Exploring the Nutritional Values of Hydrogenotrophic Bacteria as Space Food

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Abstract: **Hydrogenotrophs are organisms that are capable of metabolizing molecular hydrogen as source of energy and hence hydrogenotrophy is performed by carbon dioxide reducing organism to produce methane. Methanogens are diverse group of strict anaerobes where methanogenesis is a major route in the anaerobic mineralization of organic matter. They have very unique enzyme and cofactors involved in this metabolic pathway. The anaerobic chamber provides a convenient culture system for large-scale studies of strictly anaerobic and/or facultative anaerobic bacteria. In this study, the Hydrogenotrophic organism isolated was characterized as Methanosarcina sp. Though these hydrogenotrophs utilise carbon dioxide and grow in a minimal growth requirement, they were found to be rich in carbohydrates and protein content. Therefore, Hydrogenotroph being a good food source, can be used for future space travel where the astronauts could carry them aboard and synthesis their own food. The by-products produced by these organisms can be used to produce a protein rich meal. This system can be incorporated on to space craft where this works as a closed loop between astronauts and hydrogenotrophs, where carbon dioxide released by astronauts can be captured and filtered through a system and supplied for the growth of these microbes which in turn synthesis carbohydrates.**

***Keywords*: Hydrogenotrophs, space food, anaerobes.**

I. INTRODUCTION

Hydrogenotrophs are organisms that are capable of metabolizing molecular hydrogen as source of energy. An example of hydrogenotrophy is performed by carbon dioxide reducing organism, as they utilise carbon dioxide and hydrogen to produce methane by the metabolic pathways namely acetogenesis, sulphate reduction and hydrogen oxidation. [1]. Methanogens are the important members of microbiological consortia in natural environments, subterranean formations including petroleum reservoirs and also in marine and land animals, insects and human gut, peat bogs, waste streams, etc. Almost 80% of the earth's annual output has a biological origin, mainly from the enteric fermentations of animals and from rice paddy fields, marshlands and swamps [2]. Unidentified methanogens have been isolated from thermal environments, salt lakes, acid peat bogs, human dental plaques, termites, cockroach hind gut, rice fields and thermal reactors swine manure [3]. Thus the presence of methanogens has been demonstrated from a wide variety of ecological niches. The method of culturing methanogens can be classified as culture dependent and culture independent system. The breakthrough in an attempt for culturing anaerobic microorganism came with the Hungate anaerobic methods [4] and from there on variant modifications were made in accordance with convenience and requirements of studies [5]. Anaerobic jars are used primarily with plated media; reliable results from any anaerobic jar require adequate replacement of oxygenated environment with an anaerobic atmosphere. [6]. Anaerobic digestion is an effective biotechnological process for treatment of different organic waste with production of biogas, new pathway of bio methane production was studied based on the metabolism of hydrogenotrophic bacteria where up to 30% of biomethane can be synthesized [7]. It's almost a forgotten space technique where these hydrogenotrophic microbes grow in a closed loop where they act as supercharged carbon recyclers [8], thus giving a promising approach to exploit their potential to use them as a future space food.

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II. AIM AND OBJECTIVES

To determine the nutritional aspects of Hydrogenotrophs to be used as a food supplement in space travel

- Collection of soil sample.
- Anaerobic cultivation of Hydrogenotrophs.
- Isolation and identification of the isolates.
- Synthesis of biomass.
- Estimation of carbohydrate and protein content.

III. MATERIALS AND METHODS

Isolation of Hydrogenotrophs:
Soil samples from five different locations inside the Pigsty of Madras Christian College were collected and the serially diluted samples were plated on Minimal agar media and plates were incubated in an anaerobic jar connected to conical flask with the gas pack. (Figure.1)

Figure.1 Anaerobic jar – experimental setup

Characterization of the isolates:
The bacterial isolates were identified based on colony morphology and gram staining. Biochemical tests were performed for the confirmation of the isolates based on Bergey's manual of Determinative Bacteriology, [9].

Estimation of Carbohydrates

Qualitative method
To 200µl of sample, 100µl of Molischs reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish colour indicated the presence of carbohydrates.

Quantitative test
The total sugars produced by the isolates were determined by reaction with orcinol and sulphuric acid at 95°C and by the measurement of the resulting yellow colour at 420nm.[10]

Estimation of Protein
Biuret method is the commonly used method for determining the total protein in a sample. The principle is based on the formation of a Cu^{2+}-protein complex, which produces a violet coloured product measured by spectrophotometer at 540nm. The culture supernatant was taken by centrifuging at **2500**rpm for 3minutes and the protein content was estimated.

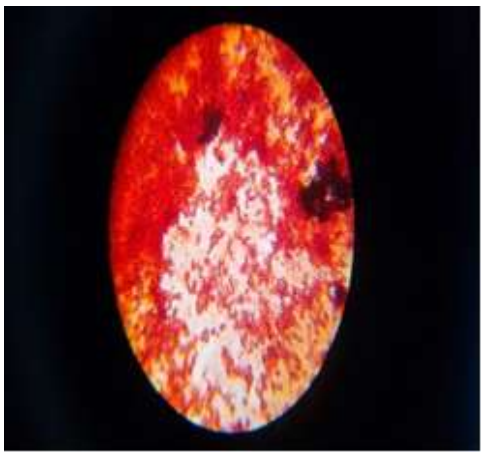
IV. RESULTS:

1. **Characterization of the isolates were based on the preliminary and Biochemical tests (Table no.1)**

Table no.1: Morphological and Biochemical characterization

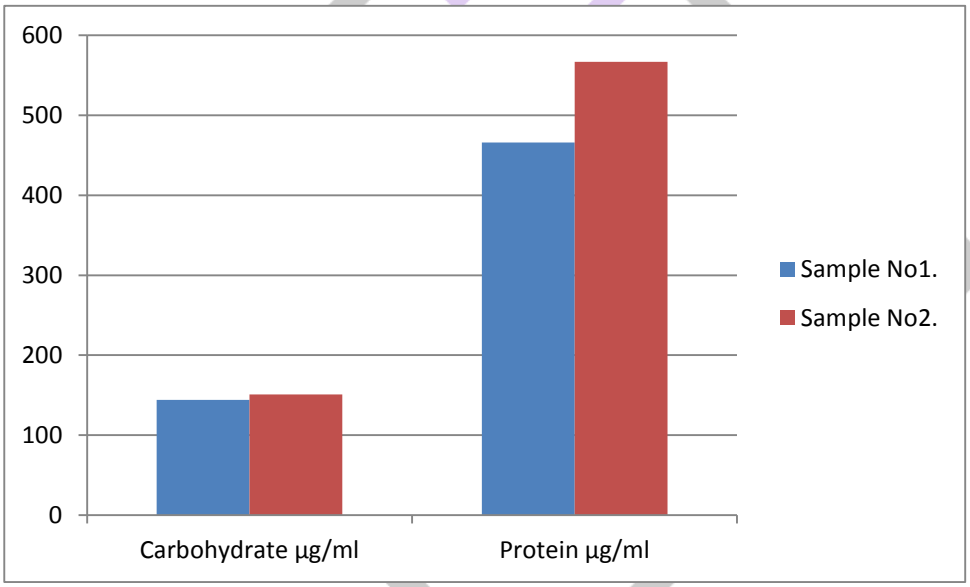
TEST	SAMPLE
Gram staining	Gram negative(sometimes Gram variable), spheroid shaped in aggregates
Colony morphology	Pink coloured colony
Motility	Non-motile
Catalase	Negative
Oxidase	Negative
Indole	Positive
Methyl red	Positive
Vogues proskauer	Negative
Spore staining	Negative
Citrate	Positive
TSI	Positive , acid and gas production
Glucose	Positive
Sucrose	Positive
Mannitol	Negative
Lactose	Positive
Organism identified	Methanosarcina

Figure: 2 Gram staining of Methanosarcina



2. Total Carbohydrates and Protein estimation

S. No.	Carbohydrate **µg/ml**	Protein **µg/ml**
Sample No 1	144	466
Sample No 2	151	567



V. DISCUSSION:

Global population is growing and is expected to reach 10 billion by 2050, and larger global population of course equals larger demands for food. We have been facing world hunger for quite sometimes now, and it is becoming clear that it is necessary to develop advanced technologies that would enable quantities of food large enough, without harming environment. Hydrogenotrophs being a good food source it can used for future space travel where the astronauts could carry them aboard and synthesis their own food .As these organism were known to utilise carbon dioxide and grow in a minimal growth requirement as suggested by Metcalf et al., [11], isolation of these organisms were done using minimal salt agar medium. Also isolation of methanogens was attempted from Pigsty of the college campus as done by Ramraj Boopathy from swine manure in the year 1996 [3]. In a study conducted by Balch etal., in the year 1976 [12], Methanosarcinabarkeri (grown on methanol) contained 41.1 nmol of CoM/mg of protein, compared to the 44.4 nmol of CoM/mg of protein found with the bioassay, while Methanobacterium thermoautotrophicum contained 7.5 nmol of CoM/mg of protein, compared to the 6.7 nmol of CoM/mg of protein found with the bioassay. The Methanospirillum hungatei data were not as consistent; this organism contained 19.4 nmol of CoM/mg of protein, compared to the 3.9 nmol of CoM/mg of protein reported previously. In the present study, Hydrogenotrophic bacterial biomass was found to be rich in carbohydrate and protein content. These organisms were able to synthesis carbohydrate content of 144 µg/ml of biomass and a protein content of 466µg/ml of biomass with such a simple supporting system.

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