Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR

A PROJECT REPORT ON –

"Isolation and characterization of agarolytic bacteria associated with Rhizosphere of SPINACH"

Submitted to,

DEPARTMENT OF BIOTECHNOLOGY

Submitted by,

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B.SC.III BIOTECHNOLOGY (ENTIRE)

Exam Seat No. – 9305

Under The Guidance of,

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Year, 2021 – 2022

"Education for Knowledge Science and Culture"

KOLHAPUR

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VIVEKANAND COLLEGE, KOLHAPUR, (AUTONOMOUS)

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Certificate

This is to certify that PRANALI PRATAPSINH BHOITE

Exam Number 9305 **has satisfactorily carried out his project report as per the syllabus prescribed by Bo'S Department of Biotechnology, Vivekanand College (Autonomous) for B.Sc- III Biotechnology (Entire).This project report represents his/her bonafied work during academic year 2021-2022.**

Place: Kolhapur

Date: 27/05/22

VIVEKANAND COLLEGE ESTD. JUNE 1964 KOLHAPUR-416 003

Examiner

Teacher in charge

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DECLARATION

I hereby declare that the project work entitled "**Isolation, characterization of agarolytic bacteria from associated with rhizosphere of spinach**" submitted to the Vivekanand College, Kolhapur for the award of the degree of "Bachelor of Science in Biotechnology" is the result of bonafied work carried out by me under the guidance of Asst/Prof Mr. A.L. Upadhye. I further declare that the results presented here have not been the basis for the reward of any other degree.

Place: - Kolhapur

Date: 27/05/2022

Bhoite

Miss. Pranali Prathpasinh Bhoite

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Bhoite.

Miss. Pranali Prathpasinh Bhoite

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INTRODUCTION

INTRODUCTION

❖ INTRODUCTION: -

Agar is the main component of the cell wall of some species of algae that belongs to the phylum Rhodophyta. These algae are commonly known as agarophytes. The percentage of agar present in the cell wall of the algae varies according to the growth and environmental conditions; it is found to be that Agar is a polysaccharide found in the cell walls of some red algae and is unusual in containing sulfated galactose monomers.

Certain marine algae of the class Rhodophyceae, called Red Sea weeds are the source of this polysaccharide. Some of the chief algal sources are Gelidium cartilagineum, Gracilaria conferroides and Pteroclaia capillcea.

The structure and composition of the agar extract of Gelidium amansii showed that it is composed of two major fractions - agarose, a neutral polymer and agaropectin, a charged, sulfated galactan (Galactose, 3-6 anhydrogalactose). The ratios of these two polymers vary widely and the percentage of agarose in agar-bearing seaweeds ranges from 50% to 90%.

The composition of agar is discussed here with along with around 60% of the dry weight of the red algae consists of agar. Agar is made up of agarose and small molecules called agaropectin. Generally red algae are the one from

APPLICATION: -

- agarase used in the pharmaceutical industry.
- agarase exhibits anticancer and antioxidant activity.
- agarase used in cosmetic industry for moisturizing and whitening of skin.
- agarase used to prepare protoplast of marine algae and recover deoxyribonucleic acid (DNA)from agarose gel electrophoresis.

• STRUCTURE OF AGARASE

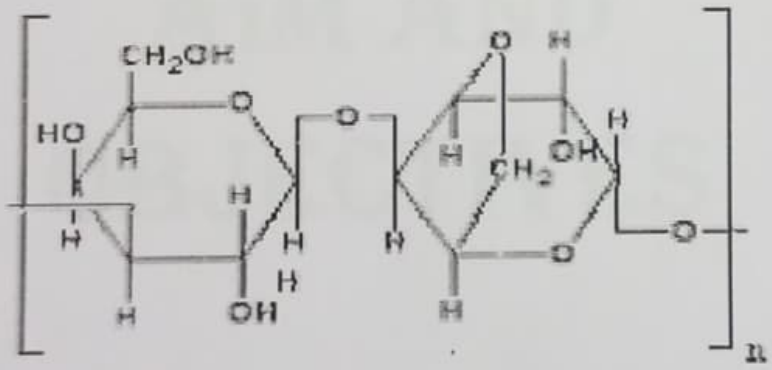

Figure : Structure of Agar and Agarose

Figure 1.

AIM AND OBJECTIVES

AIMS AND OBJECTIVES: -

1. To isolate agarolytic bacteria from soil of rhizosphere of spinach.

2. To optimization of culture condition for growth and agarase enzyme production.

3. To partially purify the enzyme by using ammonium sulfate Precipitation method.

❖ MATERIAL'S:

❖ Required Material:

- ✓ Chemicals:

  TSA medium, saline, lougols solution,2% peptone water, agar solution, distilled water.

- ✓ Glassware's:

  Conical flask, Petri plates, pipettes, test tubes, beaker, measuring cylinder, Spreader etc.

- ✓ Other requirement:

  Polythene bag, Nichrome loop, thread, cotton.

- ✓ Plant material:

  Spinach.

RESULTS AND DISCUSSION

❖ RESULT AND DISCUSSION:

- Enrichment Sample -

- Isolation Of Organisms:

  For isolation of agarolytic bacteria we used TSA medium, after 24 hrs. we got white colour colonies.

➢ Identification of organisms:

Colony character *of paenibacillus species* grown on tryptose soy agar medium for 24hrs.

Sr No.	Character	Result
1.	Size	0.2 mm
2.	Shape	Circular
3.	Colour	White
4.	Margin	Entire
5.	Elevation	Regular
6.	Consistency	Moist
7.	Opacity	Opaque

- Morphological characteristics:

Gram nature	Motility
Gram ~~negative~~ positive	motile

- Screening of agarolytic Bacteria:

- Gram staining and Motility of bacteria

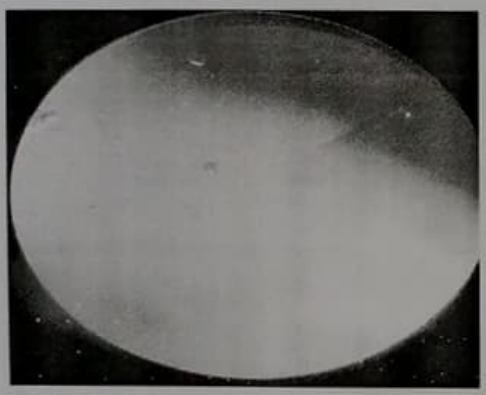

Fig. Motility

Fig. Gram staining

CONCLUSION

❖ CONCLUSION:

a. Agarolytic bacteria was successfully done.

b. Isolation of agarolytic bacteria on TSA medium was successful done.

c. Bacteria showed remarkable growth at 35 degree and pH = 7

❖ APPENDEX:

✓ Composition of TSA medium: for 100ml

a. Glucose	0.025gm
b. Peptone	0.17gm
c. K_2HPO_4	0.025gm
d. NaCl	0.05gm
e. Soybean powder	0.03
f. Agar	1.5gm
g. D/W	100ml

✓ Composition of lougal's solution:

Potassium iodide	1.0gm
Iodine	0.5gm
D/W	100ml