of Tarkarli beach, Sindhudurg, Maharashtra and Baga beach of Goa, India (Fig.1) using sterile autoclaved dilution bottles. Water sampling was performed in November 2015-

January 2016. Around 250-400µL of water, the sample was spread plated on modified nutrient agar plates (Nutrient agar plus 25% sea salt). Colonies displaying luminescence were observed on the plate after 24 hours of incubation at 22^0C in the darkroom. Highly luminescent colonies were chosen further to obtain a pure culture. Such distinct isolates have been analyzed for their morphological and biochemical characterization with the help of standard Bergey's Manual of Systematic Bacteriology [10].

Figure 1: Sampling Locations of Tarkarli beach, Sindhudurg district and Baga beach, Goa, India for isolation of Luminescent Bacterial strains

2.2. Molecular characterization of luminescent bacteria

Bacterial genomic DNA isolation was performed using Axyprep Bacterial Genomic DNA miniprep kit (Genaxy) which was confirmed by running gel electrophoresis of 0.8 % agarose gel. DNA quantification was done by NanoDrop 1000 Spectrophotometer (ThermoScientific). With the help of universal primers 8F: 5‟AGA GTT TGA TCC TGG CTC AG 3‟ and 1492R: 5‟ ACG GCT ACC TTG TTA CGA CTT 3‟ fragment of 16S rDNA gene was amplified. For bacterial 16S rDNA amplification parameters - an initial denaturation of 94°C for 3 min., 30 cycles of (94°C 30 sec., 52.7°C 30 sec., and 72°C 1.30 min), final Extension of 72°C for 5min. *In silico* analysis of 16S rDNA sequences of isolates under present investigation was performed using BLASTN search algorithm and identified for genus and species. Most accurate sequences were further aligned to obtain the phylogram using the MEGA5 software.

2.3. Immobilization of luminescent isolates

Assembly of bioluminescent beads was achieved by dissolving bacterial pellet of overnight grown luminescent culture about 10^7 cells per ml 2% of sodium alginate. The bacterial culture-alginate mixture was dropped in a dropwise manner into 1000 ml 0.15M, 0.1M, 0.05M Calcium Chloride, a crosslinking solution. Bead formation was achieved at room temperature as soon as the sodium alginate drops come in direct contact with the calcium chloride solution. The hardening of beads was achieved in 1-2 hours. The beads were washed with a fresh 0.1M crosslinking solution as well as stored in the same at 4^0C.

2.4. Exposure to Ultraviolet(UV) and Gamma radiation and their Luminometric assay

The beads of bioluminescent bacteria were subjected to exposure to Ultraviolet (UV) rays for up to six hours and Gamma rays for up to half an hour. Before exposure beads were activated for 30 min in modified nutrient broth. To estimate the effects of UV irradiation on bioluminescence, the beads of bioluminescent bacteria were then divided into 7 sets for treatment and l set as control and then subjected to exposure UV irradiation (302-365nm). One set was withdrawn at 30 min after irradiation up to 6 h and luminescence was measured on Glomax® Luminometer (Promega) to measure the luminosity as relative luminescence unit.

To estimate the effects of Gamma rays on bioluminescence, ^{60}Co source was used for Gamma irradiation. The beads of bioluminescent bacteria were then divided into 7 sets for treatment and l set as control and then subjected to exposure of Gamma ray's irradiation in the

experimental chamber at dose rates of 0.158kGay/min. One set was withdrawn at 5 min interval after irradiation up to 30 min and luminescence was measured on Glomax® Luminometer (Promega) to measure the luminosity as relative luminescence unit.

For the development of prospective biosensor, isolated strains were analyzed by comparing different parameters before and after exposure to various radiations. Hence Morphological analysis (SEM) was also performed again which exhibited no change in morphology. In the case of Biochemical tests, the same results were observed that is there was no change before and after radiation exposure. Further, Random amplified polymorphic DNA (RAPD) was carried out for observing probable genetic characterization changes in the consortium.

2.5 Random amplified polymorphic DNA (RAPD)

Bacterial genomic DNA isolation of UV and Gamma exposed sets of bio beads was performed using Axyprep Bacterial Genomic DNA miniprep kit (Genaxy). Further, different random RAPD primers were used to check the effects of UV and gamma irradiation exposed bacterial samples against unexposed bacterial samples used as Control. Each 10µl of reaction mix contained:

The following protocol was used to prepare the reaction mixture (Table 1).

Table 1: Composition of Reaction Mixture

Component	**Volume**	**Final Concentration**
Genomic DNA	3.0µl	15ng
Buffer	1.0µl	1X
dNTPs	1.0µl	0.2mM
Primer	0.6µl	0.6µM
Taq DNA pol	0.2µl	1 unit
$MgCl_2$	1.0µl	2.5mM
Water		to 10µl

To prepare mastermix (for all samples + control), all the above components except DNA were added.

- All components completely thawed and vortexed the $MgCl_2$ vigorously.
- The mastermix was vortexed to mix all components before aliquoting.

Aliquots were placed into PCR tubes and added the template DNA. Mixed well. The PCR plate carrying the reaction tubes in the sample block of the thermocycler was placed. An initial denaturation step at 94°C for 4min followed by 40 cycles with the following cycle parameters was carried out:

- Step 1 94°C for 1min
- Step 2 35°C for 1min
- Step 3 72°C for 2min. The 72°C step of the final cycle by 5min was extended.

When the amplification had finished, the loading dye was added as 3µl of each sample. A 1.2% agarose gel in 0.5X TBE buffer containing ethidium bromide (5µg/ml of gel) was prepared. The DNA length marker and the samples were loaded. The gel was kept for running in 0.5X TBE buffer at 55V for 4 h. The gel was visualized on a UV transilluminator.

3.Results and Discussion

3.1 Isolation of Luminescent Bacterial Strains

From the water samples collected more than 300 colonies were observed on the modified Nutrient Agar spread plate after 24 h of incubation out of which only prominently glowing colonies of luminescent bacterial were purified which were further subjected for morphological and biochemical analysis.

3.2 **Identification of isolated Luminescent Bacterial Strains**

The bacterial genomic DNA isolation was performed and it's PCR amplicon was electrophoreses on 1.2% Agarose Gel, as single band 1500 bp DNA has been observed when compared with 1 KB molecular marker. The consensus sequence of the 1500 bp rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the non-redundant NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using the RDP database and the phylogenetic tree was constructed using MEGA 4. This study revealed that the pure culture strains were of *Vibrio natriegens* and *Vibrio harveyi* isolated from seawater samples of Tarkarli beach, Sindhudurg, Maharashtra and Baga beach of Goa, India (Asia) respectively. 16S rRNA gene sequence of *Vibrio harveyi* and *Vibrio natriegens* have been deposited in the GenBank database with accession number KY581195 and MG386478. Further study was

carried out using these luminescent bacterial strains and the bacterial consortium of *Vibrio harveyi* with *Vibrio natriegens.*

3.2 Immobilization of luminescent isolates

Successful immobilization was achieved with 2 % sodium alginate & 0.1M Calcium Chloride as shown in Fig. no. 2. Beads were washed with a fresh 0.1M crosslinking solution as well as stored in the same at 4^0C. Bioluminescent beads were subjected further against various UV and Gamma irradiations.

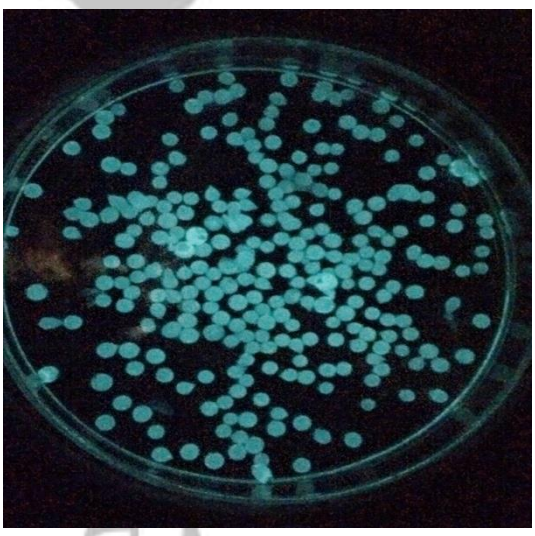

Figure 2. Biobeads of Consortium showing luminescence when observed in a dark room.

3.3 Exposure to Ultraviolet(UV) and Gamma radiation and their Luminometeric assay

Beads of bioluminescent bacteria were subjected to exposure of UV irradiation and Gamma irradiation and their luminescence were measured on GloMax® Luminometer (Promega). Figures 3 and 4 depict a gradual decrease in the luminescence of beads were observed with increasing radiation exposure time. Luminescent beads of *Vibrio harveyi* and *Vibrio natriegens* as well as their consortium showed prominent changes in luminescence when treated with UV and Gamma exposures.

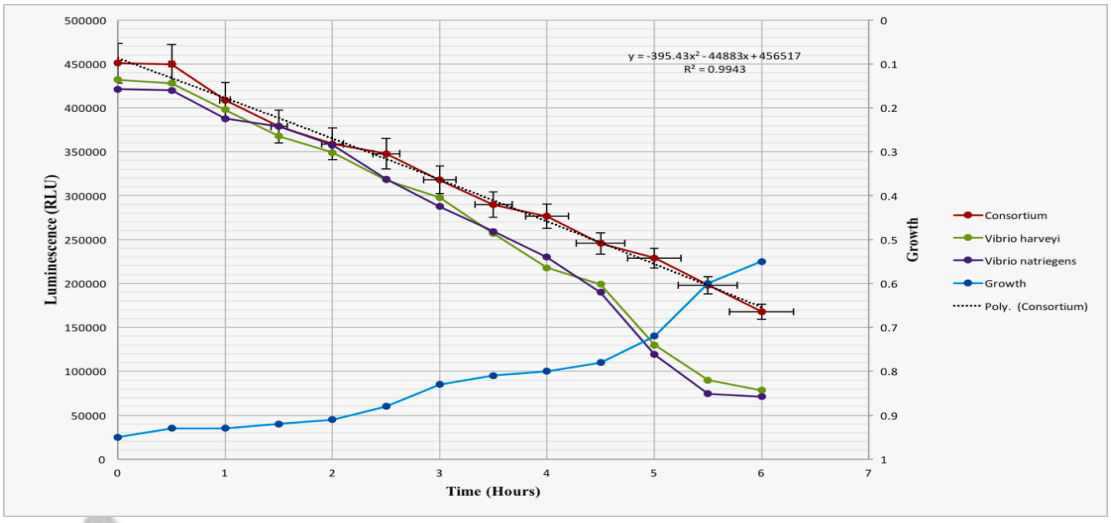

Figure 3: UV irradiation sensitivity profile of isolated *Vibrio harveyi* and *Vibrio natriegens* and their consortium against time

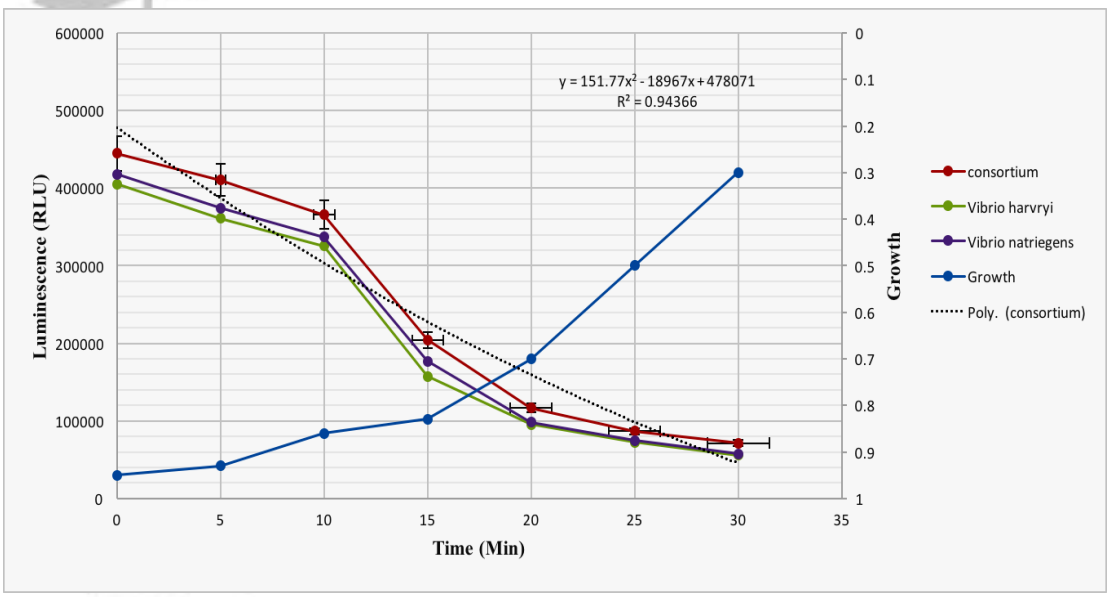

Figure 4: Gamma irradiation sensitivity profile of isolated *Vibrio harveyi* and *Vibrio natriegens* and their consortium against time.

3.4 Random amplified polymorphic DNA (RAPD)

RAPD profile was obtained by using different random primers (OPB07, OPB10, OPB17, OPL03, OPL05, OPL11, OPL12, OPL13, OPN08 & OPE09) after UV and Gamma exposures as shown in figure no. 5.

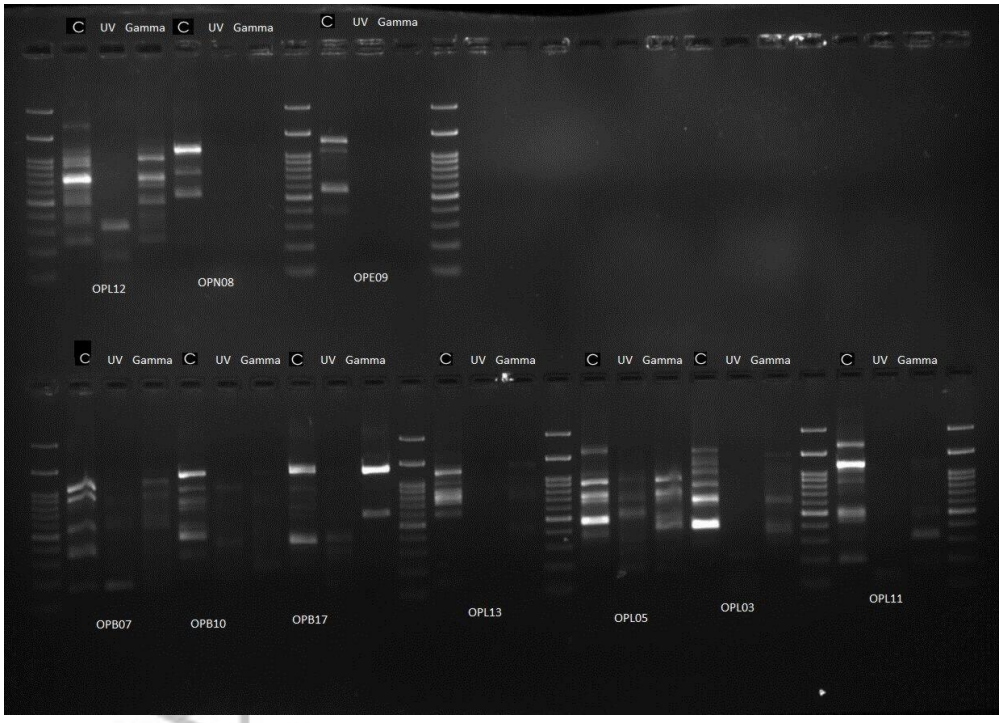

Figure 5: RAPD Profile after exposure to UV and Gamma irradiations against control.

After exposure to UV and Gamma rays RAPD profile was obtained, based on that Polymorphic Information Content (PIC) values were calculated (Table 2).

Table 2: Polymorphic Information Content values of RAPD primers

Primer Name	Expressed Locus	Null Locus	PIC
OPB07	8	7	0.497777778
OPB10	10	11	0.498866213
OPB17	7	8	0.497777778
OPB13	7	8	0.497777778
OPL05	10	14	0.486111111
OPL03	9	12	0.489795918
OPL11	11	13	0.496527778
OPL12	11	10	0.498866213
OPN08	3	6	0.444444444
OPE09	4	8	0.444444444

Evolutionary analyses were conducted in MEGA 7 software (Kumar S. *et al.,* 2016) as shown in Figure 6.

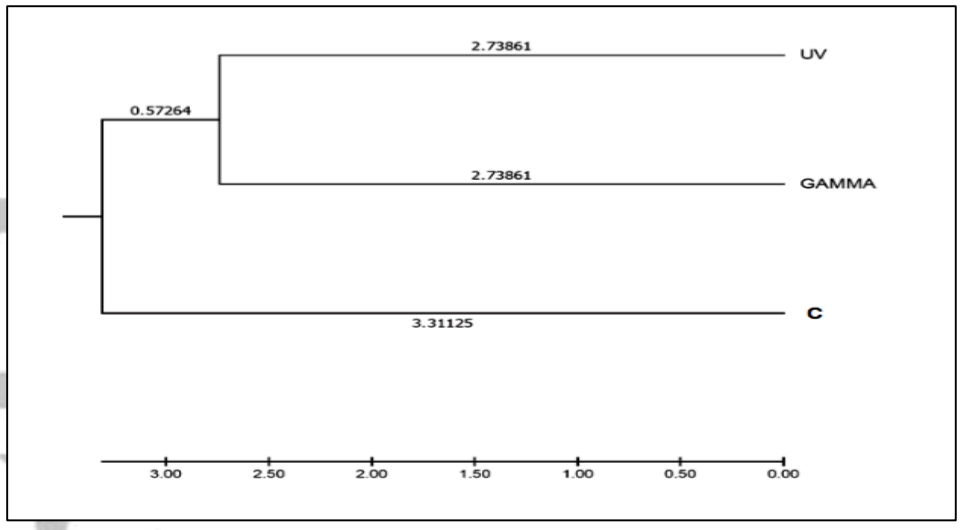

Figure 6: Evolutionary relationships of taxa with all primers

The evolutionary history was inferred using the UPGMA method [14] where C is Control that is an untreated sample as compared to UV and Gamma exposed sample. The optimal tree with the sum of branch length = 9.36111200 is shown. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analyses were conducted in MEGA 7 software [15]. The evolutionary relationships indicated that polymorphism exists between untreated and treated samples. It was observed that the primers (OPB07, OPB10, OPB17, OPL03, OPL05, OPL11, OPL12, OPL13) showed the polymorphism between treated and untreated samples but there is not much difference after UV or Gamma treatment. While in the case of primers like OPN08 and OPE09 there is no effect of treatment. This study signifies the notion of using the consortium of luminescent bacterial strains *Vibrio harveyi* and *Vibrio natriegens* as biosensing element for UV and Gamma irradiation. Biobeads used in the current investigation show property of regaining luminescence to some extent in 24 h after exposure. These beads before exposure were kept for activation in modified nutrient broth for 30 min. In this way, these beads could be reused at least twice, if stored at 4^0C in a 0.1M crosslinking solution.

Conclusion:

The current studies have explored a consortium of isolated luminescent bacterial strains *Vibrio harveyi* and *Vibrio natriegens* exhibit radiation pollutants sensing ability. After exposure to UV and Gamma rays, the RAPD profile was obtained. Further, from RAPD Profile Binary Scoring and its Primer wise PIC values were calculated to know the evolutionary relationships of taxa using the GenAlEx software. The evolutionary relationships between UV and Gamma exposed sets specified that polymorphism exists between untreated and treated

samples but there are minute alterations even after 6 hrs of UV and 30 min of Gamma treatment. Also, UV after 6 hrs of exposure and Gamma after 30 min of exposure is producing the same kind of effect on the consortium (*Vibrio harveyi* and *Vibrio natriegens*). Furthermore, it can be concluded that the bio beads of Consortium have shown significant sensitivity in terms of luminescence intensity towards radiation exposure after investigating the effects of radiation exposure by Ultra Violet and Gamma rays demonstrating it's use as potential biosensing agent. Consequently, biobeads fabrication during the present study is a preliminary attempt towards exploring novel pollution sensing biosensor.

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