5. Discussion

Solar salterns are natural or artificial ponds used for salt production and they provide ideal conditions for the growth and multiplication of halotolerant and halophilic microorganisms. In order to cope up with the harsh environmental conditions prevailing in the salterns, halophlilic microbes have several adaptive features such as efficient ion pumps, acidic proteins, internal compatible solutes and UV absorbing pigments. The pigments of halophilic microorganisms give attractive colouration to the salt pans. Pigments have wide range of applications in food, medical and neutraceutical industries. In the present study, the least explored Marakkanam salt pan, Tamil Nadu, India was investigated for pigment producing halophilic bacteria.

5.1. Isolation, identification and biochemical characterization of pigment producing halophilic bacteria

The results of the present study have shown that the availability of pigmented bacteria is related to sunlight and high salinity existing in the salt pan. The samples collected in January 2014 showed the presence of mostly white, creamy and pale coloured colonies whereas samples collected in May 2014 yielded bright coloured yellow, pink, dark orange, brown coloured colonies. Several earlier reports have stated the correlation between the sunlight and presence of pigmented bacterium in halophilic environment (Dundas and Larsen, 1962).

Khanafari *et al.*, (2010) observed red orange colouration of the salterns in Iran and isolated red pigment producing halophilic bacteria. They also reported that the red colouration of saltern in the month of May is due to the presence of extremely halophilic bacteria, archaea and algae. However due to heavy rainfall leading to low salinity in monsoon season, the population of pigment producing extreme halophiles may be low or absent leading to no colouration of saltern water. Observations of the present study corroborate the reports of Khanafari *et al.*, (2010) who noted the presence of very less number of brightly pigmented halophilic bacteria during monsoon season.

In the present study totally 48 halophilic bacteria were isolated from the sediment samples of Marakkanam salt pan. Most of the halophilic bacteria were found to be Gram positive, some were Gram negative rods, few were Gram positive cocci and few were pleomorphic forms. Similarly, Ventosa *et al*., (1983) reported the presence of 38 strains of moderately halophilic Gram-positive cocci from saline soils and the ponds of a solar saltern in Alicante (Spain). Jeon *et al*., (2008) also demonstrated the occurence of Gram-positive halophilic rod shaped bacteria from a salt lake in the Xin-Jiang Province of China. Sahay *et al*., (2011) reported the isolation and characterization of halophilic bacteria from samples collected from Pulicat Lake. Zarparvar *et al*., (2016) isolated 400 bacterial strains from Incheh Broun hypersaline wetland of Turkmenistan, of which 194 strains were Gram positive rods, 184 strains were Gram-negative rods and 22 strains were Gram positive cocci.

Pigments producing halophilic bacteria isolated from Marakkanam salt pan were highly diverse group with different biochemical characteristics. Methods used for characterization and identification of halophilic bacteria include morphological, biochemical and molecular methods (Smibert and Krieg 1994). Generally, use of morphological and biochemical techniques for identification is the traditional standard in the past; in recent years, number of biochemical tests were developed and several commercial biochemical kits are available for use.

In the present study, 25 selected pigment producing halophilic bacteria were subjected to morphological and biochemical characterization. The results have shown that most of the isolates were negative to indole, methyl red, Voges Proskauer and citrate tests. Sugar utilization of selected halophilic bacteria was found to be different and it showed that the physiology of halophilic isolates differ from each other. Chen *et al*., (2007) isolated *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium from a salt mine in Yunnan, south-west China and described the bacteria based on the biochemical characteristics.

Lee *et al*., (2004) isolated and characterized *Paracoccus haeundaensis* sp. nov., a Gram-negative, halophilic, astaxanthin-producing bacterium based on the fatty acid pattern and biochemical reaction profiles. In the present study, most of the pigment producing halophilic bacteria utilized trehalose and sucrose. Trehalose is considered as a universal stress molecule which protects an organism from high osmolarity, heat, oxidation and freezing; further it is a source of energy and signals specific metabolic pathways (Argüelles, 2000; Elbein *et al*., 2003; Paul *et al*., 2008).

5.2. Antioxidant activity and cytotoxicity of pigment producing halophilic bacteria

Free radicals are known to be associated with chronic diseases such as diabetes, cancer, autoimmune disorders and cardiovascular diseases. Antioxidant substances have the potential to quench the free radicals thereby reduce the risk of a particular disease. Especially, microbial pigments like carotenoids, melanins, phenazines, violaceins, prodigiosins, tambjamines and quinones were reported to have a strong antioxidant activity because of their biological functions (Lampila *et al*., 1985; Patel *et al*., 2007; Soleiv *et al*., 2011).

Among the twenty five selected isolates obtained in the present study, five have showed promising antioxidant activity and this may be due to the presence of biogenic pigments (carotenoids, prodigiosins and phenazines), which helps them to adapt to the excessive sunlight and harsh environmental conditions. The results of the present study corroborate the report of Rajagopal *et al*., (1997) who studied the antioxidant activity of xanthomonadin, a bacterial pigment by inhibiting photodynamic lipid peroxidation.

Another study by Kurjogi *et al*., (2010) showed that staphyloxanthin, a yellow pigment obtained from *Staphylococcus aureus* has got promising antioxidant activity against oxidative stress in swiss albino mice. Similarly, Velho-Pereira *et al*., (2015) reported the free radical scavenging ability of halophilic *Chromohalobacter* sp., *Bacillus* sp., *Salinicoccus* sp. by producing antioxidant substances.

Pinar *et al*., (2014) also reported that microorganisms produce a range of biogenic pigments such as carotenoid, ruberin and melanin which are responsible for protection of the bacteria from photooxidative damage caused by excessive sunlight. Similar studies on pigment extracts of halophilic bacteria such as *Bacillus licheniformis* and *Kocuria* sp. strain QWT-12 showed the presence of strong antioxidant activity (Mao *et al*., 2013; Rezaeeyan *et al*., 2017). Likewise, Osawa *et al*., (2010) reported antioxidant activities of rare C50 carotenoids sarcinaxanthin and sarcinaxanthin monoglucoside extracted from halophilic *Micrococcus yunnanensis*.

The results of the present study support the observations of Shindo *et al*., (2007) who reported the antioxidant activity of rare carotenoid, saproxanthin and myxol by peroxidation of lipid. Vora *et al*., (2014) reported antioxidant activity by several halophilic bacteria isolated from Kharaghoda soil. Many other studies also reported the antioxidant activity of pigment producing bacteria (de Azevedo *et al*., 2000; Duran *et al*., 2003; Patel *et al*., 2007; Antonisamy and Ignacimuthu, 2010).

The cytotoxicity assay showed the viability of cancer cells at varying concentration of pigment extracts. The viability of the cancer cells was higher with pigment extracts of *Brevundimonas* sp. S17 than the other tested pigment extracts. These kind of cellular changes were the characteristics of the apoptotic induction of cell death. The pigment extracts at 500µg/mL showed morphological changes in the HeLa cell lines. Deorukhkar *et al*., (2007) reported the cytotoxic activity of prodigiosin analogue produced by *Serratia marcescens*. Similarly, Schwartz and Shklar, (1992) showed the cytotoxic potential of beta carotene in cancer cell lines and observed morphological changes in the cell lines. The results of the present study corroborate with the findings of Rezaeeyan *et al*., (2017) who showed the cytotoxic potential of *Kocuria* sp. against breast, lung and prostate cancer cells.

5.3. Molecular characterization of selected pigment producing halophilic bacteria

Though the biochemical tests are routinely used in identification of bacteria, those methods alone may not be reliable for environmental isolates due to the scarce of information on environmental isolates. Recently, molecular characterizations have been applied for the identification of microbes, their diversity and phylogeny (Coenye *et al*., 2005). Molecular techniques would allow an assessment of the validity of the morphological species concept for common microalgae. The application of molecular markers as a useful tool in detection and identification of microorganisms has been internationally accepted. Pre genomic tools such as rDNA genes, the sequence of which is highly conserved can be used with or without full genomic information for investigating microbial communities. Nowadays, molecular phylogeny is considered as one of the important tools in identifying microorganisms from various ecological niches. Molecular techniques like RAPD, ARDRA and 16S rRNA are used in identifying and analyzing the diversity of bacteria. In specific, 16S rRNA sequences are widely employed as a powerful tool for identification of phylogenetic and evolutionary relationship among bacterial populations (Liu and Stahl, 2007).

In the present study, five selected pigment producing halophilic bacteria were subjected to 16S rRNA sequencing and the results were in agreement with the characterization by conventional methods. The sequences were compared with existing sequences using BLAST (NCBI database). This program can search through a database of thousands of entries in a minute. BLAST performs its alignment by matching up each position of search sequence to each position of the sequences in the database. For each position BLAST gives a positive score if the nucleotides match, it can also insert gaps when performing the alignment. Each gap inserted has a negative effect on the alignment score, but if enough nucleotides align as a result of the gap, this negative effect is overcome and the gap is accepted in the alignment. These scores are then used to calculate the alignment score, in ―bits‖ which is converted to the statistical E- value. The lower the E-value, the more similar the sequence found in the database is to query sequence (https://blast.ncbi.nlm.nih.gov/).

The compared sequences were identified based on their similarity to the existing sequences in NCBI. Gupta *et al*., (2015) used BLAST analysis for identification of species. The sequences was then submitted in the public database NCBI GenBank with following accession numbers: *Chromohalobacter salexigens* MF447850, *Chromohalobacter israelensis* MF447851, *Oceanobacillus manasiensis* MF447852, *Bacillus licheniformis* MF447853 and *Brevundimonas viscosa* MF447854. Gupta *et al*., (2015) demonstrated the 16S rRNA based molecular characterization of five halophilic bacteria namely *Halobacillus trueperi* SS1 and SS3, *Marinomonas* sp. SS8 *Shewanella algae* SS2, *Halomonas venusta* SS5 and isolated from Lunsu salt water body, Himachal Pradesh.

Previously, another strain of halophilic *Bacillus licheniformis* isolated from Markkanam salt pan exhibit radiotolerant activity was identified based on its 16S rRNA sequence (Paraneeiswaran, 2015). Likewise, several researchers have used 16S rRNA as a tool for the identification of halophilic bacteria from various salt pans (Lu *et al*., 2001; Nowlan *et al*., 2006; Salgaonkar *et al*., 2013; Sundaramanickam *et al*., 2015; Selvarajan *et al*., 2017).

In the present study, though all the five isolates are found in the same environment each one falls into separate clads in the phylogenetic tree clearly indicated that these organisms differ from each other. Wright *et al*., (2006) reported the phylogenetic relationship between the halobacteriales inferred from 16S rRNA gene sequences. Similarly, Roohi *et al*., (2014) demonstrated the presence of halophilic bacteria like *Bacillus* sp., *Oceanobacillus* sp., *Halobacillus* sp. and *Salinicoccus* sp. and its phylogenetic relationship between the isolates based on 16S rRNA sequences.

The secondary structure of the five pigment producing halophilic bacteria showed variations in their secondary structure, thus confirmed that these isolates were different from each other. Moreover, the bacteria also differ in their free energy values and stem and loop numbers. Sloma and Mathews, (2015) showed the application of secondary structure prediction in molecular analysis. In the present study, the restriction enzymes created a different type of fragments in all the halophilic bacteria indicating that they belong to different species.

5.4. Optimization of pigment production using one variable approach in *Bacillus licheniformis* S15

Basically two biotechnological strategies are used when producing microbial pigments; i) Search for novel sources and ii) enhancing the pigment yield through optimization of cultural conditions or by strain improvement (Venil *et al*., 2013). In the present study, salinity, metal ions, carbon source, pH and temperature was chosen for optimization analysis. The study showed that carbon sources, pH and temperature highly influenced the pigment production. Khodaiyan *et al*., (2007b) reported that carbon sources like glucose, fructose and sucrose positively influenced the quantity of canthaxanthin production by *Dietzia natronolimnaea*.

Khanafari *et al*., (2010) reported that addition of 1% sucrose to the growth medium increase the production of red pigments in halophilic bacteria isolated from solar salt pan at Imam Khomeini port, Iran. Kirishna *et al*., (2014) have optimized the cultural conditions for the production of red pigment (prodigiosin) by *Vibrio* sp. The nutritional control of pigment and isoprenoid compound formation in *Halobacterium cuti-rubrum* and *H. halobium* were studied; it showed that addition of 0.1% glycerol to the medium increased the growth but lowered the pigment production whereas incorporation of glucose to the medium showed increase pigment production (Gochnauer *et al*., 1972).

Asker and Ohta, (1999) reported that canthaxanthin production was higher at pH 7.2 in *Halobacterium* sp. isolated from a salt farm in Alexandria, Egypt. Khanafari *et al*., (2010) found that neutral pH favoured pigment production whereas acidic or alkaline pH inhibited bacterial growth and pigment production in orange and yellow pigment producing halophilic bacteria from solar salt Lake of Iran. The present study was in agreement with Kirishna *et al*., (2014) i.e., an increase in red coloured pigment production at pH 7 when compared to pH 6 and 8 by *Vibrio* sp.

isolated from Nellore coastal region, Andhra Pradesh. Mulik *et al*., (2017) reported that the carotenoid content of halophilic *Kocuria* sp.BRI 36 was high at pH 7.5.

The study by Khanafari *et al*., (2010) reported that at 25˚C, the halophilic bacteria from solar salt Lake of Iran produced higher quantities of yellow and orange pigment. A halophilic archaea, *Halorubrum* sp. showed an increase in total carotenoid production at 35˚C (Pathak and Sardar, 2012). The pigment production was reported to be high in *Serratia marcescens* at 27˚C whereas no growth and pigment production was observed at 35˚C (Giri *et al*., 2004). The optimum temperature for pigment production i.e., carotenoids by *Halorubrum* sp.TBZ126 was recorded at 31˚C and 32˚C (Hamidi *et al*., 2014).

It was reported that *Flavobacterium* sp. cultured in a nutrient medium containing glucose or sucrose, sulphur-containing amino acids such as methionine, cystine or cysteine, pyridoxine and bivalent metal ions selected from the group consisting of Fe^{2+}, Co^{2+}, Mo^{2+} or Mn^{2+} produced canthaxanthin to the level of 190mg/L (Dufossé, 2006). On the contrary, in the present study the addition of metal ions to the medium lowered pigment production. These results are in agreement with the observation of Bau and Wong, (1979) wherein the addition of zinc is detrimental to pigment production by *Monascus*. Similarly, addition of iron to the culture medium lowered the astaxanthin production by *Phaffia rhodozyma* (An *et al*., 2001).

In the present study also, the culture medium without the addition of metal ions favoured higher quantity of pigment to those compared with metal ion supplemented cultures. It was inferred that metal ions negatively affected the pigment production in *Bacillus licheniformis* S15. The present study also showed that sucrose, temperature and pH played a major role in pigment production by *Bacillus licheniformis* S15.

The optimization process using one variable at a time approach gives non-reliable results and also the interactive effects of different variables for production cannot be resolved by this approach. Statistical experimental approaches including factorial design and response surface methodology are more reliable than conventional experiments (Khuri and Cornell, 1987). RSM provides a large amount of information and is more economical approach because a limited number of experiments are performed for monitoring the interaction of the independent variables on the response. The equation of the model easily clarifies the effects for binary combinations of the independent variables. Many types of response surface designs are used for optimization like Central composite, Doehlert, and Box–Behnken. Box–Behnken design is preferable to the Central composite designs because it requires fewer test runs and is rotatable (Sathiyanarayanan *et al*., 2013).

Central composite design is one of the most common experimental designs among different classes of RSM and this strategy particularly help us to predict the better concentration of substrates with less errors (Sathiyanarayanan *et al*., 2013). Statistical optimization methods have been successfully employed for the optimization of many bioproducts. Arun *et al*., (2014) optimized the extracellular polysaccharide production in *Halobacillus trueperi* AJSK using RSM. EPS production in bacterium through fermentation process was optimized using RSM (Fang *et al*., 2013).

Statistical approach was used for optimizing the cultural conditions that could support maximum pigment production. Similarly, Hamidi *et al*., (2014) employed statistical experimental design response surface methodology and central composited design to study the total carotenoid production by *Halorubrum* sp. TBZ126 with three factors such as temperature, pH and salinity. The optimal culture conditions for carotenoid production were found to be at temperature 31ºC and 32ºC, pH 7.51 and 7.94 and NaCl (w/v) 18.33% and 20.55% respectively.

Abdelhafez *et al*., (2016) investigated the influence of lactose, sucrose, pH, inoculum concentration for β-carotene production by *Serratia marcescens* and optimized the production process using Plackett-Burman Design and central composite design. Zhou *et al*., (2009b) reported the RSM based optimization of yellow pigment production with various concentrations of peptone, NH_4NO_3 and

KH_2PO_4 by *Monascus* sp. in submerged fermentation. The canthaxanthin production by *Dietzia natronolimnaea* HS-1 was optimized by means of response surface methodology (RSM) in order to achieve high-level production of canthaxanthin (Khodaiyan *et al*., 2007a).

5.5. Purification and characterization of pigment from *Bacillus licheniformis* S15

Pigment from *Bacillus licheniformis* S15 was purified and a single pinkish orange coloured band was obtained using silica gel column chromatography. Previous reports showed the purification of pigments in column chromatography with ethyl acetate as a solvent (Williams *et al*., 1956). The famous carotenoid chemist, Liaaen-Jensen, (1995) suggested three procedures for identification and structure elucidation of carotenoids such as UV-Visible spectroscopy (UV-Vis), Mass spectroscopy (MS) and Nuclear Magnetic Resonance spectroscopy (NMR).

The UV-Visible spectrophotometric analysis showed that the absorption maximum of the purified pigment was about 535nm. Similarly, Asker and Ohta, (1999) characterized the carotenoid pigment from the halophilic bacteria isolated from soil samples of salt farm in Alexandria, Egypt. The Rf value of purified pigment analysed by TLC was 0.96 and this reports were similar to the studies of Mulik *et al*., (2017) who reported the red-orange pigment producing halotolerant bacteria, *Kocuria* sp. BRI36 from cold oceanic regions of Antarctica. The results of the UV-Vis spectrophotometry and TLC indicated that this purified pigment belong to carotenoid group. These results were similar to the studies of Kleinegris *et al*., (2010) who showed that the carotenoid absorption ranges from 400-550nm. Carotenoid pigments of extremely halophilic bacterium *Salinibacter ruber* was extracted and purified by thin layer and column chromatography (de Lourdes Moreno *et al*., 2012).

FT-IR is a sensitive technique used to identifiy the organic molecules and it reveals the functional group of a compound. The representative band at 1459cm^{-1} seems to be the bending vibration of CH_2 – methylene group whereas the peaks between 1381 cm^{-1} and 1365cm^{-1} matched the peaks of carotenoid (Rohman *et al*., 2010). Similarly, Biswas *et al*., (2016) showed the isolation of a halophilic archaeon, *Haloferax* sp. from solar salterns of West Bengal and purified the bacterioruberin pigment. Likewise, the FT-IR analysis of purified pigment showed the peaks relevant to carotenoid.

Previous works on the structural analysis of carotenoids, including several novel methods, were mass spectroscopy, chemical analysis, HPLC and very limited NMR characterization. In the presnt work, much more convincing support for the proposed structural identifications lies in the observation of chemical shifts associated with methoxyl groups on carbon C3 and also on chemical shifts associated with protons of the methoxyl group. The ^{1}H NMR spectra of the pigments revealed several characteristic features of carotenoid spectra. The spectra of ^{1}H revealed a number of resonances in the region of 6.0–7.0 ppm, which represent different olefinic protons along the p-electron conjugated chain.

COSY, homonuclear correlation spectroscopy elucidates spin-couplings between protons that are connected through a single bond. The differences in the chemical shift of the proton associated with C3 and C30 also support the proposed identifications. The assignment of the 3.93 ppm resonance to the proton in the C3 position for pigment is confirmed by the COSY interactions. The COSY spectrum of purified pigment showed signals between 7-7.8 ppm and it was similar to the study by Englert, (1985) who characaterized the carotenoid pigment using COSY spectrum. Lutnaes *et al*., (2002a) investigated the extremely halophilic bacterium *Salinibacter ruber*, identified a novel pigment salinixanthin and assigned its structure based on the MS and NMR spectrum.

Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy is used to determine long-range ^{1}H-^{13}C connectivities. Since it is a long-range chemical shift correlation experiment, HMBC provides the information about the chemical shift of carbon atoms that are about 2-3 bonds away from the proton to which they correlate so that the quaternary carbon atoms can also be detected. The HMBC spectrum showed the chemical shift of carbon atoms of the purified pigment obtained in the present study. Previously, Lutnaes *et al*., (2003) investigated the charge localized beta, beta carotene dication.

Chattopadhyay *et al*., (1997) characterized the bacterioruberin pigment from psychrotrophic *Micrococcus roseus* isolated from Antarctica. Similarly Nugraheni *et al*., (2010) reported diadinoxanthin from a sea grass symbiotic bacteria, *Bacillus licheniformis*. Till date, only few *Bacillus licheniformis* strains are reported to produce pigments. Lutnaes *et al*., (2002b) reported the rhodovibrin, 2-ketorhodovibrin, anhydrorhodovibrin and chlorobactene from green phototrophic and purple bacteria using ^{13}C NMR and ^{1}H NMR analysis.

Hertzberg *et al*., (1976) studied the structural analysis of pigment produced by *Corynebacterium autotrophicum* and the pigment was identified as zeaxanthin based on the ^{1}H NMR spectral analysis. Similarly, Shindo *et al*., (2006) isolated three orange and yellow pigment producing marine bacteria and showed the presence of rare carotenoids such as (3R, 2 S) - myxol and (3R) – saproxanthin using ^{1}HNMR spectral data. The various NMR analysis data were supported by the results of NMR studies described in previous reports (Lutnaes *et al*., 2002b).

In the present study, the data obtained from the various spectroscopic analysis of the pinkish orange pigment with FT-IR, MS, ^{1}HNMR, ^{13}C NMR, COSY NMR, HSQC NMR and HMBC NMR very clearly testify that the pigment produced by *Bacillus licheniformis* S15 is a novel carotenoid pigment.

5.6. Bioactivity of the purified pigment from *Bacillus licheniformis* S15

Diabetes mellitus is a chronic disease, and its incidence is tremendously increasing globally. Decreasing postprandial hyperglycemia by retarding glucose absorption through inhibiting carbohydrates digesting enzymes (α-amylase and α-glucosidase) is one of many approaches used for the management of this disease. The inhibition of carbohydrate digesting enzymes (α-amylase and α-glucosidase) activity, as well as delaying the digestion, and absorption of carbohydrates from the small intestinal tract is considered as one of the major treatment options for Type II DM.

By inhibiting these key enzymes, minimal amounts of glucose would be absorbed into the blood stream, hence the plasma glucose will not spike after a meal. Hyperglycemia alone does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycemia, which is mediated and complicated through oxidative stress (American Diabetes Association, 2010). Several *in-vivo* studies have also shown the antidiabetic activity of carotenoids such as astaxanthin, lycopene, beta-carotene and canthaxanthin (Yanai *et al*., 2008).

Postprandial hyperglycemia was significantly suppressed by oral administration of astaxanthin, which significantly lowered the postprandial AUC. Therefore, it can be said that astaxanthin is a useful natural agent in treating diabetes. In the present study, the novel pinkish orange carotenoid pigment showed the inhibition of α-amylase and α-glucosidase enzymes which was supported by Gopal *et al* ., (2017) who showed both *in-vitro* and *in-vivo* inhibitory activity of α-amylase and α-glucosidase by lactucaxanthin, a carotenoid isolated from lettuce.

Inflammation is defined as a complex biological reaction of vascular tissues to any harmful stimuli. It is also considered as a protective mechansim of an organism to remove the harmful external stimuli and initiate the healing process. At the onset of an inflammation, the cells undergo activation and release inflammatory mediators. These mediators include histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), the kinin system, the fibrinolytic system and prostaglandins. These mediator molecule works together to increase permeability and vasodilatation of blood vessels. Thus, leading to increased blood flow, migration of leukocytes, mainly neutrophils outside the blood vessels into the injured tissues and exudation of plasma proteins and fluids (Kawata *et al*., 2018).

The purified pigment from *Bacillus licheniformis* S15 showed activity against pathogenic *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*.

The results indicate that the purified pigment has antimicrobial potential. Similarly the antimicrobial potential of pigment were reported by Mulik *et al*., (2017) who showed antimicrobial activity of red pigment from *Kocuria* sp. against Gram positive and Gram negative bacteria.

The present study showed that the novel carotenoid pigment extracted from *Bacillus licheniformis* S15 has membarane stabilization activity and nitric oxide scavenging activity. Thus, the study has shown the anti-inflammatory potential of novel carotenoid pigment obtained from *Bacillus licheniformis* S15. Earlier reports indicate the anti-inflammatory activity of carotenoid extract from dried pepper (Hernández-Ortega, *et al*., 2012); membrane stabilization activity of the carotenoid, lutein (Vidya *et al*., 2015); anti-inflammatory activity of beta carotene and lycopene (Kawata *et al*., 2018) which are supported by the findings of the present study.

The present study gives an idea about the distribution of pigment producing halophilic bacteria in Marakkanam salt pan, Villupuram district, TamilNadu, India. The pigment producing halophilic bacteria were characterized using morphological, biochemical and molecular aspects. The pigment of the selected isolate, *Bacillus licheniformis* S15 was extracted, purified and characterized. The bioactivity of the purified pigment extract was studied.

On the basis of the investigation made on *Bacillus licheniformis* S15, the present work concludes as follows:

- *Bacillus licheniformis* can produce pigments in hypersaline habitats.
- The pigment production was influenced by varying ranges of salinity, various carbon sources, pH and temperature in which pH, temperature and sucrose was found to be significant in increasing pigment content of *Bacillus licheniformis* S15.
- The pigments were extracted, purified and characterized by FT-IR, MS and NMR techniques.
- The purified pigment has antidiabetic, antiinflammatory and antimicrobial activity.