Production, Detection and Characterization of Betaine

A. Materials and Methods

5.1 Bacterial Culture:

The complex medium used for the preservation and maintenance of *Halomonas* sp. contained 5g Tryptone, 10g Yeast extract, 3g Sodium Citrate, 2g KCl and 20g MgSO₄.7H₂O dissolved in 1litre of distilled water and pH was adjusted to 7.5 and incubated at 37°C for 48 hours.

5.2 Optimization of growth parameters

5.2.1 Effect of pH on growth

Production medium was adjusted to different pH ranging from 5 to 13 at difference of 1 pH using 0.1% Glucose with 10% NaCl (halo bacterium medium) was incorporated as a substrate after autoclaving and the effect was recorded as optical density at 600nm.

5.2.2 Effect of temperature on growth

The optimum temperature for the growth and production of betaine by halophilic bacteria was determined in production medium, by keeping it in temperature controlled shaker at 140 rpm from 30°C to 55°C with a increment of 5°C at 7.5 pH for 24 hrs.

5.2.3 Effect of NaCl on growth

The effect of NaCl was studied on growth and betaine production using halobacterium medium, containing different concentration of NaCl (0, 5, 10, 15, 20
and 30 % w/v) incubated the broth under shaker temperature 37 °C, pH adjusted to 8.0 for 24 hrs.

5.3 Production of Betaine

The Production of compatible solute by *Halomonas sp.* was first grown in 100 ml of seed culture medium in a 250 ml flask at pH 7.5 and 37 °C for 18 hrs at 200 rpm. This culture broth was used to inoculate 500 ml batch cultivation medium with 7% NaCl after 18 hrs. Cells were harvested from the culture medium by centrifugation at 6000 rpm for 10 min at 4°C.

5.4 Purification of Betaine

Samples from the fermented broth were mixed at 4°C with 5 ml of distilled water and extracts were dried under reduced pressure at 35 °C and purified sample was detected by UV scan absorbance from 230-281nm for the presence of betaine and was further characterized by FTIR.

5.5 Characterization of Betaine by FTIR analysis

The analysis of betaine was performed by FTIR, from the 400 to 4000 cm⁻¹, KBR was mixed with the sample, and compressed and KBR-Sample pellet was prepared for the analysis.
B. Results and Discussions

5.6 Production of osmolyte Betaine

Reports on isolation of novel isolates from alkaline habitats were very few with respect to the organic osmolytes. Today, many of this alkalophilic isolates were of considerably industrial important, for use in pharmaceutical and cosmetic industries and also in anti-aging molecule, anti-stress molecule. In our study, selected strain showed most promising for the production betaine with optimum activities at pH 8.0 and temperature of 37°C upto 15% salt concentration which was desirable one for the industrial application. Complex media components, such as yeast extract and peptone, contained considerable amounts of betaine (1 - 3% of the dry weight) that could be taken up by heterotrophic bacteria (Galinski and Truper, 1994).

5.7 Screening and characterization of Betaine

5.7.3 Effect of pH and Temperature on production of Betaine

In *Halomonas* sp. betaine production was observed at pH 8 and temperature 37°C at 18 hrs as the pH increased the growth and production of betaine increased as showed in Fig. 5.1 and 5.2. Betaine was detected up to pH 10 later on it was not detectable and it was also true for the growth of the *Halomonas* sp. Hypersaline conditions of the growth medium, influenced the accumulation of Betaine extremely in high concentrations in their cytoplasm, archae bacterium *Methanohalophilus* Z7302 reported to accumulate 4.1 M betaine at 37°C and pH 7 (Lai and Gunsalus, 1992). Sulphur reducing extremely halophilic bacteria *Ectothiorhodospira halochloris* synthesizes 2.5 mol betaine at 37°C at pH 7.5 (Galinski and Herzog, 1990).
Fig. 5.1. Effect of pH on production of Betaine from *Halomonas* sp.

Fig. 5.2 Effect of temperature on production of Betaine from *Halomonas* sp.
5.7.2 Effect of NaCl on production of Betaine

The betaine was detected at very lower concentration of Salt 7% and the growth also increased with increase in salt concentration as shown in Fig. 5.3, this finding suggest that Halomonas sp. has evolved a sophisticated mechanism to regulate the accumulation of compatible solutes to survive and grow in habitats with extremely gradient saline concentrations. As the salt concentration increased the presence of betaine could not be detected above 20 % salt and also the growth decreased.

The halophilic microbial world is tremendously diverse, and novel types of halophiles are being discovered at a high pace. Cyanobacteria accumulated betaine under high salt concentration, halophilic cyanobacterium Synchocystis DUN52 produced 3.0 M betaine when the bacterium is cultivated at 200 g sea salt l\(^{-1}\) (Mohammed et al., 1983). Whereas the halophilic strains could synthesize the sugars and glycosyl glycerol under salt stress conditions (Mackay et al., 1984). Eubacteria halophilic Methanogenic archaebacteria (Methanothalophilus-strains) also synthesized betaine as their osmolyte (Lai et al., 1991).

5.8 Detection of betaine by UV scan and FTIR analysis

5.8a UV scan

The purified betaine in double distilled water produced by Halomonas sp. was detected by using UV Vis spectral reading, it was scanned from 200 nm to 800 nm. At 281 nm the single peak was observed which was correlated to the presence of Betaine as shown in Fig 5.4 by referring it with the standard betaine curve. The single peak formed indicated the presence of pure betaine.
Fig. 5.3 Effect of Salt on production of Betaine from *Halomonas* sp.

Fig. 5.4 UV Visible scan for the detection of betaine from *Halomonas* sp.
5.8b FTIR analysis of betaine

In present study, the betaine accumulation was observed to accumulate the higher concentration of pure betaine which was further characterized by FTIR analysis, on comparison with the NIST data for FTIR, as shown in Fig.5.5 and 5.6 respectively. By referring with standard NIST data it was confirmed as betaine accumulated by *Halomonas* sp. and it could tolerate 15 % w/v NaCl concentration. NIST provided the standard reference spectrum of several chemical entities and also considered as most useful resource for the comparative studies of the molecules.
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Fig. 5.5 FTIR analysis of Betaine Purified from *Halomonas* sp.

Fig. 5.6 Ref: NIST Chemistry Web Book (http://webbook.nist.gov/Chemistry)