ISOLATION AND CULTURING OF PHOTOBIONTS FROM ANTARCTIC LICHEN

Project Work submitted to the Central University of Punjab

For the award of

Masters of Sciences

In

Life Sciences

Specialization in Plant Sciences

BY

SAYANYA ACHARYA

Supervisor

Dr. Felix Bast

Department of Plant Sciences

School of Basic and Applied Sciences

Central University of Punjab, Bathinda

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DEDICATIONS

To My Father Amal Acharya and
My Mother Sriparna Acharya

CERTIFICATE

I declare that the project work entitled "**Isolation and Culturing of Photobionts from Antarctic Lichen**" has been prepared by me under the guidance of Dr. Felix Bast, Assistance Professor, Department of Plant Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this project work has framed the basis for the award of any degree or fellowship previously.

SAYANYA ACHARYA

Department of Plant Sciences,

School of Basic and Applied Sciences,

Central university of Punjab, Bathinda-151001.

Date:

CERTIFICATE

I certify that SAYANYA ACHARYA has prepared her project work entitled "**Isolation and Culturing of Photobionts from Antarctic Lichen**", for the award of M.Sc. degree of the Central University of Punjab, under my guidance. She has carried out this work at the Department of Plant Sciences, School of Basic and Applied Sciences, Central University of Punjab.

Dr. Felix Bast

Department of Plant Sciences,

School of Basic and Applied Sciences,

Central University of Punjab, Bathinda-151001.

Date:

ABSTRACT

ISOLATION AND CULTURING OF PHOTOBIONTS FROM ANTARCTIC LICHENS

Name of the Student- Sayanya Acharya

Registration no.-16mslsps06

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Name of Centre- Plant Sciences

Name of School- Basic and Applied Sciences

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Lichen is the symbiotic association in between algal and fungal partner. Lichens having various economic importance. The main objective is to isolate and culture of the photobionts from the lichen samples collected from Antarctica. *Trebouxia* sp. is extremely psychrophilic and fastidious in growth, found in the lichen samples. Both samples belong to genus *Umbilicaria*, *Umbilicaria antarctica* and *Umbilicaria aprina*. In the lichen samples Gyrophoric acid is found as secondary metabolites. First to isolate the photobionts, we use micromethod and for the culturing use 3M BBM media for 45-60 days at 22-25^{o} C temperature. Then subculturing process is done and followed by DNA extraction. The extracted DNA was kept for further use in sequencing purpose. The isolated and cultured photobionts can be further studied using DNA sequencing, SEM, and confocal microscopy to get a clear idea about the photobionts and its internal organelles; these will help in proper identification of the photobionts as well as the lichen. Further phylogenetic studies of photobionts and co-evolutionary studies between photobionts and mycobionts which may be done by extensive taxa sampling and through use of molecular systematics techniques.

Sayanya Acharya

Dr. Felix Bast

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Sayanya Acharya

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LIST OF ABBREVIATIONS

Abbreviation	Name
f	Foot (feet)
µl	Micro liter
ml	Milliliter
mg	Milligram
cm	Centimeter
M	Molar
mM	Millimolar

CHAPTER 1

INTRODUCTION

Lichen is an organism where fungi (mycobiont) and a photosynthetic partner (photobiont) associate symbiotically. The photosynthetic partner may be algae or cyanobacteria or both. From the genetic and evolutionary perspective, lichens can certainly not be regarded as an individual entity and for this reason; there are many implications for many investigations such as developmental and reproductive studies. On the other hand, its ecology is unique from all others that are why some author considered that lichen as a miniature ecosystem or micro-ecosystem.

The nature of the lichen symbiosis is widely debated and deserves further investigation. Most refer lichens as a classical example of mutualism where all partners benefit. Alternatively, lichens are regarded as an example of controlled parasitism because the mycobiont partner seems to obtain most of the benefits and the photobiont may grow more slowly in the lichenized state than when free-living (Ahmadjian 1993). The lichen symbiosis typically involves a close physiology. The dominant mycobiont is heterotrophic and derives its nutrition from photobiont, the flux of carbohydrates, as polyols in case of green algal lichen and glucose in case of cyanolichens (Smith and Douglas 1987). Another result of close physiological integration is the occurrence of a wide range of secondary metabolites which are unknown in free-living fungi and that’s why their occurrence adds to the uniqueness of the lichen symbiosis.

The morphology of lichen is strongly influenced by photobiont, though in nature there are few cases, where the same mycobiont (using molecular techniques) is able to form two very different, interconnected thalli, respectively, cyanobacteria and a green alga (Armaleo and Clerc 1990). This different morphology is named as Photosymbiodemes. The mycobiont may completely envelop the photobionts and particularly in the case of green algae, penetrate the surface of the photobiont with a structure called haustoria because haustoria are sometimes along with the

phycobiont cells and because parasitic fungi often procedure haustoria (Ahmadjian 1993), that's why it interprets lichenization as an example of controlled parasitism.

Lichen can grow almost all surfaces and for this reason, they can help in primary succession. Approximately 8% of Earth's surface is covered by lichen (Hait, 2010) and 20,000 species are found till date. Lichens having a wide range of shape and forms and according to the shapes and forms, lichens are grouped by thallus type.

Common grouping of lichen thallus growth forms are (Nash, 2008):

a. FRUTICOSE - growing like a tuft, leafless small shrub, upright or hanging down, 3D branches with nearly round cross-section or flattened.
b. FOLIOSE - growing in 2-dimensional, flat, leaf-like lobes.
c. CRUSTOSE - crust-like, adhering tightly to a surface like a thick coat of paint.
d. SQUAMOSE - formed of small leaf-like scales crustose below but free at the tips.
e. LEPROSE – powdery e.g., (*Lepraria*)
f. GELATINOUS – jelly like
g. FILAMENTOUS – tough or like matted hair
h. BYSSOID – wispy, like teased wool
i. STRUCTURELESS

On the basis of the substratum, the lichens are grouped in the followings (Bhat et al., 2011)

a. CORTICOLOUS - grows on the surface of trees (e.g., cryptothecia)
b. FOLLICOLOUS – grows on the surface of leaves (e.g., *Eugenia racemoides)*
c. SAXICOLOUS – grows on the rock surfaces (e.g., *Rocella tinctoria*)
d. TERRICOLOUS – grows on the soil (e.g.,*Peltigera*)
e. MUSCICOLOUS – grows on the mosses (e.g.,*Cladonia rangiferina*)

f. LIGNICOLOUS – grows on wood from which bark has been stripped (e.g., *Cheiromycina*)

Photobionts:

Lichen mycobints provide shelter to the photobionts and photobionts provide mycobionts simple sugar. Photobionts are normally belonging to cholorophyta or cyanobacteria. 90% of all lichens associated with a green-algal photobiont and only 10% are with cyanobacteria. Till date about 100 species of photobionts are identified and the most common are from four main groups- green alga (*Trebouxia* & *Trentepohlia*) and cyanobacteria (*Nostoc* & *Scytonema*). Mycobionts are specialized on particular photobionts. Typically lichen fungi only associated with a very narrow range of related species, though mycobiont may associate flexibly with different photobionts on the changing of their environmental situation.

The economic importance of lichens (www.biologydiscussion.com)**-**

1. Ecological significance:
   a. Pioneer colonizers- lichens are said to be the pioneers in establishing vegetation on bare rocky areas (lithosere). Lichens play an important role in nature in the formation of soil by forming acids (oxalic acid, carbonic acid etc.). This phenomenon is called as pedogenesis.
   b. Role in environmental pollution- lichens are very delicate to environmental pollutants; the lichens can be used as reliable biological indicators of pollution.
2. Lichen as Food:

   Cetraria islandica (Iceland moss) is taken as food in Sweden, Norway, Scandinavian countries, Iceland etc.

   Lecanora esculanta is used as food in Israel & *Umbilicaria esculanta* in Japan.

In France, the lichens are used in confectionery for making chocolates and pastries.

3. Lichen as fodder:

   Cladonia rangiferina (reindeer moss) is main food for reindeers in polar countries.

   Cetraria islandica is used as fodder for horses.

   Everia, Parmelia, and *Lecanora* are also used as fodder.

4. Source of medicines:

   Lichens are used to cure jaundice, fever, diarrhea, epilepsy, hydrophobia and other skin diseases. Lichens having great medicinal value-

   Table 1. Some lichens with their medicinal uses:

Lichen names	**Diseases remedy**
Lobaria pulmonaria & *Cetraria* spp.	In respiratory diseases particularly tuberculosis.
Usnea barbata	For strengthening hair and for uterine ailments.
Xanthoria parietina	For jaundice
Cladonia spp.	For a whooping cough
Peltigera canina (dog lichen)	For hydrophobia
Roccella montagnei (Erythrin)	In angina, a serious heart disease
Parmelia saxatilis	For epilepsy
Evernia spp. & *Cladonia* spp.	To control fever

   Usnic acid (from *Usnea* and *Cladonia*) used as antibiotic and treatment for various infection. Also effective against as gram-positive bacteria and also having anti-tumor property.

5. Lichen used in industrial purpose:

   a. Tanning & dyeing:

      Cetraria islandica and *Lobaria pulmonaria* show the astringent property.

Orchil, a blue dye obtained from *Roccella* and *Lecanora*, used to dye woolen articles and silk fabrics. Purified Orchil (Orcum) used as a biological stain.

Litmus, which is used as an acid-base indicator, is a dye obtaining from *Roccella tinctoria* and *Lasallia pustulata*.

b. Cosmetics and perfumes:

Evernia, Ramalina, Pseudorina having volatile oils, used as perfumes.

c. Brewing and distillation:

Cetraria islandica contain carbohydrates in the form of lichenin, and from this alcohol is produced in Sweden and Russia.

d. Minerals:

Lecanora esculanta is found in lime stone deserts and yields a large amount of calcium oxalate crystals. These are 60% of its dry weight.

6. Natural products:

Table 2. Some lichens and the natural products found from them-

Products	**Produced from**
Salazinic acid	*Ramalina siliquosa*
Squamatic acid	*Cladonia crispate*
Lecanoric acid	*Parmelia subrudecta*

7. Poisons from lichens:

Table 3. Some lichens and the poisons produced-

Lichen	**Poisonous due to**
Letharia vulpina (wolf moss)	Vulpinic acid (used as a poison for wolves)
Cetraria juniperina	Pinastrinic acid
Parmelia molliuscula	Selenium
Xanthoria parietina	Beryllium
Evernia furfuracea	Chlorine

Harmful aspects of lichen:

1. Lichens growing on young fruits trees and sandal trees are harmful to plant.
2. During summer some species of lichens (e.g. *Usnea barbarata*) become so dry and inflammable that they often help in spreading forest fire.
3. Some lichens act as allergens.
4. The commercial value of glass and marble stone is reduced because of itching of their surface by lichens.
5. Some lichens e.g., *Cladonia rangiferina*, *Cetraria islandica* accumulate large quantities of radioactive strontium and caesium from atomic fall-outs. The radioactive heavy elements may be incorporated in the food chain, lichen->reindeer-> man, leading to their accumulation in human tissues.

Antarctica is world's south most part, and this is one of the coolest places in the whole world. The lowest temperature is recorded -89.2^{o}C till now, being a cold dessert the average rainfall is 166mm. There are 163 research station in Antarctica till dated. Biodiversity of Antarctica is very low, terrestrial vertebrates (like springtails, lice, nematodes etc.), sea life (e.g. penguin, blue whales, orcas, colossal squids and fur seals etc.), fungi about 1,150 types, among which 750 non-lichenized and remaining lichen-forming. Plants largely consist of bryophytes (e.g. *Colobanthus quitensis, Deschampia antarctica* etc.); other organisms are mostly alga, phytoplankton, multicolored snow algae, and diatoms.

Three main types of lichen exist in Antarctica, crustose (*Rhizocarpon geographicum*), foliose (*Umbilicaria esculanta*) and fruticose (*Cladonia rangiferina*). In the most favorable conditions in the maritime Antarctica, growth rates reach 1cm or more per 100 years (too much slow growth rate of lichens). In the harsher environment of the continental Antarctica, growth is much slower. 500 lichen species are identified from Antarctica and South Georgia till now.

Lichens have been collected from as far south as $86^{0}\,30^{/}$.

In continental Antarctica, many lichens are able to absorb water vapour from show and ice.

OBJECTIVES

1. Morphological, chemical and anatomical analysis of the collected lichen samples.
2. Isolation and culturing of photobiont from Antarctic lichens.

CHAPTER 2

REVIEW OF LITERATURE

2.1 National status of the review

According to my best knowledge, there are just two studies have been done till date on photobiont identification on the basis of DNA sequencing data. The studies state that *Asterochloris* sp. is in association with *Cladonia* sp.(Ridka et al.,2014) & Foliicolous lichen with Chlorococcaceae (Shravan & Krishnamurthy,2016).

1.2 The international status of the review

There are many reports on isolation and culture of photobionts from lichens at the international level. The spray method was the first developed method by Wiedeman et al. in 1964. Micropipette method was then developed (Ahmadjian, 1967) and it was the first successful and one of the most used method till now. In this method the photobionts isolated from lichen by slicing & crushing and homogenate on agar medium. The cutting method (Yoshimura et al., 2002), Sucrose-KI gradient centrifugation method (Fontaniella et al., 2002). Gasulla et al.(2010), was the most accepted method still, was developed for isolation of photobionts including homogenization of thallus, centrifugation through Percoll, washing with Tween-20 and sonication.

Photobionts of various lichens have been identified worldwide by morphological studies of vegetative cells, plastids, pyrenoids using light microscope and Transmission Electron Microscope (TEM) and molecular systematics techniques.

Table 4. List of mycobionts with their photobionts from Antarctica:

Mycobionts	Photobionts	References
Acarospora macrospora	*Chlorococcoid*	Lindsay,1978
Bryoria fremontii	*Trebouxia sp.*	Boluda et al., 2015
Buellia russa	*Trebouxia sp.*	Lindsay,1978
Caloplaca carina	*Trebouxia gigantea*	Amhadjian, 1993
Cladonia mitis	*Trebouxia glomerata*	Czeczllga, 2010
Cladonia rangiferina	*Trebouxia irregularis*	Ridka et al.,2014
Chenotheca phaeocephala	*Trebouxia arboricola*	Tibell L. & Beck A.,2002
Cetraria aculeate	*Trebouxia jamesii*	Farnandez-mandoza et al.,2011
Evernia sp.	*Trebouxia sp.*	Tschaikner et al., 2007
Lobaria pulmonaria	*Nostoc sp. & Dictyochloropsis reticulata*	Eymann et al.,2017
Pannaria lurida	*Nostoc sp.*	COSEWIC assessment & status report, 2016
Peltigera canina	*Nostoc punctiformis*	Czeczllga,2010
Peltigera didactyle	*Nostoc sp.*	Lindsay, 1978
Physcia sp.	*Trebouxia sp.*	Ahmadjian,1993
Placopsois contortuplicata	*Nostoc sp.*	Lindsay, 1978
Rhizocarpon atroflavescens	*Trebouxia gigantea*	Ahmadjian,1993
Rinodina sp.	*Trebouxia sp.*	Nadyeina et al.,2011
Usnea desopoga	*Trebouxia excentrica*	Ahmadjian,1993
Xanthoria parietina	*Trebouxia arboricola*	Ahmadjian,1993

CHAPTER 3

METHODOLOGY

3.1. SAMPLE COLLECTION

Lichen specimens are collected from Antarctica by Dr. Felix Bast. Samples were collected from Antarctica (fig-1). The moisture was soaked from the fresh samples by blotting sheets and kept in -20^0 C after neatly wrapping in aluminum foil.

3.2. SAMPLE IDENTIFICATION

The samples from Antarctica were identified based on the morphological study, chemical analysis, and anatomical studies. The results of these studies were analyzed and then the genus and species names were assigned to the collected samples. The herbarium were prepared and deposited in the University's herbarium.

Table:5 Lichen samples with the herbarium accession number:

S.No.	Sample	Sampling site	Herbarium accession no.
1.	*Umbilicaria antarctica* Frey & I.M Lamb	Antarctica	863-1
2.	*Umbilicaria aprina* Nyl.	Antarctica	994-1

3.2.1. MORPHOLOGICAL IDENTIFICATION

The samples were observed under Magnus MSZ-TR microscope and various external features on the upper and lower surface of the thallus were observed and photographs were taken. The whole thallus was photographed by Sony DSLR camera.

3.2.2. CHEMICAL ANALYSIS

3.2.2.1. **Chemical spot test** (Nayaka S., 2014)-

The chemical reagents commonly used for color spot test are aqueous potassium hydroxide (designated as K), bleaching powder or saturated aqueous solution of calcium chlorohypochlorite Ca(OCl)Cl (designated as C) and an aqueous solution of p-phenyl diamine (designated as Pd).

K test is performed on both upper surface and medulla by exposing with blade and C & Pd solution is placed on medulla. The color changes due to presences of particular lichen substances in lichen thallus.

In addition to these, KC solution (first KOH aq.solution then common bleach aqueous solution) is also applied, if the above all chemicals giving negative tests.

3.2.2.2. **Thin layer chromatography (TLC)** (Nayaka S., 2014)-

Many lichen samples are undetectable in color spot test. In such case, TLC must be performed. Lichens contain various secondary metabolites and other chemical substances, and these substances can be identified by doing TLC. First, 50mg of lichen samples were taken in acetone eppendrofs for 5mins. Thin aluminum plates which already precoated by silica gel were used as TLC plates, and loading line was drawn at 2cm from the base. 4 dots (2 for control at both ends and 2 for each sample). Solvent system A (Toluene: Dioxane: Acetic acid = 180:45:5) was used. The TLC plate was placed in TLC tank having a solvent system and allowed to run till the solvent touches the finishing line. After that, the plate was taken out and 10% sulfuric acid was sprayed and kept it in a hot air oven at 110^0 C for 5-10 minutes till dry. The spots were distinguished by their color and distance they traveled from the loading point. Rf value were calculated and compared with the control and TLC for lichens were referred. (Fig-2)

3.2.3. ANATOMICAL IDENTIFICATION

The thin vertical section (V.S.) of the thallus of the samples were cut by the blade manually and observed under 10X, 20X, 40X and 100X objectives of Olympus microscope CX41 and photographs were taken.

3.3. Protocols for isolation of the photobionts (Gasulla et al., 2010) -

Isolation is done through micropipette method. The protocols are as follows-

a. Weigh the samples (15-25 mg). Wash the samples tap water and distilled water thoroughly.

b. Lichen thallus were homogenized in sterile isotonic buffer (0.3M sorbitol in50mM, pH7.5) and resuspended them in a homogenizer and take them in 2ml eppendrof.

c. Filter the homogenized sample through sterile 'muslin' clothes. And then centrifuge the filtrate at 4000rpm for 5mins.

d. Resuspend the pallet in 200µl of sterile isotonic buffer and then add 1.5ml sterile Percoll (80%) in isotonic buffer.

e. Centrifuge at 13,500 rpm for 10mins. Three-layer formed (upper green layer, middle white layer, and lower thick debris), which took the middle layer which is just below the upper green layer.

f. The taken layer was diluted 2 folds and then centrifuges it in 6000rpm for 10mins.

g. Discard the supernatant and resuspended the pellet in 2ml of sterile distilled water and add a drop of tween 20.

h. The suspension was sonicated at 40 KHz for 1min and then centrifuge at 4000rpm for 5mins. Repeat the step for 5times.

i. The final pallet is composed of algae. Resuspend the cell muss and then count the number of cells through Haemocytometer.

3.4. Protocols for preparing media for photobionts culture (Bischoff, H.W. & Bold, H.C.; 1963)

BBM (Bold's Basal Media) was used for the culture of the photobionts. The composition of the media is as follows:

	STOCKS	**FOR 50ml SOLUTION**
1.	Sodium nitrate – $NaNO_3$ (3N)	3750mg
2.	Magnesium sulfate heptahydrate – $MgSO_4 . 7H_2O$	375mg
3.	Sodium chloride – NaCl	125mg
4.	Potassium phosphate monobasic – KH_2PO_4	875mg
5.	Potassium phosphate dibasic – K_2HPO_4	375mg
6.	Calcium chloride dihydrate – $CaCl_2 . 2H_2O$	125mg
	(Trace Elements Solutions for 10ml solution)	
7.	Zinc sulphate heptahydrate – $ZnSO_4 . 7H_2O$	88.2mg
	Manganese chloride tetrahydrate – $MnCl_2 . 4H_2O$	14.4mg
	Molybdenum trioxide – MoO_3	7.1mg
	Cupper sulfate pentahydrate – $CuSO_4 . 5H_2O$	15.7mg
	Cobalt nitrate hexahydrate – $Co(NO_3)_2 . 6H_2O$	4.9mg
8.	Boric acid – H_3BO_3	114.2mg
9.	EDTA	500mg
	KOH	310mg
10.	Ferrus sulfate heptahydrate – $FeSO_4 . 7H_2O$	49.8mg
	Conc. H_2SO_4	10µl

Table 6. For the preparation of the media:

STOCK	STOCK QUANTITY		
	Per 1lt.	**Per 500ml**	**Per 250ml**
Stock 1-6	10ml	5ml	2.5ml
Stock 7-10	1ml	500µl	250µl
Agar (1.2%)	12gm	6gm	3gm

The final cell density of photobiont suspensions was made up to 5 X 10^3 cells/ml by adjusting with sterile distilled water to a cell density of photobionts cells. 50µl of the suspension was taken as inoculation and spread on previously prepared Petri plates containing 3M BBM media with 1.5% agar and 5 Petri plates were prepared for each sample. Petri plates were kept in 12 hours photoperiod at a 20-25^{o}C temperature (avg.). After 45 days the number of colonies grown were counted. For subculturing of photobionts, colonies were picked and placed onto another Petri plates by using a sterile needle. The whole process was maintained in sterile condition.

Table 7. Name of lichen samples, their respective sampling sites with collection date, latitude-longitude, and altitude:

S. No.	Sample No.	Species	Collected by	Sampling site	Collection date	Latitude and longitude	Altitude (f.)
1.	863-1	*Umbilicaria antractica* Frey & I.M.Lamb	Dr. Felix Bast	Antarctica	14.01.2017	69° 23' 26.862" S 76° 14' 27.9636" E	592
2.	994-1	*Umbilicaria aprina* Nyl.	Dr. Felix Bast	Antarctica	07.03.2017	70° 45' 41.2236" S 11° 46' 45.1955" E	250

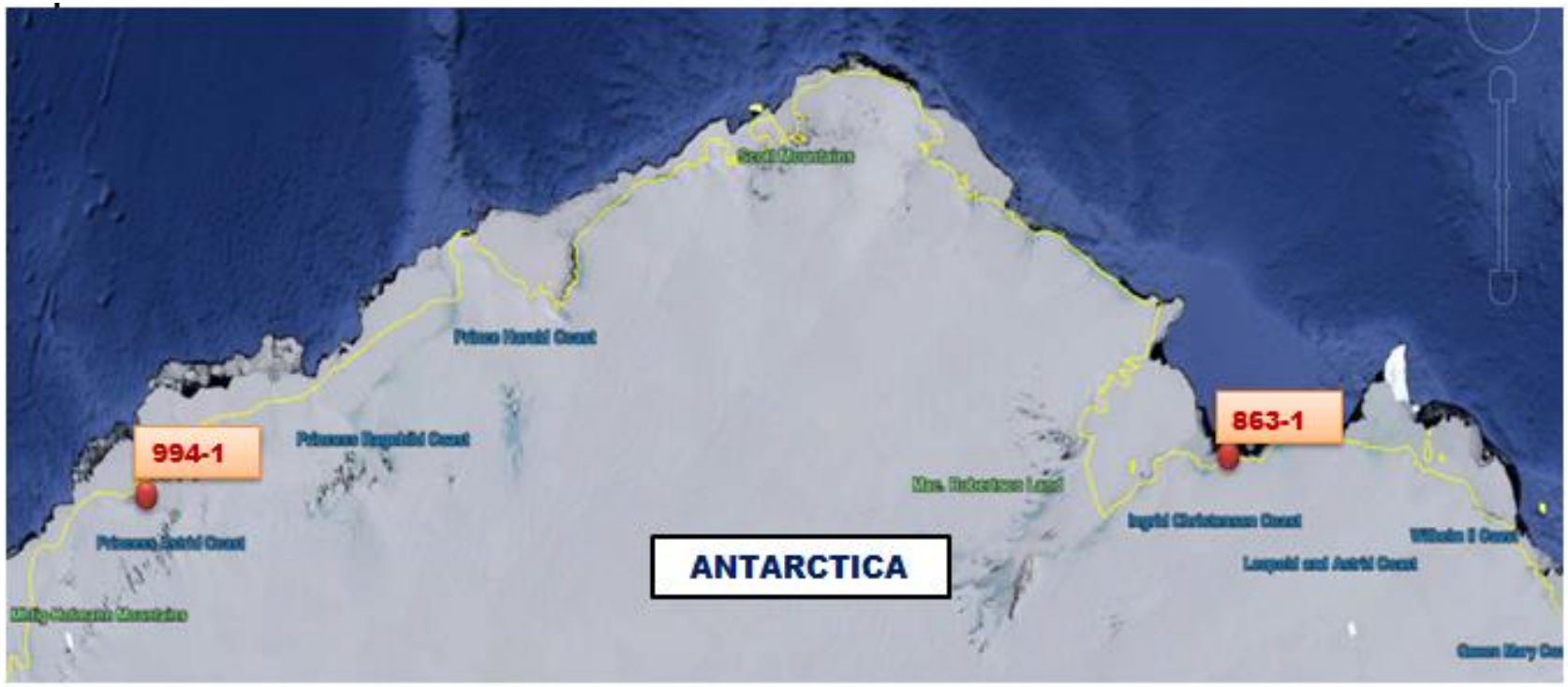

Figure 1.- Showing the sampling sites of the lichens in Antarctica

CHAPTER 4

RESULTS

4.1. Morphological identification of the samples:-

The morphological studies of the collected samples consisted of both micro and macroscopic observation of the thallus to note the relevant morphological and anatomical features. Color spot test, TLC, and morphological study of the isolated and cultured photobionts. The morphological studies of the collected samples are as follows:

4.1.1. Important identifying characteristics of *Umbilicaria.*

4.1.1.1. Morphological characters-

Thallus foliose, umbilicate, corticated on both sides. Upper side smooth or wrinkled. Lower side either smooth or with rhizines or ridges, in most species with thallospores. A cosmopolitan genus, growing on rocks (called rock lichen) or very rarely on wood, occurring mostly in cold and mountains. This genus is widespread and often found in Antarctica region (but surprisingly rare on South Georgia).

4.1.1.2. Anatomical characters-

Photobionts are green in color. Ascomycota apothecia, laminal, without thalline exciple, black; lecidine, gyrose or umbonate. Asci with an apical dome, 8 spored. Ascospores are simple or muciform, colourless or brownish. Hamathecium of paraphyses, simple or branched. Conidia cylindrical. Thallospores, simple to multicellular.

4.1.2 *Umbilicaria antarctica* Frey & I.M. Lamb (863-1)-

Systematics position of this lichen:

Kingdom- Fungi

Division- Ascomycota

Class- Lecanoromycetes

Family- Umbilicariaceae

Genus- *Umbilicaria*

Species- *U. antarctica*

Morphological features:-

1. Thallus irregular, to 30 or more cm. across, but usually 5-15 cm, usually monophyllous.
2. Upper surface grey to brown-grey. Lower surface black, with rhizines and thallospores.
3. Rhizines simple to dichotomously branched, a few strap-like. Thallospores simple, 5-8 µm in diameter, produced in columns.
4. Widespread and abundant, especially on sheltered moist rock faces, where it often provides almost completely cover, predominantly in coastal sites, also commonly on dry boulders and stones in scree, boulder fields, moraines and fellfield habitats.

Photobionts:- Green, spherical in structure and cell wall is prominent, unicellular. Photobiont is *Trebouxia* sp.

4.1.3 *Umbilicaria aprina* Nyl. (994-1) -

Systematic position of the lichen:

Kingdom- Fungi

Division- Ascomycota

Class- Lecanomycetes

Family- Umbilicariaceae

Genus- *Umbilicaria*

Species- *U. aprina*

Morphological features:-

1. Thallus irregular, usually monophyllous, to 5-10 cm across.
2. Upper surface pale to dark gray to brown-grey, smooth to rugulose to weakly ridge.
3. Lower surface dark grey to black, sometimes motted, with rhizines and thallospores.
4. Rhizines simple to branched, never strap-like, but sometimes broadened in connection with ramifications, dark in basal part and pale in the upper part.
5. Thallospores evenly developed, usually unicellular but sometimes bi-cellular, 4-6µm in diameter.
6. Widespread and locally abundant on a rock, usually boulders and stones. Typically in habitats flushed by melt water, and at margins of temporary melt streams. From low altitude coastal sites to high altitude in land sites in continental Antarctica.

Photobionts: Green, unicellular, ellipsoidal in shape. The photobiont belongs to genus *Trebouxia* sp.

4.1 **Table 8. Results of color spot-test of lichen samples:**

S.no.	Sample no.	Species names	Color spot tests				Secondary metabolites present
			10% K^+	C^+	KC	Pd	
1.	863-1	*Umbilicaria antarctica*	_ve	_ve	_ve	_ve	Gyrophoric acid
2.	994-1	*Umbilicaria aprina*	_ve	_ve	_ve	_ve	Gyrophoric acid

4.2 **Table 9. Results of TLC test:**

S. No.	Sample no.	Specimen	Types	TLC result
1.	863-1	*Umbilicaria antarctica* Frey &I.M. Lamb	Foliose	Gyrophoric acid Rf-3
2.	994-1	*Umbilicaria aprina* Nyl.	Foliose	Gyrophoric acid Rf-3

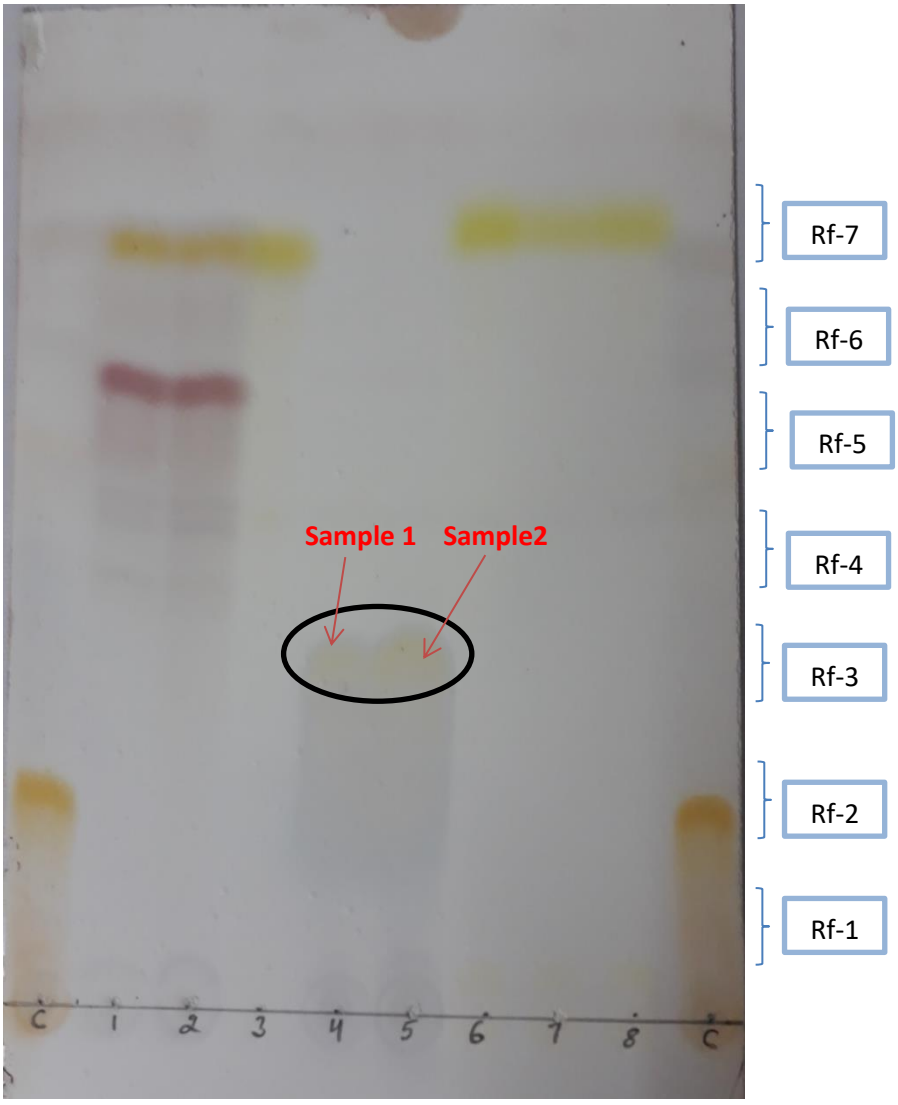

Figure 2. Showing TLC plate. The plate was ran using solvent system A for 40 minutes. Control sample (C) – *Parmelinella wallichiana,* spot 4 – *Umbilicaria antarctica*, and spot 5 – *Umbilicaria aprina*.

Morphological and anatomical images of *Umbilicaria antarctica* (863-1)

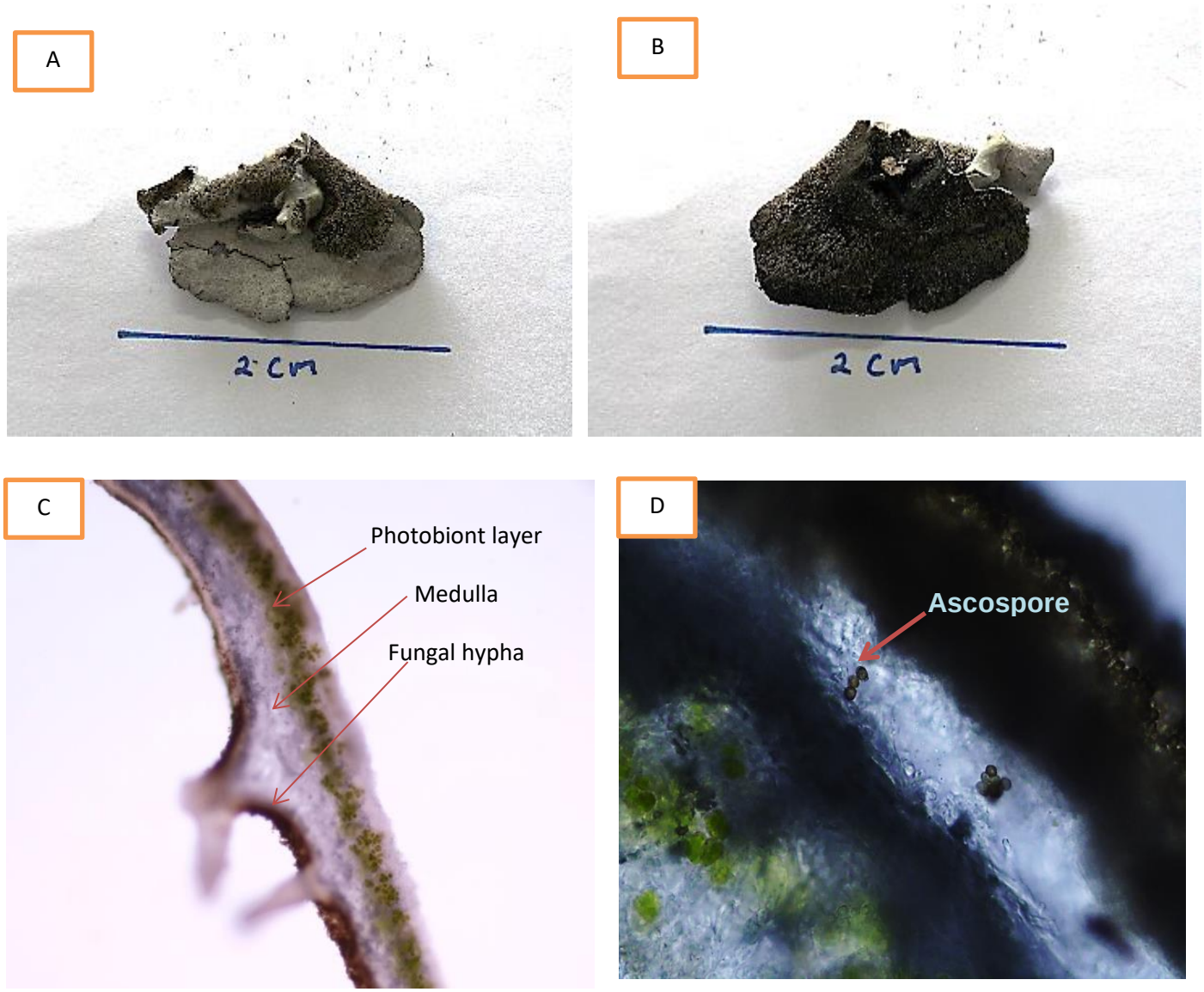

Figure3. *Umbilicaria antarctica*: A–showing upper portion of the thallus; B – showing the lower portion of the thallus with rhizines and umbilicate structure. C – showing an anatomical section of thallus; D – showing photobionts and spores in an anatomical section of the thallus.

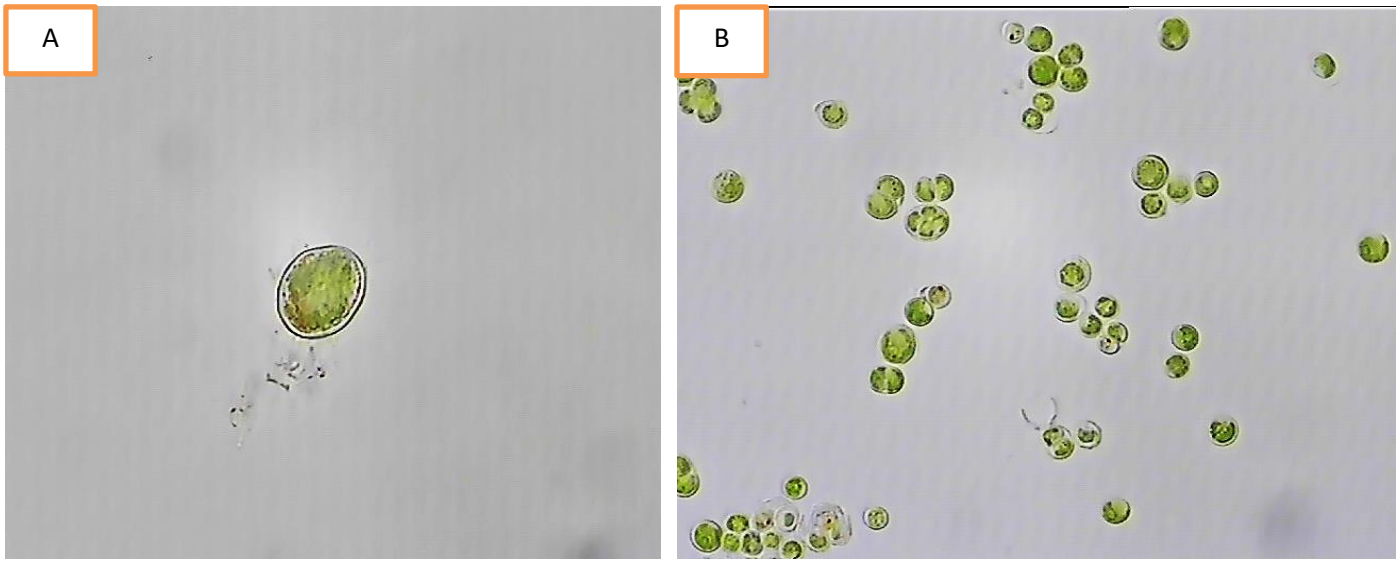

Figure4. A and B showing isolated and cultured photobionts of *Umbilicaria antarctica.*

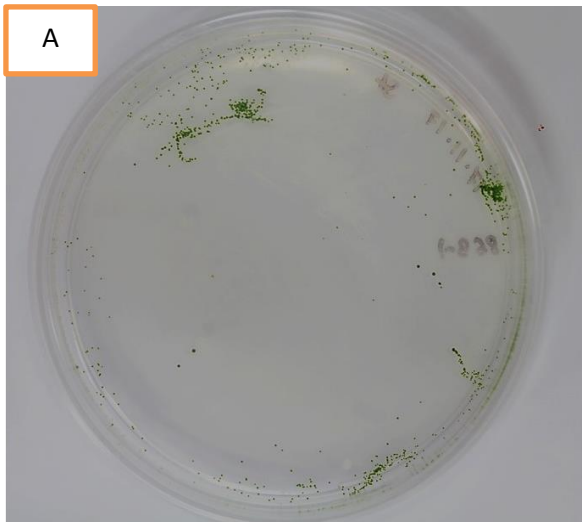

Figure 5. A. showing primary cultured petri dish of photobionts from *Umbilicaria antarctica*

Morphological and anatomical images of *Umbilicaria aprina* (994-1)

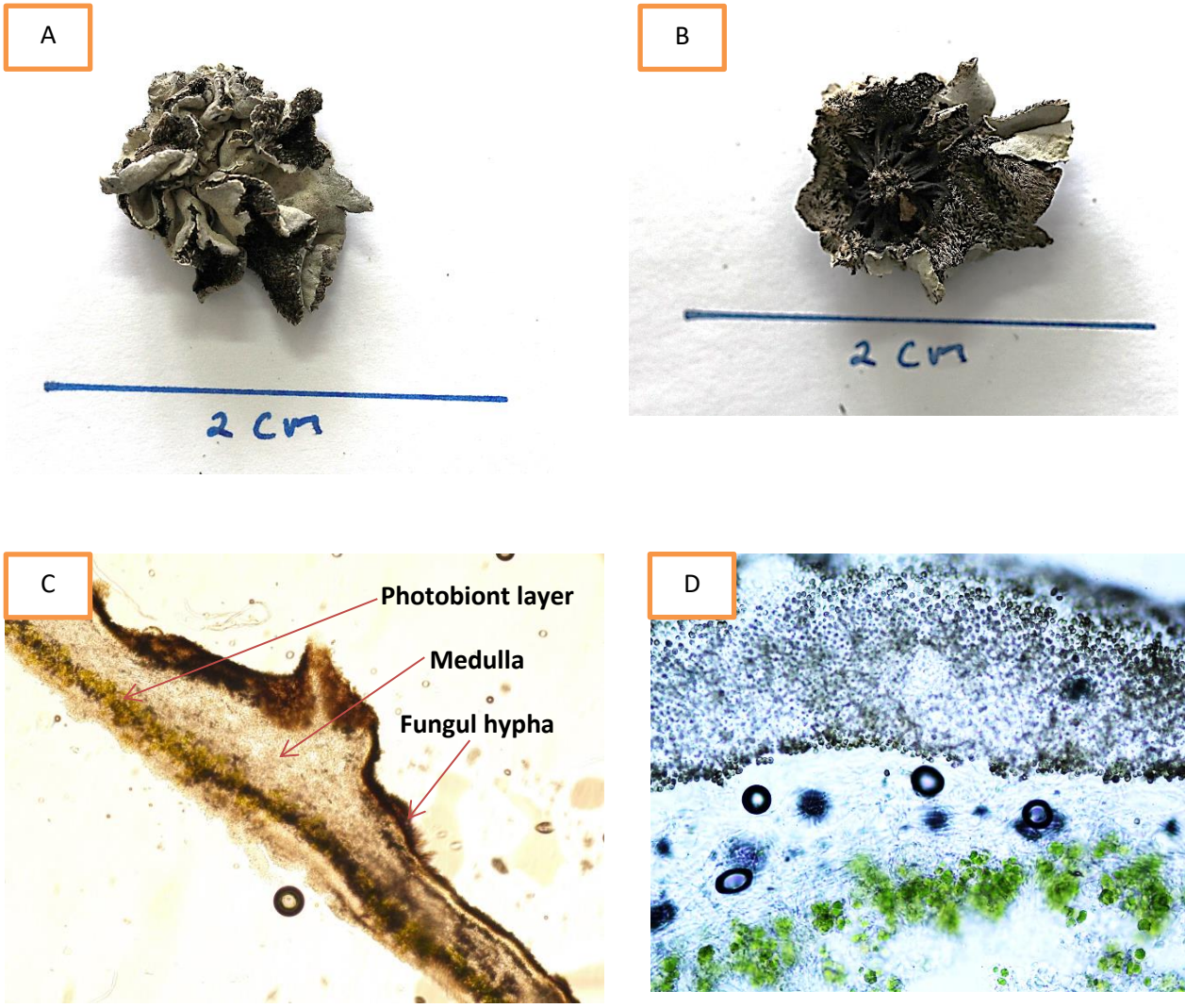

Figure 6. *Umbilicaria aprina*: A – showing upper portion of the thallus; B – showing the lower portion of the thallus with rhizines and umbilicate structure. C – showing an anatomical section of thallus; D – showing photobionts in an anatomical section of the thallus.

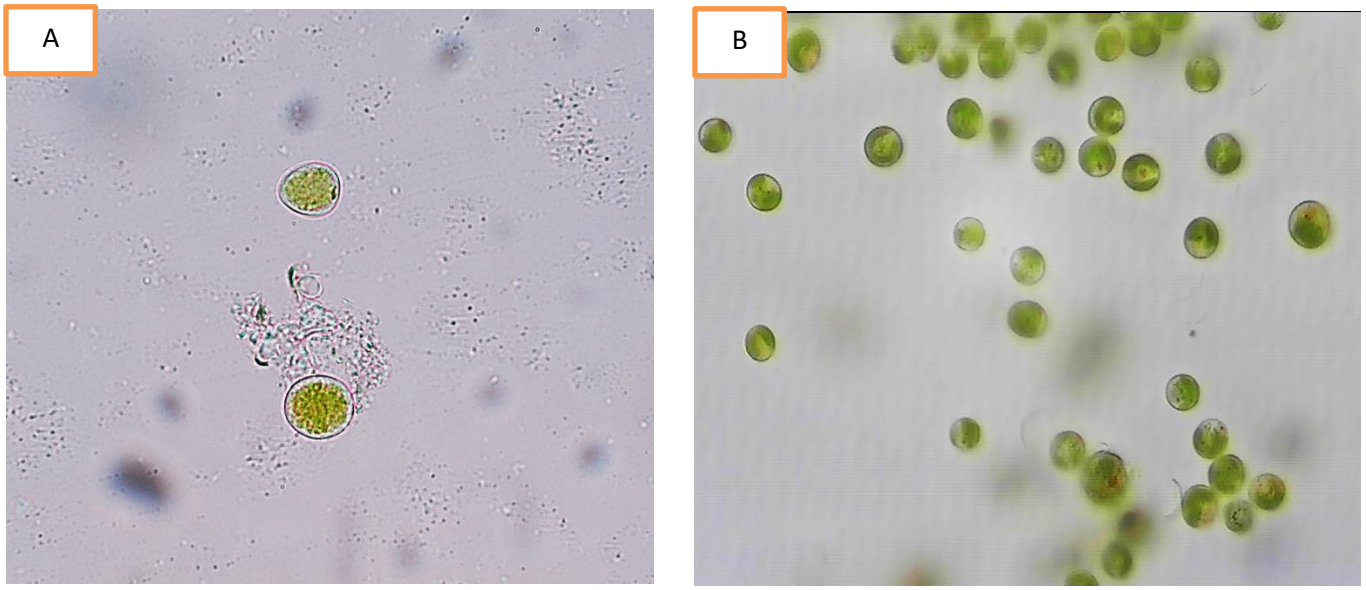

Figure7. A and B showing isolated and cultured photobionts of *Umbilicaria aprina.*

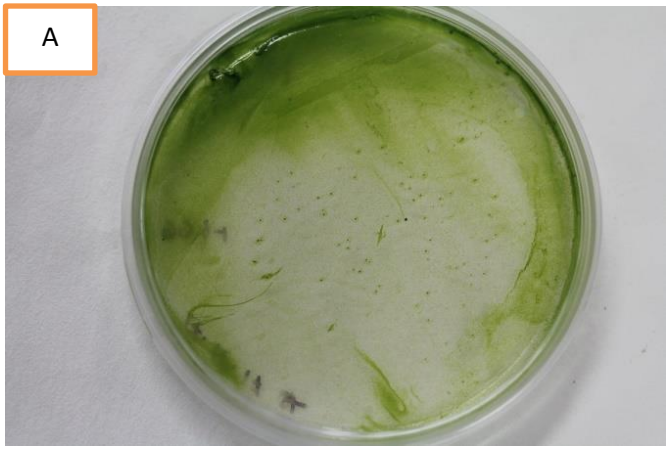

Figure8. A. showing primary cultured petri dish of photobionts from *Umbilicaria aprina.*

CHAPTER 5

DISCUSSION

The lichen samples were foliose type belongs to ascomycetes fungi. Umbilicaria grows on rock (rock lichen), and having umbilicate structure; the lower side of umbilicate having rhizins, this is why this lichen is named as *Umbilicaria* sp. Gyrophoric acid was found in the lichen, and formerly known as *Gyrophora* sp. Photobionts were isolated from the lichen samples and cultured and DNA was extracted from the photobiont samples. Photobiont is *Trebouxia* sp. (Romeike et al.,2002). *Trebouxia* is very fastidious to grow in-vitro, optimum temperature and conditions should be maintained. First, the fungal or bacterial contamination should be removed and then standardize a protocol to culture *Trebouxia* sp.. *Trebouxia sp.* can easily grow in 3N BBM agar media and this genus is unicellular, oviform and spherical shaped cells, cell size normally 5-7µm in diameter. Results of color spot tests were negative in the lichen *Umbilicaria sp.* two photobionts were cultured and identified. Both the lichens and photobionts already identified, but separately identified, not association between the specific genus of *Umbilicaria sp.* and *Trebouxia sp.*

FUTURE PERSPECTIVE

The isolated and cultured photobionts can be further studied using DNA sequencing, SEM, and confocal microscopy to get a clear idea about the photobionts and its internal organelles; these will help in proper identification of the photobionts as well as the lichen. Further phylogenetic studies of photobionts and co-evolutionary studies between photobionts and mycobionts which may be done by extensive taxa sampling and through use of molecular systematics techniques.

CHAPTER 6

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