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OPTIMIZATION OF BACTERIORHODOPSIN PRODUCTION BY *HALOSTAGNICOLA LARSENII* IBS (MCC 2956)

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ABSTRACT

Haloarchaea are salt loving microorganisms isolated from saline environments including ancient salt deposits and rock salt. A few haloarchaeal species have been reported to produce bacteriorhodopsin (BR) which works as a light driven proton pump. A haloarchaeal strain *Halostagnicola larsenii* IBS (MCC 2956) isolated from commercial Indian black salt (IBS) sample was employed for optimization of BR production. The organism produced 0.288 g l^{-1} BR under optimum conditions as 3.42 M NaCl, pH 7-8, incubation temperature of 45 °C and incubation period of 9 days. The present report appears to be the first report on optimization of BR production using *Halostagnicola larsenii* IBS strain.

KEYWORDS: **Haloarchaea, Bacteriorhodopsin production, Optimization, Indian black salt, *Halostgnicola larsenii***

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INTRODUCTION

Haloarchaea are salt loving organisms that inhabit hypersaline environments. They are well adapted to saturating sodium chloride concentrations. Halophilic archaea are found distributed all over the world in natural hypersaline brines in coastal regions, artificial salterns, salt lakes, salted food products, ancient salt sediments, salt pans, rock salt and rarely from sea water[1]. Recently, halophilic microorganisms including haloarchaea have been isolated from Arabian sea water and saline soil near salt pan, Shiroda, West Coast of Maharashtra, India[2]. Among the various saline environments, rock salt has been looked upon as a source of halophilic microorganisms by researchers over the globe. The presence of viable microorganisms from ancient rock salt was described[3-4]. Haloarchaeal strains of *Haloarcula, Halorubrum*, and *Halococcus salifodinae* were reported from rock salt in Winsford salt mine[5] and from Permo-Triassic salt deposit respectively[6]. The origins of halophilic microorganisms and haloarchaea were proposed to be in ancient salt deposits and ancient rock salt[7]. *Halococcus dombrowskii* sp. nov[8] and *Halobacterium noricense* [9] were reported from alpine Permo-Triassic salt deposit. Middle-Late Eocene rock salt were studied as a source of haloarchaea viz. *Halobacterium* and *Halolamina* [10.] Black Salt is a type of rock salt commonly called as Himalayan Black salt. It is salty, pungent smelling condiment used in South Asia. The Black Salt contains sodium chloride, trace impurities of sodium sulphate, sodium bisulphate, sodium bisulphide, iron sulfide and hydrogen sulphide. Due to presence of iron sulphide, it forms brownish pink to dark violet translucent crystals when whole. When ground into powder, it looks light purple to pink in colour [11-12]. Black salt is considered as a cooling spice in Indian Ayurvedic preparations. It helps in relieving intestinal gas, heartburn, hysteria etc. [11-13]. Since there were many reports regarding occurrence of haloarchaeal strains in different rock salts and the commercial Indian black salt looks pink in colour, it was thought to be a source of haloarchaea. *Halostagnicola larsenii* IBS (MCC 2956) was isolated from Indian Black salt procured from local market by enrichment of the sample in Sehgal and Gibbons (SG) medium[14] containing 3.42 M NaCl[15]. Pure culture obtained from well isolated pink-red colony was identified based on morphological, physiological, biochemical characterization and 16 S rRNA gene sequencing[15]. Bacteriorhodopsin (BR) is a retinal protein of haloarchaea, first reported from *Halobacterium salinarum*. It converts light energy of 'green light' (500-600 nm, max 568 nm) into an electrochemical energy to produce ATP. It functions as a light driven proton pump transporting protons out of the cells. Production of bacteriorhodopsin has been extensively studied using a model haloarchaeon *Halobacterium salinarum*[16-19]. Presence of BR in other haloarchaeal genera namely in *Haloarcula japonica* strain TR-1 [20], *Halostagnicola larsenii* isolated from rock pit sea water, West Coast of India[21] and *Haloferax larsenii* isolated from red rock, Malvan, West Coast of India[22] was reported. Very few researchers studied optimization of BR production [23-25]. In all these studies, *H. salinarum* strain was employed. Till date research on optimization of BR production using another haloarchaeon is lacking. The present paper describes optimization of production of BR by *Halostagnicola larsenii IBS* (MCC 2956) isolated from commercial Indian black salt.

MATERIALS AND METHODS

Cultivation of Halostagnicola larsenii IBS (MCC 2956) for production of BR

Halostagnicola larsenii IBS (MCC 2956) isolated from commercial Indian Black salt[15] was used for study. The culture was grown in Sehgal and Gibbon's (SG) medium[14] containing g/l: yeast extract, 10 g; casamino acids, 7.5 g; trisodium citrate, 3 g; potassium chloride, 2 g; magnesium sulphate, 20 g; sodium chloride, 200 g (3.42 M) ; pH 7.2. The culture was grown in shaker incubator at 37 °C, 100 rpm for 8 days.

Production of BR

The culture was grown in SG medium with 3.42 M NaCl for 8 days at 37 °C under shaking (100 rpm) condition. The cell pellet obtained after centrifugation at 8000 rpm was used for estimation of BR by BR assay [19,21.]

Optimization of production of BR

Inoculum Preparation

The inoculum was prepared by growing *Halostagnicola larsenii* IBS (MCC 2956) in 25 ml SG medium containing 3.42 M NaCl for 4 days at 37 °C, 100 rpm. The culture having optical density 1.6-1.7 at 600 nm was used as an inoculum.

Effect of different environmental and cultural conditions on growth and BR production

Effect of different environmental and cultural conditions on growth of the isolate and on production of BR was assessed by checking growth in terms of optical density and BR production in g l^{-1}. The 4 days grown inoculum of the isolate was inoculated in 25 ml sterile SG medium in 100 ml flask and the culture was grown at different cultural and environmental conditions viz. different concentrations of NaCl (3.42, 4.28, 5.13 M), pH of SG medium (pH 7, 8, 9), incubation period (Day 1-Day 10), incubation temperature (30 °C, 37 °C, 40 °C, 45 °C) by 'one variable at a time' approach while keeping other conditions constant. After appropriate incubation, cells were centrifuged and BR assay was performed[19]. Production of BR was then studied under all optimum conditions. All experiments were run in triplicate and the means and standard deviations were calculated.

RESULTS AND DISCUSSION

Detection of bacteriorhodopsin (BR) in the isolate IBS

The isolate IBS was found to produce 0.265 g l^{-1} BR within 8 days of incubation which was comparable to the rock pit sea water isolates of *Halostagnicola Larsenii* (0.035-0.258 g l^{-1}.)[21]. *Halobacterium* sp. isolated from the Dead Sea produced 0.4 nmol BR /mg of protein[16]. The haloarchaeon *H. halobium* was reported to produce 0.282 g l^{-1} BR within 10 days of incubation[19].

Effect of different cultural and environmental conditions on growth and production of BR by isolate IBS

Effect of concentration of sodium chloride in SG medium on growth of the isolate IBS and production of BR within 8 days of incubation is shown in Fig. 1. The concentration of 3.42 M NaCl was found to be optimum for growth and production of BR (0.265 g l^{-1}) however the high salt concentration i.e. 4.28 and 5.13 M affected growth as well as BR production. Studies on effect of pH revealed that at pH 7 and 8, the isolate grew well and produced 0.187 g/l BR within 8 days of incubation (Fig. 2). Thus pH range of 7-8 was found to be optimum. The BR production by isolate IBS was maximum (0.206 g l^{-1}) at 45 °C. Thus incubation temperature of 45 °C was found to be optimum (Fig. 3). Effect of incubation period on growth and BR production by the IBS isolate is depicted in Fig.4. The isolate started production of BR from day 3. Maximum BR production was observed on day 9 (0.288 g l^{-1}). Thus day 9 was found to be optimum for BR production. It was noted that the isolate produced maximum BR during stationary phase. The optimization studies showed that NaCl concentration of 3.42 M, pH 7-8, temperature of 45 ° C and incubation period of 9 days were the optimum conditions for production of BR. Under all these optimum conditions, the BR production was found to be 0.288 g l^{-1}. Bacteriorhodopsin is a unique protein of halophilic archaea which was discovered in purple membrane of *Halobacterium halobium* [26]. It functions as a light driven proton pump. Since BR is a source of bioenergy, enhancing yield of BR becomes necessary. Hence a few researchers have carried out optimization studies. The production of BR by *H. halobium* using cell recycle culture method resulted in yield of 0.282 g l^{-1} within 10 days of incubation [19]. Optimization of the production of bacteriorhodopsin by *Halobacterium salinarum* PTCC 1685 using 'one factor at a time' approach and 'Taguchi design' resulted in maximum BR production of 0.197 g l^{-1} at 38 °C temperature and pH 7.5 [24]. *Halorubrum sodomennse* A01 was studied for the production of BR (0.0123 g l^{-1}) [27]. 11 strains of *Halostagnicola larsenii* isolated from rock pit sea water, Rock garden, Malvan, India were found to produce BR in the range of 0.035-0.258 g l^{-1} [21]. In comparison to above reports, the isolate IBS produced 0.249-0.288 g/l BR within 4 to 9 days of incubation. Thus, this optimization study will be useful for scale up production of BR using *Halostagnicola larsenii*.

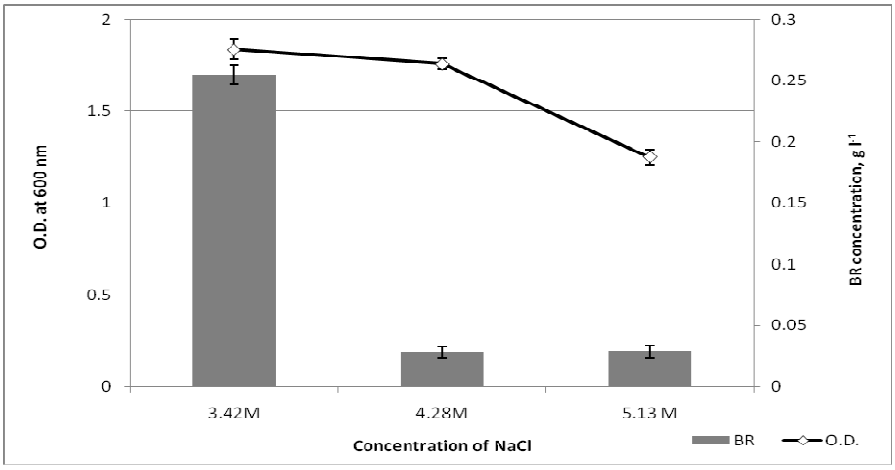

Figure 1
Effect of concentration of NaCl on growth of IBS isolate and production of BR

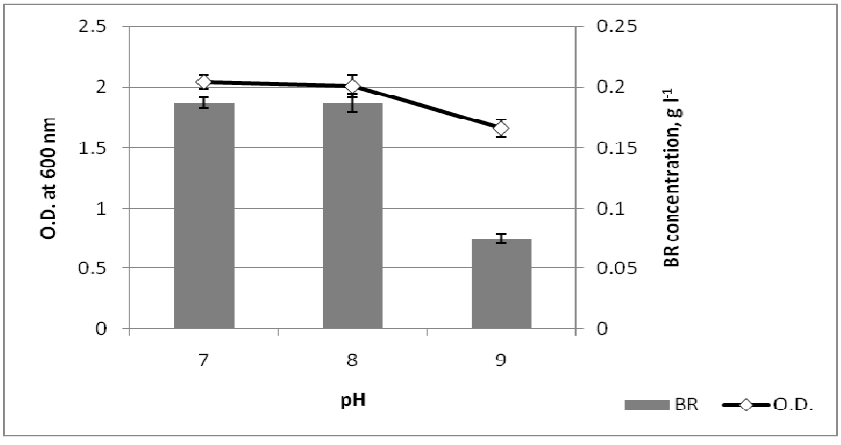

Figure 2
Effect of pH of SG medium on growth of IBS isolate and production of BR

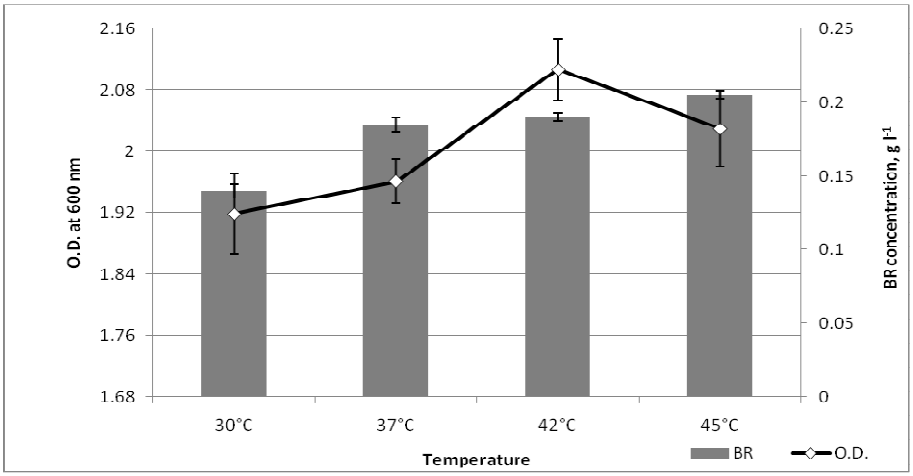

Figure 3
Effect of incubation temperature on growth of IBS isolate and production of BR

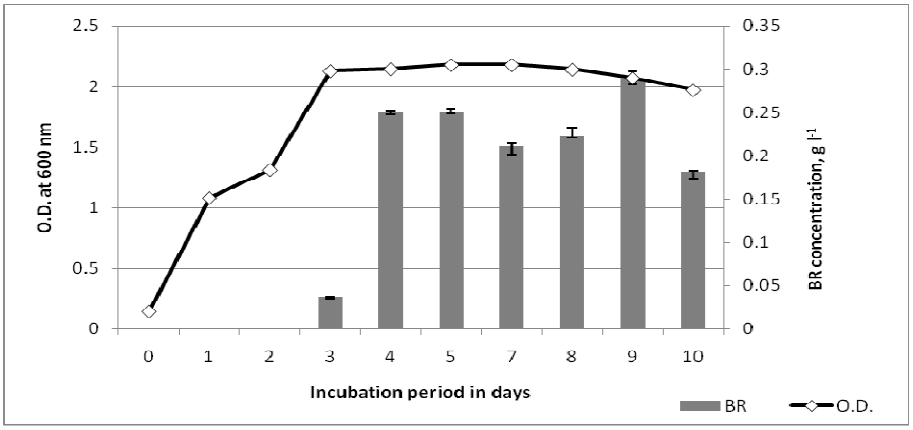

Figure 4
Effect of incubation period on growth of IBS isolate and production of BR

CONCLUSION

The haloarchaeon *Halostagnicola larsenii* IBS (MCC 2956) isolated from commercial Indian black salt sample was studied for production of BR. The optimum conditions for production of BR by the isolate IBS were NaCl concentration of 3.42 M, pH 7-8, incubation temperature of 45 °C and incubation period of 9 days yielding 0.288 g l^{-1} BR. To the best of our knowledge, this is the first report of optimization of culture conditions for production of BR by *Halostagnicola larsenii*.

CONFLICT OF INTEREST

Conflict of Interest declared none.

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