ISOLATION AND CHARACTERISATION OF THERMOPHILIC BACTERIA FROM TATTA PANI HOT SPRINGS.

By

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CERTIFICATE

This is to certify that the work entitled, "**ISOLATION AND CHARACTERISATION OF THERMOPHILIC BACTERIA FROM TATTA PANI HOT SPRINGS.**" submitted by **NISHTHA AWASTHI (051525), SHRUTI HANDOO (051510)** in partial fulfillment for the award of degree of Bachelor of Technology in **BIOINFORMATICS** of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

21/05/09

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We take this opportunity to thank our seniors and colleagues for their help and support throughout the process.

At the end we would like to dedicate this work to our parents and all the teachers. Their inspiring words will be a guiding force in all our endeavors to attain greater height.

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1.	DNA	-	Deoxyribonucleic Acid
2.	RNA	-	Ribonucleic Acid
3.	rRna	-	Ribosomal Ribonucleic Acid
4.	tRNA	-	Transporter Ribonucleic Acid
5.	mRNA	-	Messenger Ribonucleic Acid
6.	A+T	-	Adenine + Thymine
7.	G+C	-	Guanine + Cytosine
8.	ΔG_f	-	Free Energy
9.	PCR	-	Polymerase Chain Reaction
10.	TDS	-	Total Dissolved Solids
11.	rpm	-	Rotations per minute
12.	nm	-	nanometers
13.	CMC	-	Carboxy Methyl Cellulose
14.	DNS	-	Di-Nitro Salicylic Acid

ABSTRACT

The hot springs at Tattapani located on the right banks of river Satluj in Himachal Pradesh are amongst the unexplored hot springs regions in the world. They provide us with a huge potential for isolation of thermophilic microorganisms which have a wide range of Industrial applications. Three strains of bacteria have been isolated from the hot springs of Tattapani. Their identification systems are based on morphological characteristics, biochemical tests and optimization of growth conditions. Also the strains have been screened for their antibacterial potential against *E.coli*. Two out of the three colonies had antibacterial potential

CHAPTER I

INTRODUCTION

1.1 THERMOPHILES

Thermophiles are a type of extremophiles which thrive at relatively high temperatures, above 45°C. Many thermophiles are archaea. Thermophiles are found in various geothermally heated regions of the Earth such as hot springs and deep sea hydrothermal vents (Beal,2007), as well as decaying plant matter such as leat logs and compost. As a prerequisite for their survival, thermophiles contain enzymes that can function at high temperatures. Some of these enzymes are used in molecular biology (for example heat-stable DNA polymerases for polymerase chain reaction), and in washing agents. Thermophiles are classified into:-

- Obligate thermophiles require such high temperatures for growth,
- Facultative thermophiles can thrive at high temperatures but also at lower temperatures (below 50°C).

Proteins from thermophiles have characteristic amino acid compositions as compared to mesophiles. That is mainly because of the differences in composition on the protein surface. The surface regions of thermophilic proteins have fewer (non-charged) polar amino acids and more charged amino acids, and these charged residues result in an increased number of intramolecular salt bridges. Decreased sequence length is another feature of thermophilic proteins. They tend to be shorter than their mesophilic homologs, and the main cause of this length reduction is the deletions in the loop regions.

Thermal adaptations of RNA molecules are also known. rRNA and tRNA molecules of thermophilic bacteria have higher G+C contents than mesophiles .Because the GC base pair forms more hydrogen bonds than the AT base pair, higher G+C contents in the double-stranded stem region improves thermo stability of the RNA molecules. Additionally, mRNA of thermophiles contains more purine nucleotides.

A likely explanation of this phenomenon is that it is to prevent aggregation of mRNA molecules. Adaptation of genomic DNA is not as simple as RNA. There is no apparent correlation between the G+C contents of the DNA and the optimal growth temperature of the organism. Instead, several thermophiles have special proteins that bind to DNA and raise its melting temperature significantly. Furthermore, the cell membranes of thermophiles contain an increased ratio of saturated lipid and maintain their fluidity.

Also,Lipids from thermophilic archaea have a dramatically different chemical structure

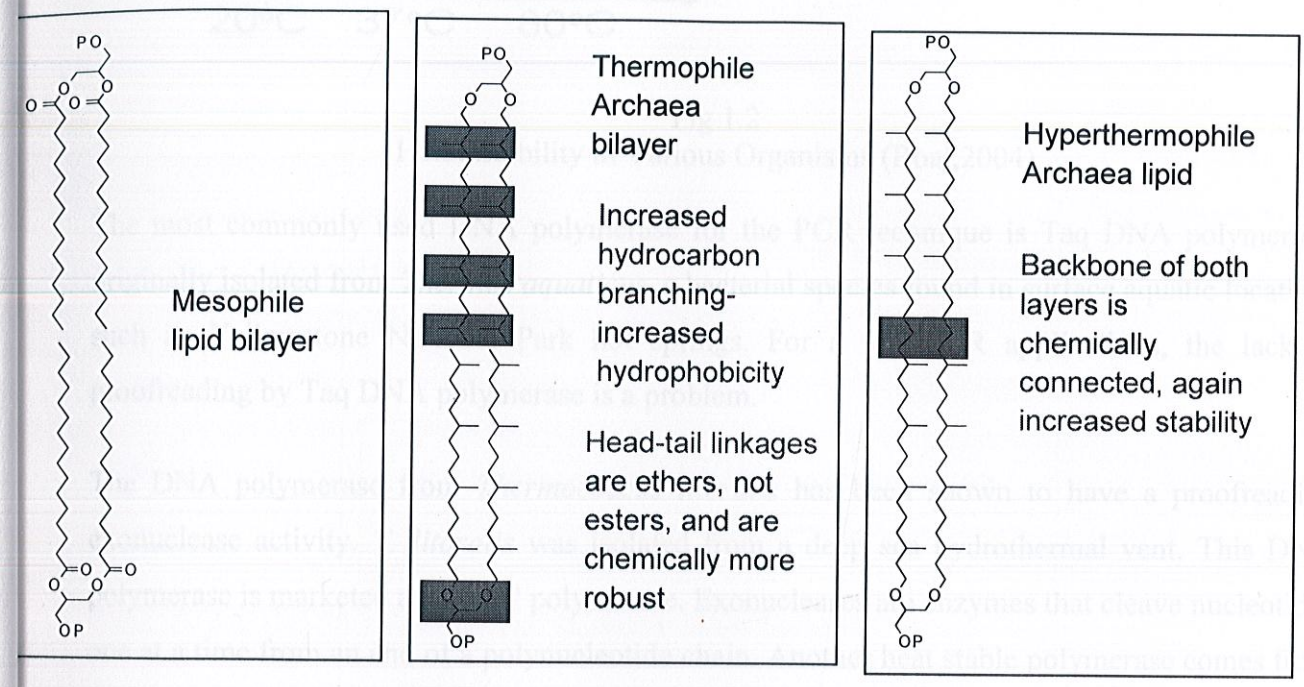


Fig 1.1
Different chemical structures of lipids from various sources (Boal, 2004)

Thermophilic helices are more stable. Calculated ΔG_f values indicated that helices of thermophilic origin were more stable than mesophilic helices. Eight of the thermophilic helices were found to be more stable- these helices are likely to be related to structural stability

No change was found for two helices, both of which are directly involved in interactions with DNA and other proteins, these helices likely need to retain flexibility for functional stability.

Interestingly, total helix stability was found to be the same value if the optimal temperature for protein activity is taken into account- this is again related to the need for molecular flexibility.

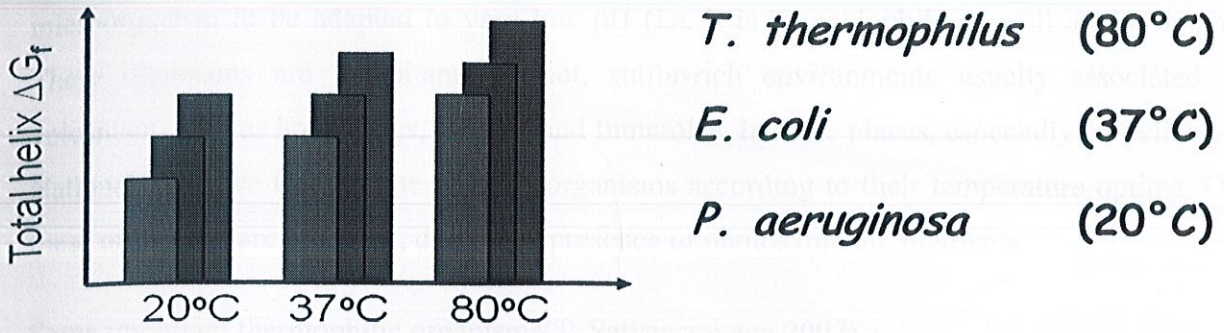


Fig 1.2
Helix Stability in Various Organisms (Boal,2004)

The most commonly used DNA polymerase for the PCR technique is Taq DNA polymerase, originally isolated from *Thermus aquaticus*, a bacterial species found in surface aquatic locations such as Yellowstone National Park hot springs. For a few PCR applications, the lack of proofreading by Taq DNA polymerase is a problem.

The DNA polymerase from *Thermococcus litoralis* has been shown to have a proofreading exonuclease activity. *T. litoralis* was isolated from a deep sea hydrothermal vent. This DNA polymerase is marketed as "Vent" polymerase. Exonucleases are enzymes that cleave nucleotides one at a time from an end of a polynucleotide chain. Another heat stable polymerase comes from the organism *Pyrococcus furiosus*, (Pfu). This organism grows optimally at 100°C, making it a hyperthermophile. Taq DNA polymerase is adequate for most PCR, but one study (Hamilton *et al*, 2001) reported that higher fidelity thermostable DNA polymerases such as Vent account for as much as 30% of DNA polymerase sales. In addition, the study of proteins from thermophilic organisms has provided important insight into the mechanism of protein folding because these proteins must be stable at temperatures that would denature typical proteins. Therefore, understanding how thermophilic proteins have evolved to be stable can yield information about the functional modulation of folding landscapes.

Many of the hyperthermophile Archaea require elemental sulfur for growth. Some are anaerobes that use the sulfur as an electron acceptor during respiration instead of oxygen. Some are lithotrophs that oxidize sulfur to sulfuric acid as an energy source, thus requiring the microorganism to be adapted to very low pH (i.e. it is an acidophile as well as thermophile). These organisms are inhabitants of hot, sulfur-rich environments usually associated with volcanism, such as hot springs, geysers and fumaroles. In these places, especially in Yellowstone National Park, we find a zone of microorganisms according to their temperature optima. Often these organisms are coloured, due to the presence of photosynthetic pigments.

Some important thermophilic organisms(T. Satyanarayana,2007)

- *Pyrococcus furiosus*
- *Thermus aquaticus*
- *Thermus thermophilus*
- *Chloroflexus aurantiacus*
- *Thermococcus litoralis*
- *Pyrodictium abyssi*
- *Bacillus stearothermophilus*
- *Thermobifida fusca* (bacterium often found in decaying plant matter)

The Pompeii worm survives the scalding temperatures surrounding deep-sea hydrothermal vents thanks to a symbiotic relationship with thermophilic bacteria.

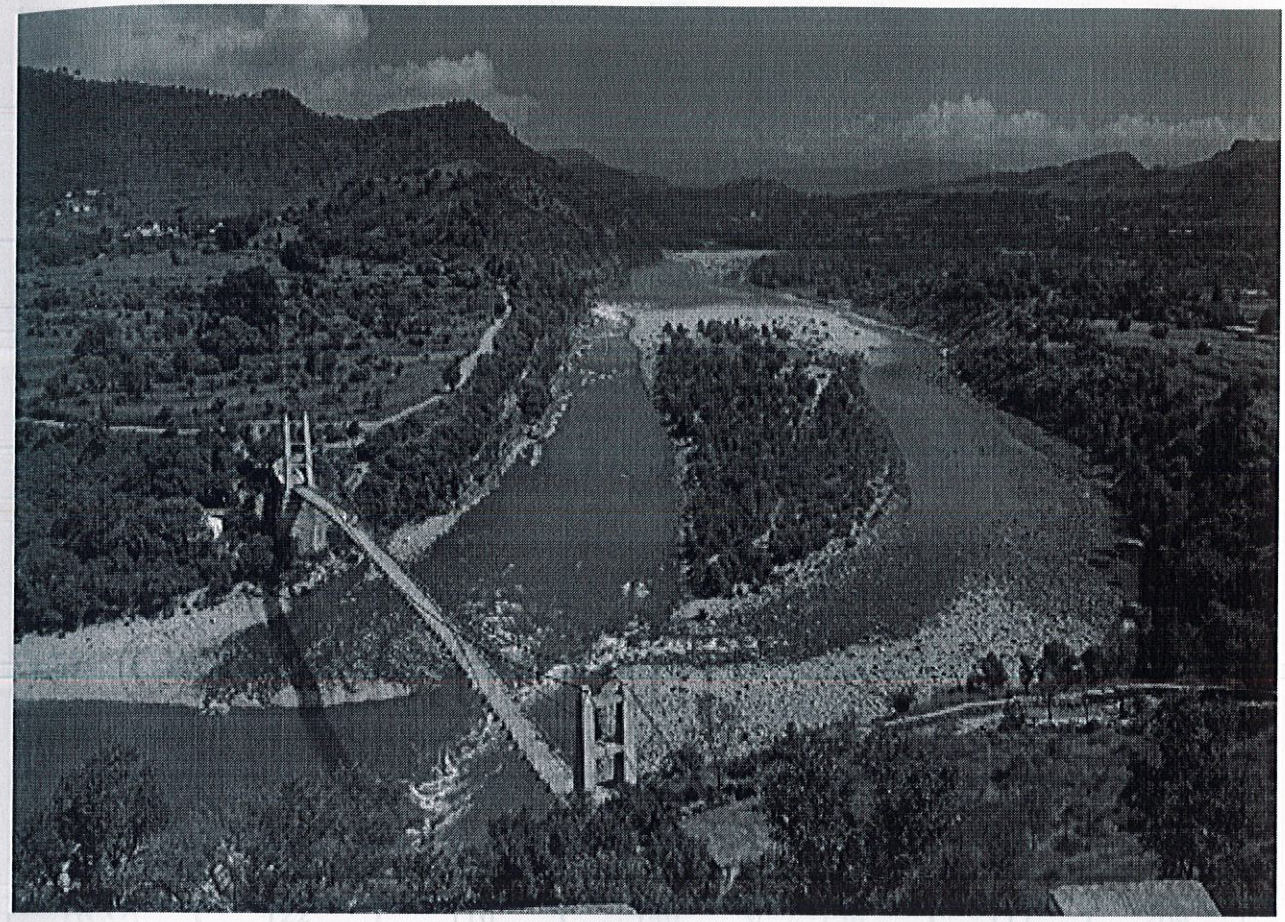


Fig 1..3
Aerial view of Tattapani

Tattapani means "Hot Water". Tattapani is 51 km from Shimla and 29 km from Naldera. It is a famous tourist resort located in district Mandi, Himachal Pradesh on the right bank of river Satluj at an altitude of 656 meters. This natural sulphur spring is pure and has curative power for various kings of bodily ailments. The water level of the spring keeps changing with the fluctuation in the water level of the river. The hot water ponds though, are different from springs. They simply vanish when the level of the river rises in June-July and reappear in November-December. The hottest convective water emerges at Tattapani at near boiling point for water at atmospheric pressure (90^0C) in association with an N_2-rich gas phase of clear meteoric signature. Since such fluids do not carry any corrosive components, they can be conveniently exploited for industrial purposes, such as drying processes.

The chemical and isotopic composition of Tattapani and associated gas phases have been investigated by Department of Earth Sciences, IIT, Bombay, India in collaboration with CNR (Italian Council for Research), Center of Study for Minerogenesis and Applied Geochemistry, Florence, Italy.

Table 1.1(Minissale et al, 2000)
Chemical and isotopic composition of water samples from various locations of Tattapani

Properties	Tattapani I	Tattapani II	Tattapani III	Tattapani IV	Tattapani V	Tattapani VI
Type	Thermal well	Thermal well	Thermal Spring	Thermal Spring	Cold Well	Cold Well
Temperature	93	90	64.5	87.6	30.1	29
TDS(mg/kg)	464	475	459	458	159	602
pH	8.49	8.39	8.23	8.19	6.49	7.82
pCO_2	-3.08	-3	-2.87	-2.7	-1.4	-2.39
Na (mg/kg)	143	146	141	140	10	154
K (mg/kg)	7.5	9.4	7.7	7.8	1.5	3.7
Ca (mg/kg)	4	4.4	3.1	3.1	23	24
Mg (mg/kg)	0.23	0.13	0.07	0.05	5.7	3.9
HCO_3	122	116	140	138	111	264
CO_3 (mg/kg)	3.7	2.8	n.d.	n.d.	n.d.	n.d.
SO_4 (mg/kg)	38	40	40	40	2.5	90
Cl (mg/kg)	150	158	128	130	6.3	64
SiO_2 (mg/kg)	104	115	138	125	75	117
NH_4 (mg/kg)	3.1	<0.1	3.7	3.4	1.3	0.36
B (mg/kg)	0.17	0.11	0.17	0.17	<0.01	0.07
Li (mg/kg)	0.22	0.24	0.23	0.23	0.04	0.1
NO_3 (mg/kg)	<0.05	<0.05	<0.05	<0.05	6	2.3
Br (mg/kg)	0.52	0.52	0.5	0.5	0.07	0.25
F (mg/kg)	20	21	20	20	0.7	14

Table 1.2(Minissale et al, 2000)
Chemical and isotopic composition of gas phase at various locations at Tattapani

Properties	Tattapani I	Tattapani II	Tattapani III	Tattapani IV	Tattapani V	Tattapani VI
He	—	0.54	1.41	1.54	—	—
Ar	—	1.51	1.44	1.6	—	—
Ne	—	0.0013	0.0013	0.0011	—	—
N_2	—	88.5	92.2	93.7	—	—
O_2	—	4.25	2.75	0.692	—	—
CO_2	—	2.88	0.179	0.485	—	—
CO	—	<0.0001	<0.0001	<0.0001	—	—
H_2S	—	<0.005	<0.005	<0.005	—	—
H_2	—	0.2653	0.2087	0.0015	—	—
CH_4	—	2.04	1.84	1.98	—	—

The fact that the flow rate is high, and that they emerge near the boiling point for water at atmospheric pressure ($T>90^0C$) but in association with a gas phase of clear meteoric signature, suggests the presence of a very well developed convective circuit.

Such a circuit at the near boiling point for water would very likely provide, if exploited, a huge quantity of hot fluid. Such fluid could be used for industrial purposes such as drying processes. The lack of corrosive components, such as H_2S, is a warranty for a long duration of eventual industrial plants that could be established in such provinces.

OBJECTIVES

- Isolation Of Thermophillic Strains From Sample.
- Morphological and Biochemical Characterization of Strains.
- Optimization of Growth Conditions.
- Determination of Antimicrobial Activity.

CHAPTER II

2.1 MATERIALS

2.1.1 ***Water Sample*** - The water sample was collected, in a mineral water bottle, from a hot spring in Tatta Pani.

2.1.2 ***Chemicals Used*** – Nutrient Agar, Nutrient Broth, Thermus Agar, Sulphur-Free Media(Defined), MacConkey Agar, Skim Milk Agar, Triple Sugar Iron Agar, Methyl Red Indicator, Starch Agar, Simmon's Citrate Agar,

* The chemicals used were manufactured by S.D. Fine Chemicals, Qualigens Fine Chemicals Ltd. and Merck Ltd.

* The various agar media used were manufactured by HiMedia Laboratories Pvt. Ltd.

2.2 METHODS

2.2.1 ***Isolation of thermophilic strains from water samples***

2.2.1.1 The pH of the spring water sample was measured using a pH meter. The water was found to be slightly alkaline with a pH of 7.8.Thermus agar, nutrient agar, and nutrient broth were prepared and their pH, as well, was set to 7.8.

The composition of Thermus Agar is:-

- NaCl 0.5%
- Peptone 0.5%
- Beef extract 0.4%
- Yeast extract 0.2%
- Agar 2%

2.2.1.2 50µl of water sample was taken from the spring water and inoculated into a flask containing nutrient broth. The broth was left at 35^0C and 120 rpm for 24hrs.After 24hrs, the presence of turbidity showed positive growth in the broth. An inoculum of 2µl was spread onto a nutrient agar plate. The plate was incubated at 37^0C to check for isolated colonies.

2.2.2 ***Screening based on Temperature tolerance***(Elnasser, Maraqa, Owais, Khraisat,2007)

2.2.2.1 20µl from the previous broth was inoculated into a new broth and set at a temperature gradient of 5^0C after every 24hrs, till no turbidity was observed, and 2µl from each broth was spread on a nutrient agar plate.

2.2.2.2 At 55^0C, three colonies were isolated which were later subjected to biochemical and morphological studies, for their characterization.

2.2.3 ***Characterization of Strains Obtained***

2.2.3.1 Morphological Study

2.2.3.1.1 Colony Characteristics

2.2.3.1.2 Margin/Edge

2.2.3.1.3 Surface Texture

2.2.3.1.4 Elevation

2.2.3.1.5 Consistency

2.2.3.1.6 Optical features

2.2.3.1.7 Pigmentation

2.2.3.1.8 Gram's staining

Cells were stained with crystal violet dye. Next, a Gram's iodine solution (iodine and potassium iodide) was added to form a complex between the crystal violet and iodine. A decolorizer such as ethyl alcohol or acetone was added to the sample. A counter stain, such as the weakly water soluble safranin, was added to the sample, staining it red. Since the safranin was lighter than crystal violet, it does not disrupt the purple coloration in Gram positive cells. However, the decolorized Gram negative cells are stained red.

2.2.3.1.8.1 KOH Test (A secondary test for identification of Gram's nature) A drop of 3% KOH was placed on a slide, mixed in cells, and kept for a minute for reaction to occur.

2.2.3.2 Biochemical Test

2.2.3.2.1 Catalase Activity : 3% H_2O_2 solution was made in distilled water. One drop of H_2O_2 solution was put over colony or the suspension culture and was observed for effervescence.

2.2.3.2.2 Starch hydrolysis : Starch agar plates were prepared and inoculated with thin suspension of the culture. After 48hrs of incubation the surface was flooded with Gram's Iodine. The plates were then observed for a colorless zone surrounding the colonies.

2.2.3.2.3 Casein Hydrolysis : Skimmed Milk Agar plates were prepared and inoculated with thin suspension of the culture. After 48hrs of incubation the plates were observed for a zone of clearing around the bacterial growth.

2.2.3.2.4 Citrate Test : Citrate Agar plates were prepared and inoculated with thin suspension of the culture. After 48hrs of incubation the plates were observed for development of a Prussian blue color.

2.2.3.2.5 MacConkey Agar Test:Maconkey Agar plates were prepared and inoculated with thin suspension of the culture. After 48hrs of incubation the plates were observed for development of a pink color.

2.2.3.2.6 Triple Sugar Iron Agar Test: Triple Sugar Iron Agar plates were prepared and inoculated with thin suspension of the culture. After 48hrs of incubation the plates were observed for decoloration.

2.2.4 *Optimization of Growth Conditions*

2.2.4.1 Determination of Optimum Temperature(Elnasser, Maraqa, Owais, Khraisat,2007)

2.2.4.1.1 The growth of the strains was studied individually, at 55^0C, 60^0C, 65^0C, 70^0C, by measuring O.D. at 660 nm using a spectrophotometer.

2.2.4.1.2 Flasks were inoculated by using 20ul of 24-hour old culture.

2.2.4.1.3 Also, a comparative analysis of growth of the strains on Sulphur-free defined media and nutrient broth was done at 55^0C and 60^0C.

2.2.4.1.4 Growth was determined in 24 hour interval by measuring the O.D at 660nm.

2.2.4.2 Growth Kinetics in Defined Sulphur Free Media(Goorissen, Boschker, Stams and Hansen)

Composition of Sulphur free media:

KH_2PO4	2.44g/L
Na_2HPO_4	5.57g/L
NH4Cl	2.0g/L
$MgCl_2$	0.2g/L
$CaCl_2.2H_2O$	0.001g/L
$FeCl_3$	0.001g/L
Glucose/Glycerol	20.0g/L

2.2.5 *Growth with CMC (Ibrahim and diwany, 2007)*

2.2.5.1 The isolates are inoculated in Bushnell Hass media with CMC as the carbon source. They are then grown on their optimal temperature in an orbital shaker running at the optimal rpm. Optical Density at 660 nm was examined at 24 hour interval.

2.2.5.2 Also, to test for the utilization of reducing sugars present in the media DNS assay is was performed and the Optical Density at 540 nm was examined at 24 hour interval.

2.2.6 *Antibacterial Activity (Venugopalan et al,2008)*

2.2.6.1 The isolates were cultivated in nutrient broth for 48 hours. The cell free supernatant of all three colonies is was tested for antibacterial activity on E. coli by the Disc Method.

2.2.6.2 Results of the colonies were compared to the results from the use of antibiotic discs on E. coli.

CHAPTER III

RESULTS AND DISCUSSION

Colonies Isolated from Water Sample at 55^0C:

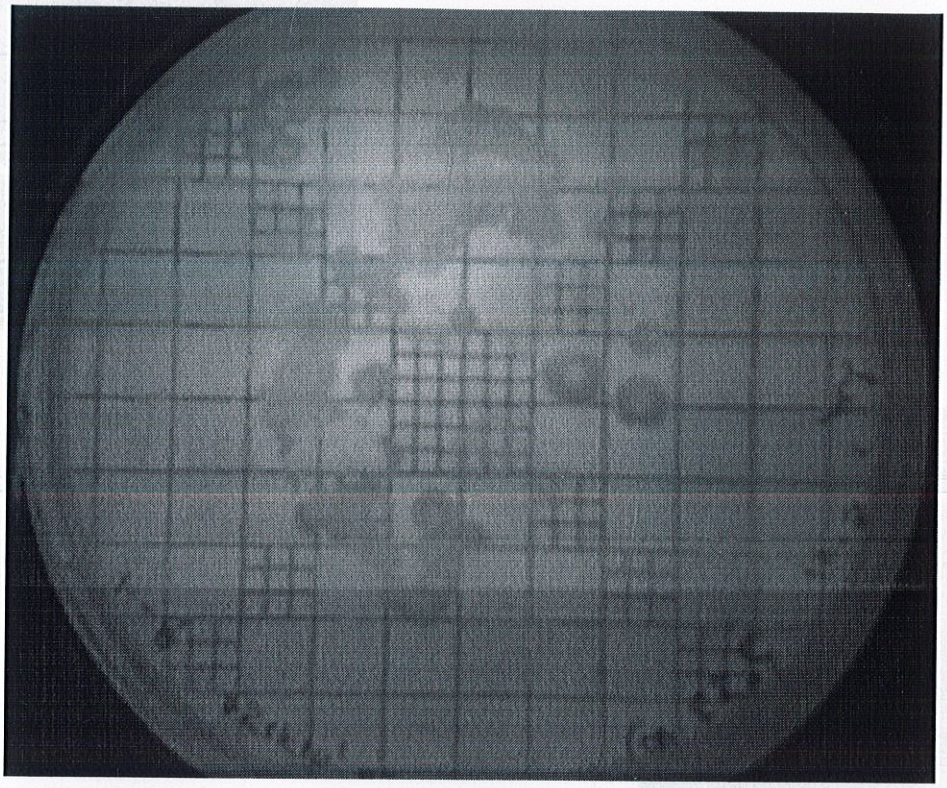


Fig 3.1
A Mixed Culture obtained at 55^0C

In fig 3.1, the plate contains a mixed culture of the three colonies obtained at 55^0C. These colonies were used for further study, throughout the project.

Isolated Colonies:

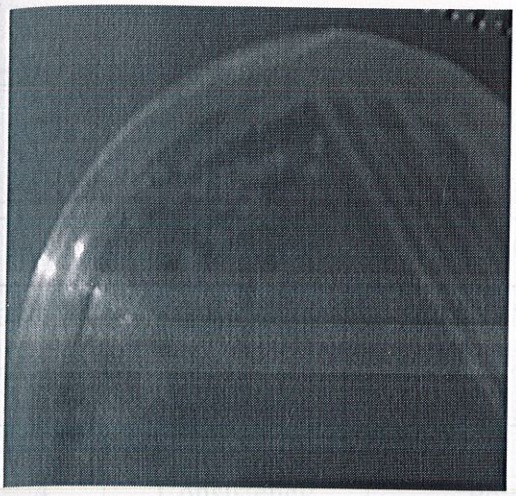


Fig 3.2
Colony I

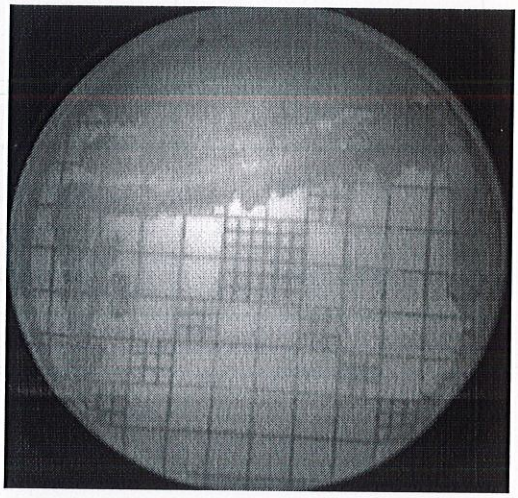


Fig 3.3
Colony II

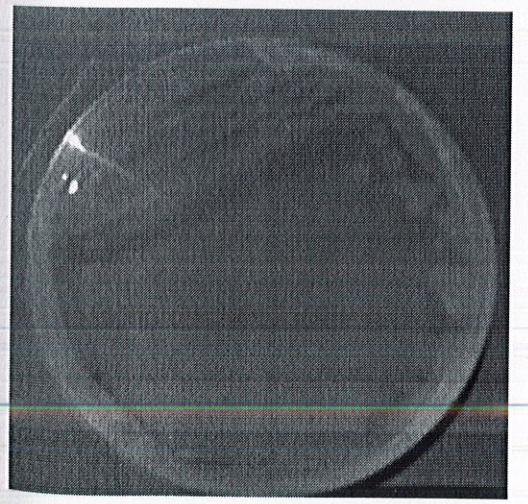


Fig 3.4
Colony III

Colony Characteristics

Table 3.1
The results of general morphological tests.

S.No.	Characteristics	Colony		
		I	II	III
1.	Size(diameter)	0.6cm	2.4cm	1.8cm
2.	Margin/Edge	Irregular, rough, rhizoid edges	Irregular, smooth, lobate edges	Regular, smooth, granular edges
3.	Surface Tension	Wrinkled, shiny	Dull	Shiny
4.	Consistency	Powdery	Mucous	Mucous
5.	Elevation	Flat	Flat	Flat
6.	Optical Features	Translucent	Translucent	Opaque
7.	Pigmentation	Off-white/pale yellow	Off white	Off-white
8.	Gram's Nature	Positive	Negative	Negative
9.	Shape of microbe	Bacillus	Bacillus	Bacillus

Biochemical tests:

Table 3.2
Results of various Biochemical tests

Tests	Colony		
	I	II	III
Citrate Agar Test	Positive	Positive	Positive
Skimmed milk Agar Test	Positive	Negative	Positive
Mac Conkey Agar Test	Negative	Positive	Positive
Starch Agar Test	Negative	Negative	Negative
Triple Sugar Iron Agar Test	Red/Yellow	Yellow with black precipitate	Yellow

Table 3.3
Reference table for Triple Sugar Iron Agar test:

Results (slant)	Interpretation
Red/yellow	Glucose fermentation only; Peptone catabolized
Yellow/yellow	Glucose and lactose and/or sucrose fermentation
Red/red	No fermentation; Peptone catabolized
Red/no color change	No fermentation; Peptone used aerobically
Yellow/yellow with bubbles	Glucose and lactose and/or sucrose fermentation; Gas produced
Red/yellow with bubbles	Glucose fermentation only; Gas produced
Red/yellow with bubbles and black precipitate	Glucose fermentation only; Gas produced; H_2S produced
Red/yellow with black precipitate	Glucose fermentation only; H_2S produced
Yellow/yellow with black precipitate	Glucose and lactose and/or sucrose fermentation; H_2S produced

According to the results of the various biochemical tests (table 3.2, 3.3), we conclude that:

- Colony I is a rod shaped, gram positive, catalase and casease positive bacteria. It is able to ferment only glucose and not sucrose or lactose and lacks amylase activity.
- Colony II is a rod shaped gram negative, catalase positive bacteria. It shows no casease or amylase activity. it is able to ferment glucose, lactose and/or sucrose during which H_2S is produced.
- Colony III is a rod shaped gram negative, catalase and casease positive bacteria. It shows no amylase activity. It is able to ferment glucose, lactose and/or sucrose but no H_2S is produced.

Optimisation Of Growth Conditions:

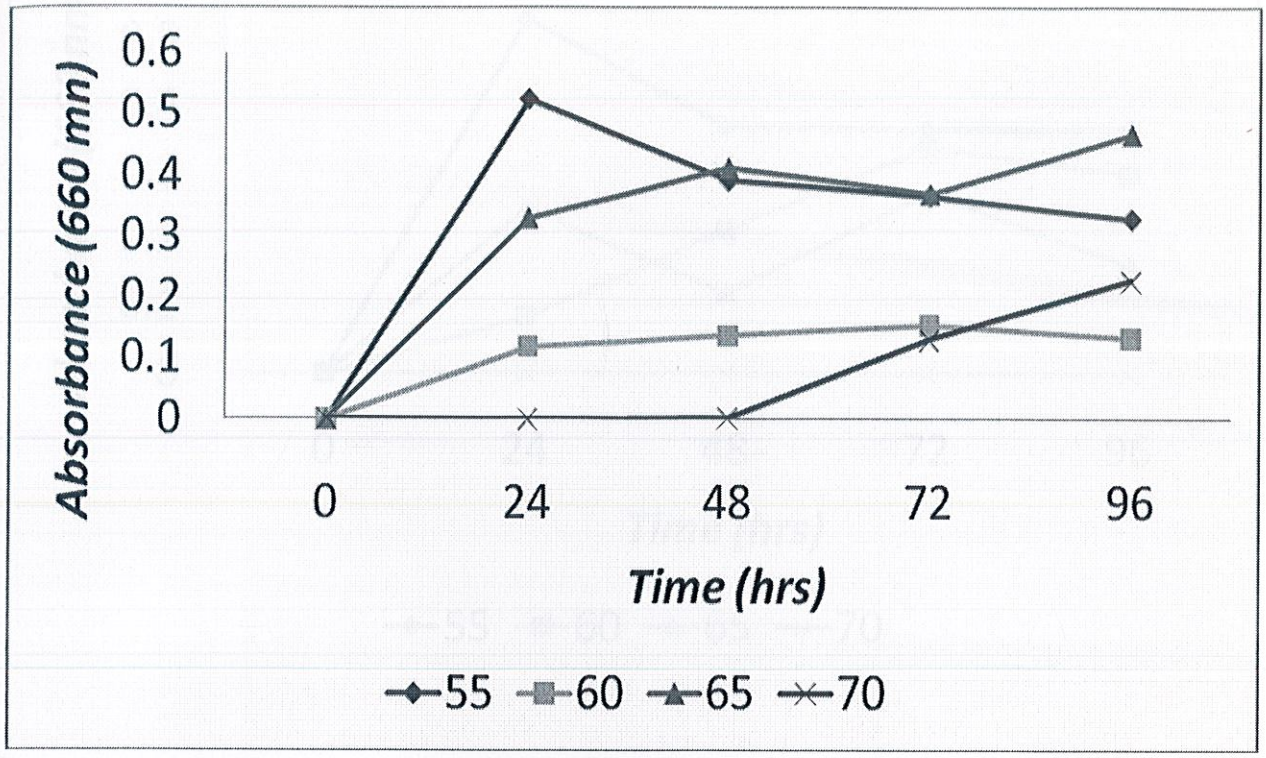


Fig 3.5
Growth of Colony I at various temperatures

The above graph shows the growth pattern of Colony I, isolated from the spring water sample, at 55^0C, 60^0C, 65^0C, 70^0C respectively.

At 55^0C, though there is a steep rise in the first 24 hrs, but it follows a steep decline.

At 60^0C, the growth is uniform and steady but very low.

At 65^0C, there is a moderate rise within 24 hrs, and thereafter also the changes are gradual

At 70^0C, the growth is negligible for 48 hrs, after which there is a steep rise.

Thus, according to the graph, Colony I grows best at 65^0C.

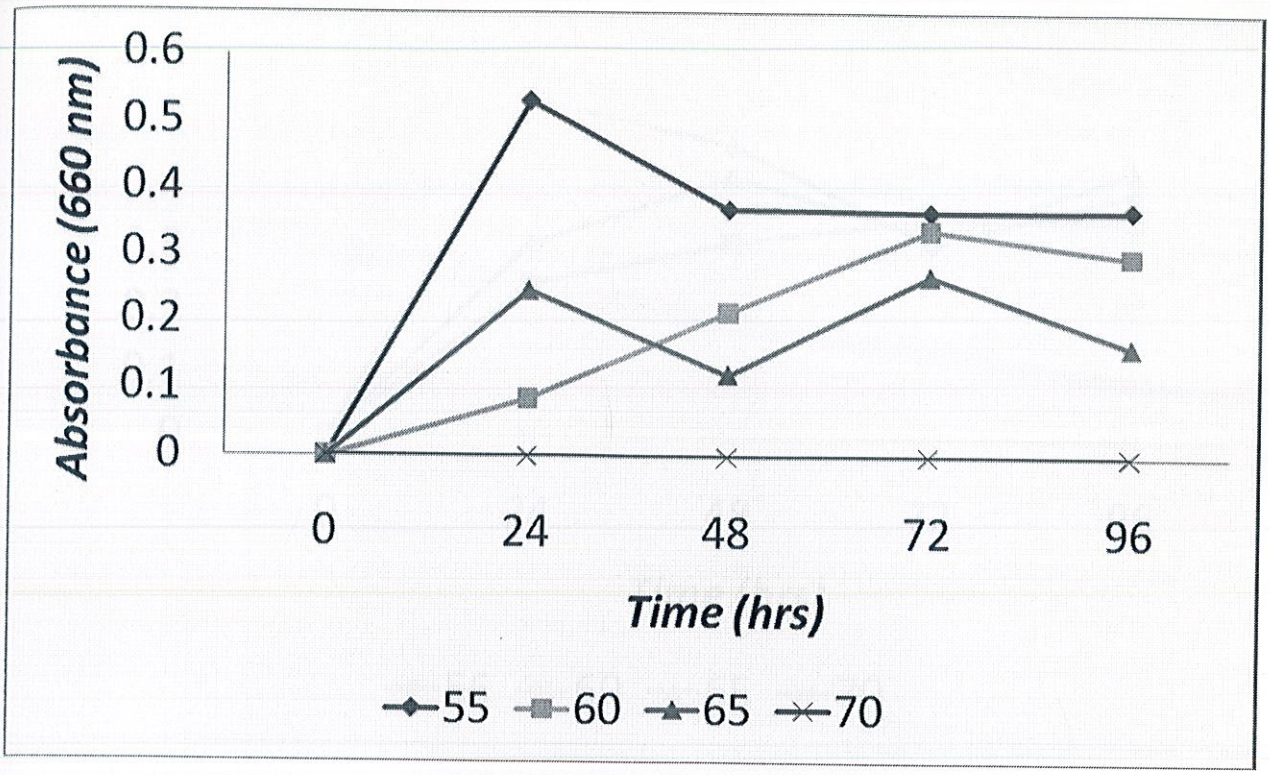


Fig 3.6

The above graph shows the growth pattern of Colony II, isolated from the spring water sample, at 55^0C, 60^0C, 65^0C, 70^0C respectively.

At 55^0C, though there is a steep rise in the first 24 hrs, but it follows a stationary phase.

At 60^0C, the growth is uniform and steady but very low.

At 65^0C, the growth is very haphazard.

At 70^0C, there is no growth at all.

Thus, according to the graph, Colony II grows best at 65^0C.

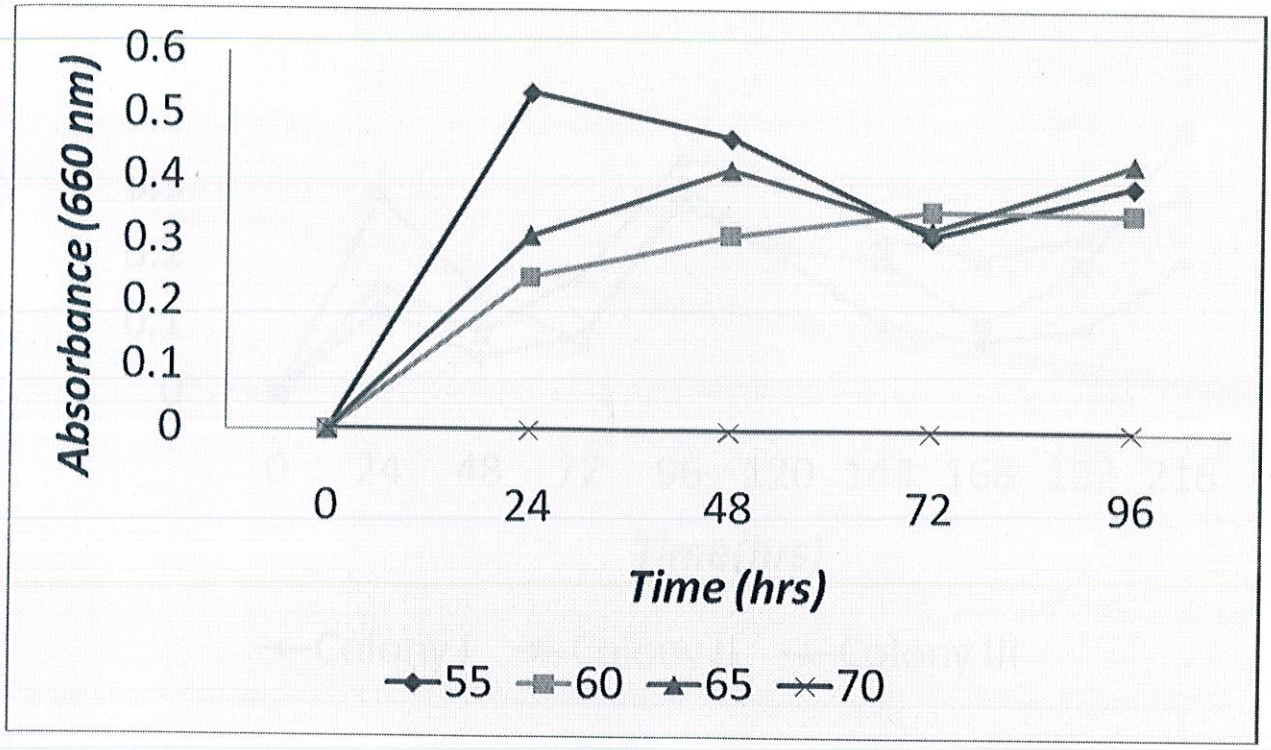


Fig 3.7

The above graph shows the growth pattern of Colony III, isolated from the spring water sample, at 55^0C, 60^0C, 65^0C, 70^0C respectively.

At 55^0C, though there is a steep rise in the first 24 hrs, but it becomes random thereafter.

At 60^0C, the growth is uniform and steady.

At 65^0C, there is a moderate rise within 48 hrs, but it becomes random.

At 70^0C, there is no growth at all.

Thus, according to the graph, Colony III grows best at 60^0C.

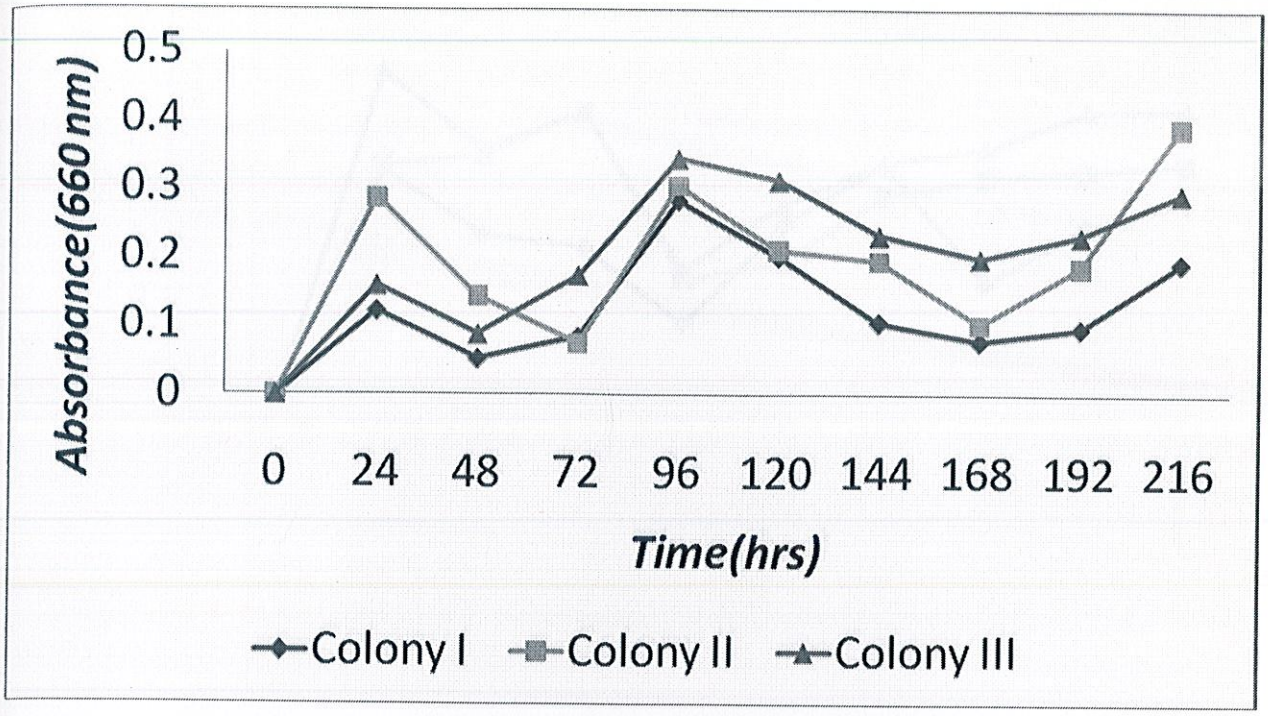


Fig 3.8
Growth of all three colonies in nutrient broth at 55^0C

Here all 3 colonies were kept in separate flasks, and their growth was observed at 55^0C.

All the growth patterns follow approximately the same trend – a sudden initial rise (exponential phase), followed by the stationary phase.

However, Colony III exhibits the best growth pattern as compared to colonies I and II as its growth is most consistent.

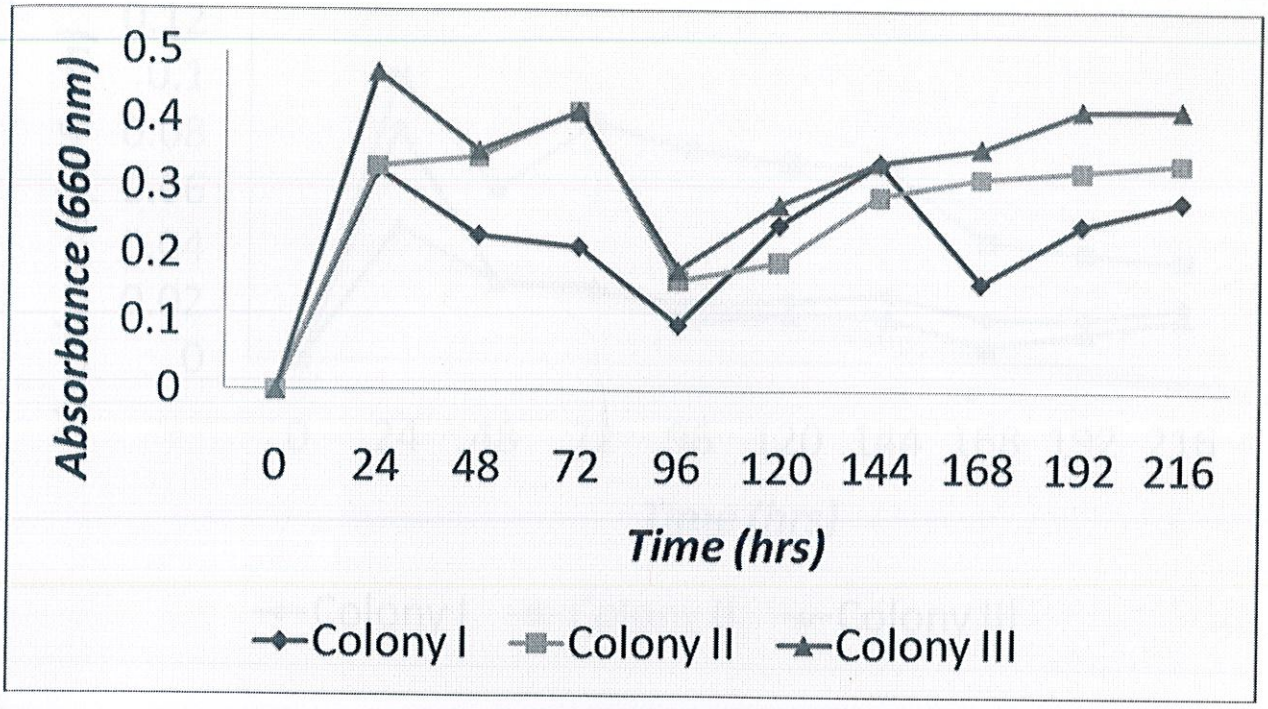


Fig 3.9
Nutreint broth at 60^0C

Also, all three colonies were observed at 60^0C.

Here, the colonies show a quick arrival to the exponential phase following which the growth pattern becomes somewhat stable.

Again, Colony III proves to be better out of all three colonies, with a comparatively stable and slightly higher growth.

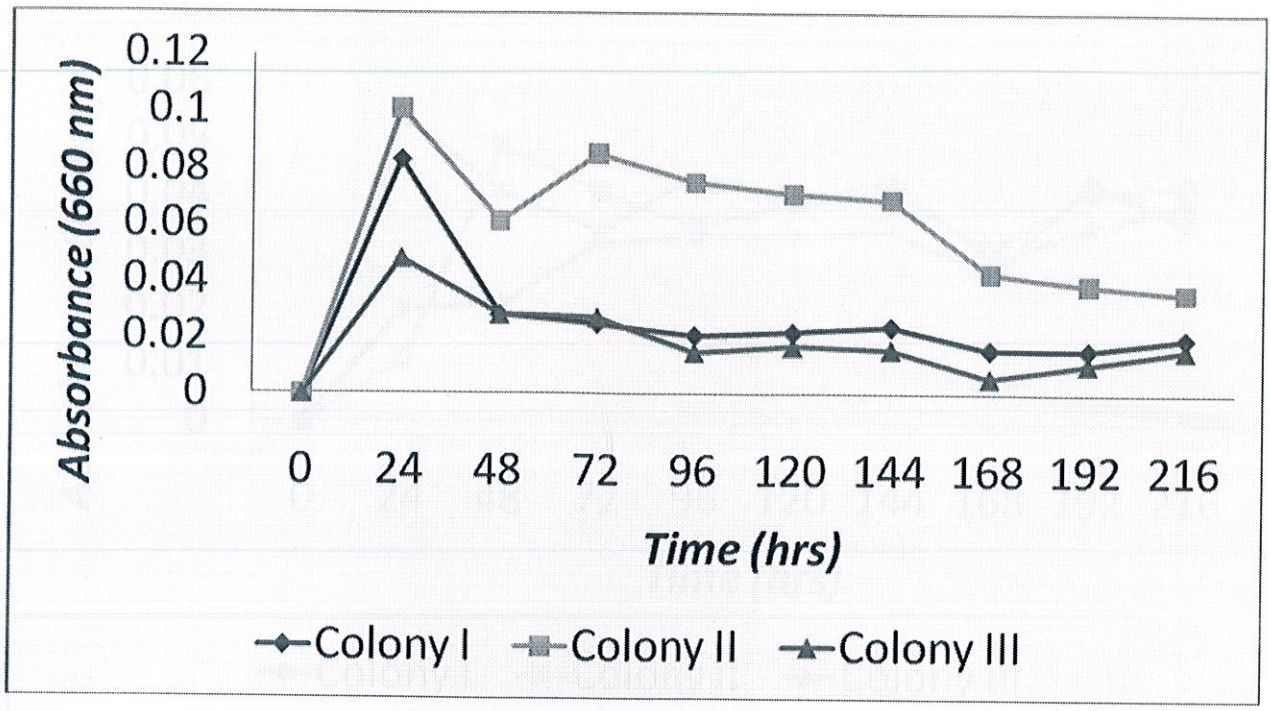


Fig 3.10
Sulphur-free Media at 55^0C

Colonies were also grown on the Sulphur –free media, for a comparative analysis with nutrient broth, first at 55^0C and later at 60^0C.

Here too, the colonies arrive quickly at the exponential phase, but there's a decline in the growth patterns in all three colonies and also the growth isn't as high as the growth in nutrient broth.

However, colony II proves to be growing better than the other two colonies.

Thus we can conclude that, Colony I and III are relatively more dependant on sulphur for their normal growth, whereas, Colony II has better chances of proliferation without sulphur.

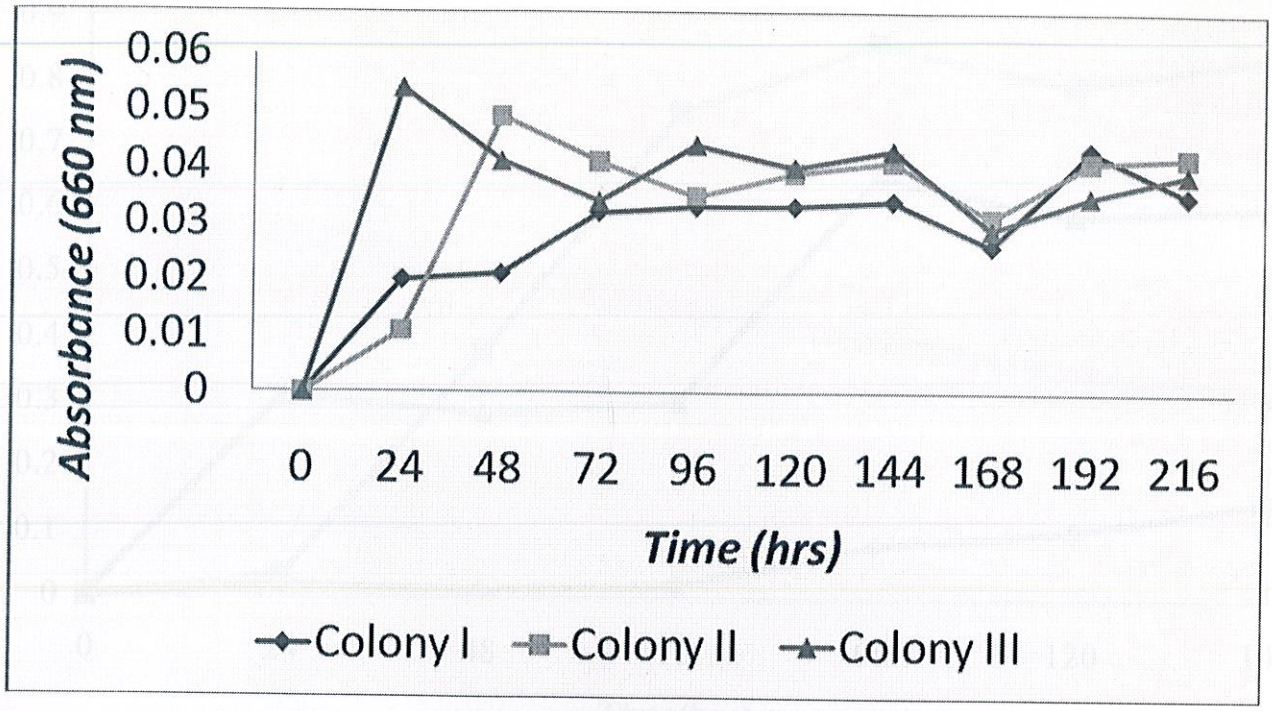


Fig 3.11
Sulphur-free Media at 60^0C

Comparing this result to the result in fig 3.8, 3.9 and 3.10, we see the same trend being repeated.

Although a the difference in this result is that in stead of gradally declining, the growth rate becomes more stable.

Thus, we can say that these colonies grow better without sulphur at a higher temperature.

Here too, colony II has the best growth pattern out of all three colonies.

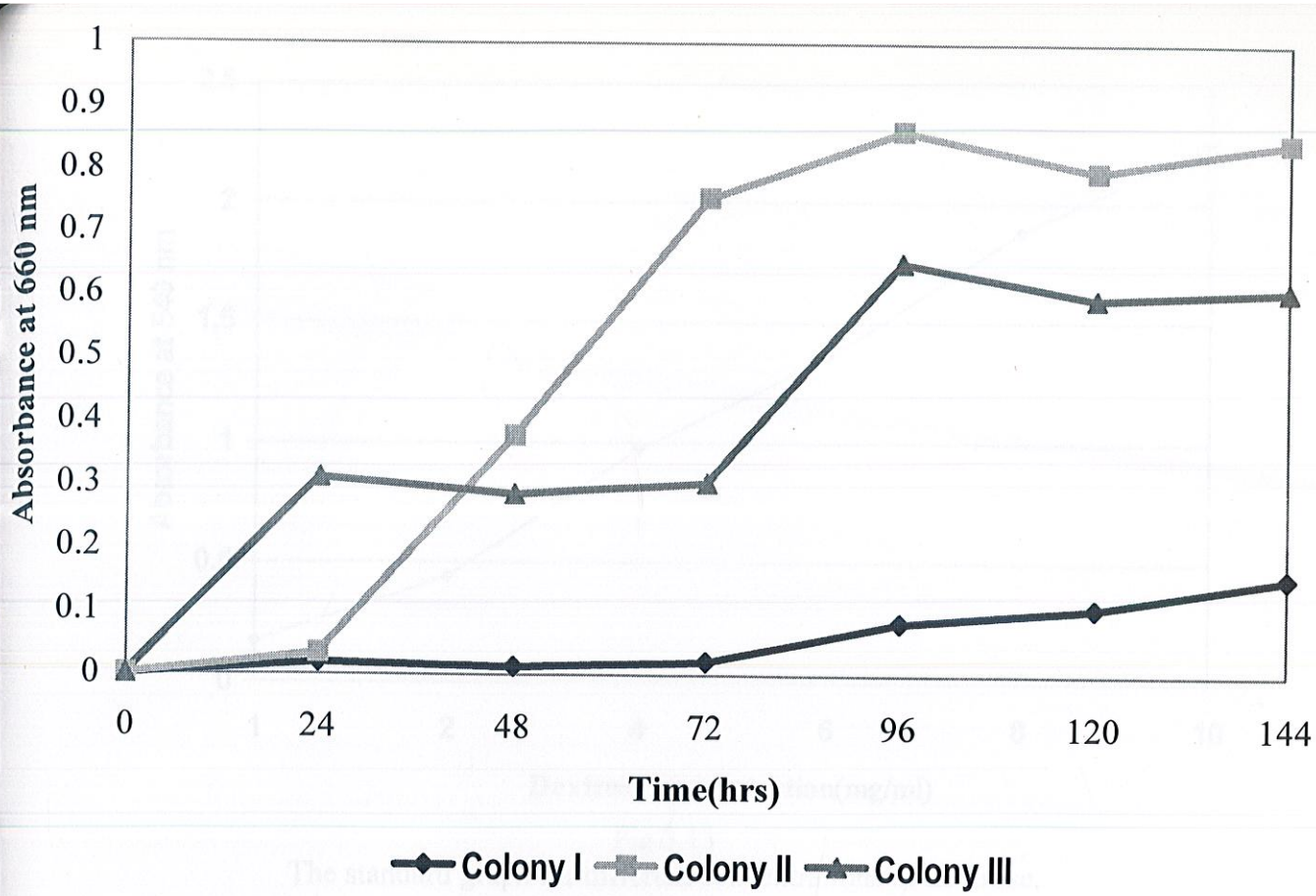


Fig 3.12
Growth in Bushnell Haas media, with CMC as carbon source:

The above graph shows the growth patterns of all three colonies in Bushnell Hass media with CMC as carbon source.

Colony I shows very low growth rate, thus implying that CMC as a carbon source is not sufficient for it growth.

Colony II and III have better growth rates showing that they are able to utilize CMC as a carbon source.

However, the growth of the three colonies in Bushnell Hass is very low as compared to their growth in nutrient broth. Thus we conclude, that the colonies are unable grow in minimal media.

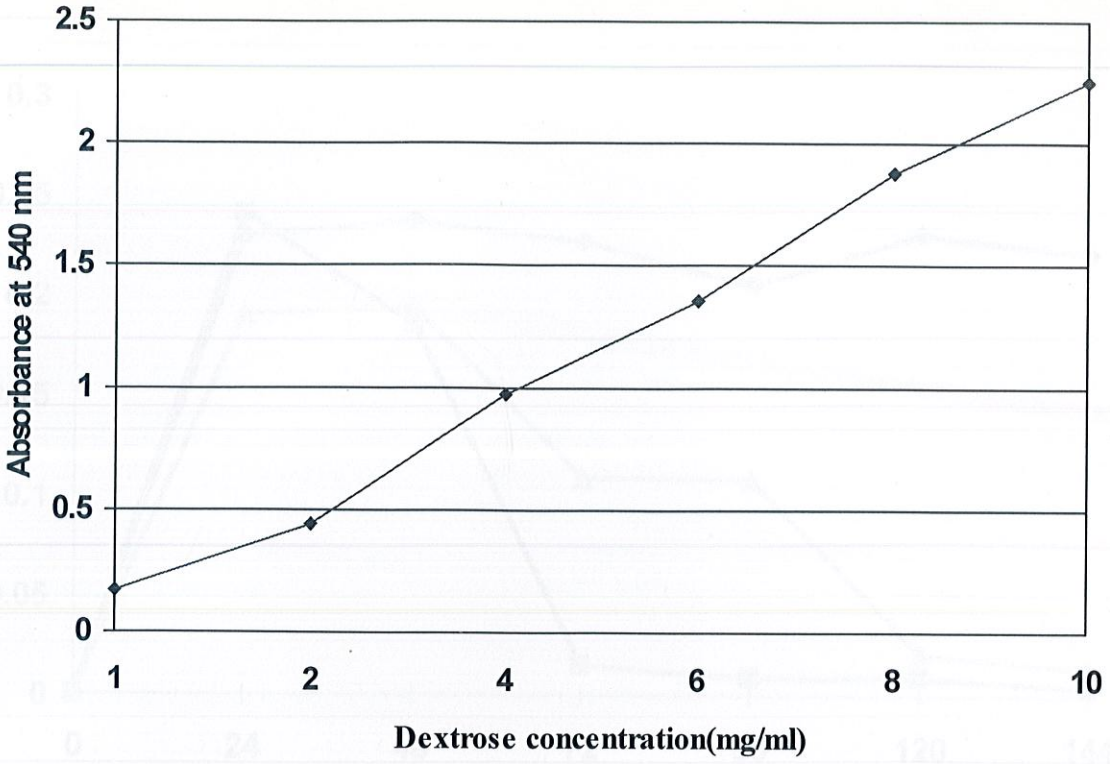


Fig 3.13
The standard graph for different concentrations of dextrose.

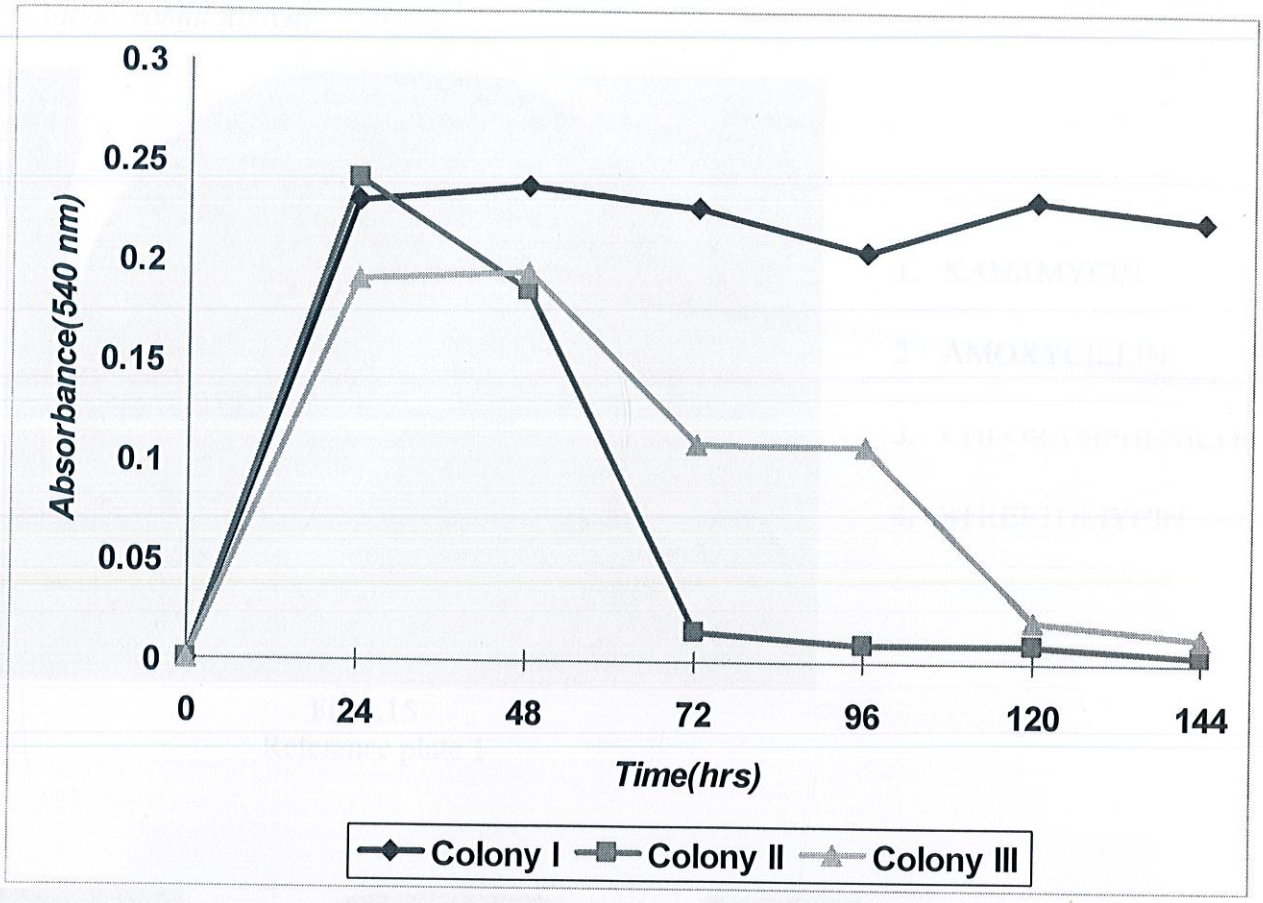


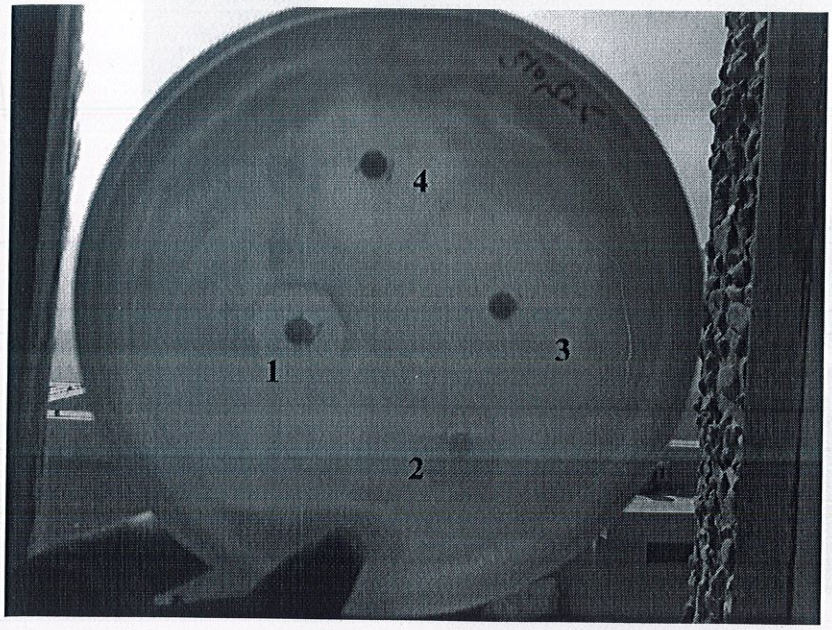
Fig 3.14
Growth in Bushnell Haas Media, DNS Assay result:

In this graph, the level of cellulose was analysed in the three colonies, by means of DNS assay.

Since, colony I showed lowest growth in Bushnell hass media with CMC, therefore it hasn't been able to utilize cellulose and thus, the level of cellulose hasn't shown a noticeable decline.

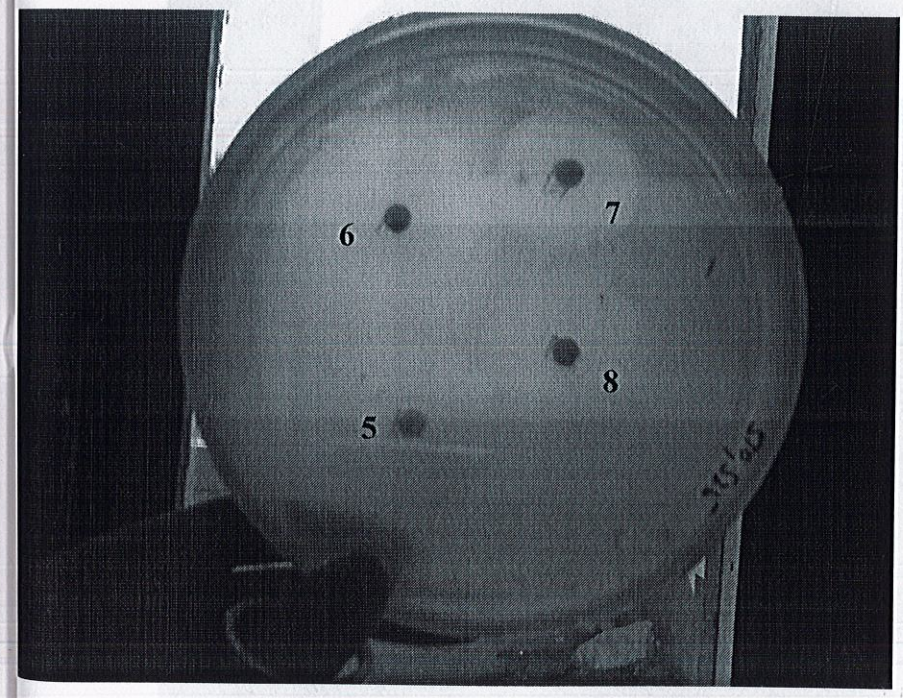
But, on the other hand colonies II and III gradually utilize all the cellulose present in the media.

Antimicrobial Activity:



1. KANAMYCIN
2. AMOXYCILLIN
3. CHLORAMPHENICOL
4. STREPTOMYCIN

Fig 3.15
Reference plate 1



5. RIFAMPICIN
6. OFLOXICIN
7. ERYTHROMYCIN
8. TETRACYCLINE

Fig 3.16
Reference plate 2

Fig 3.17
Colony I

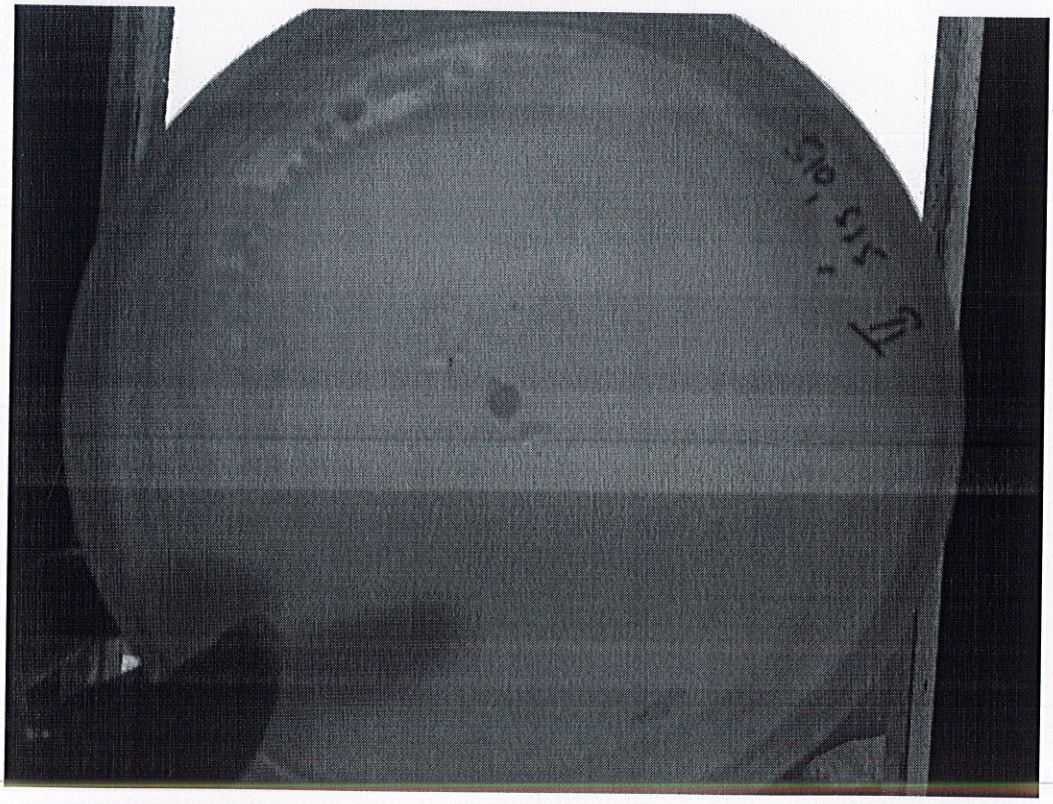


Fig 3.18
Colony II

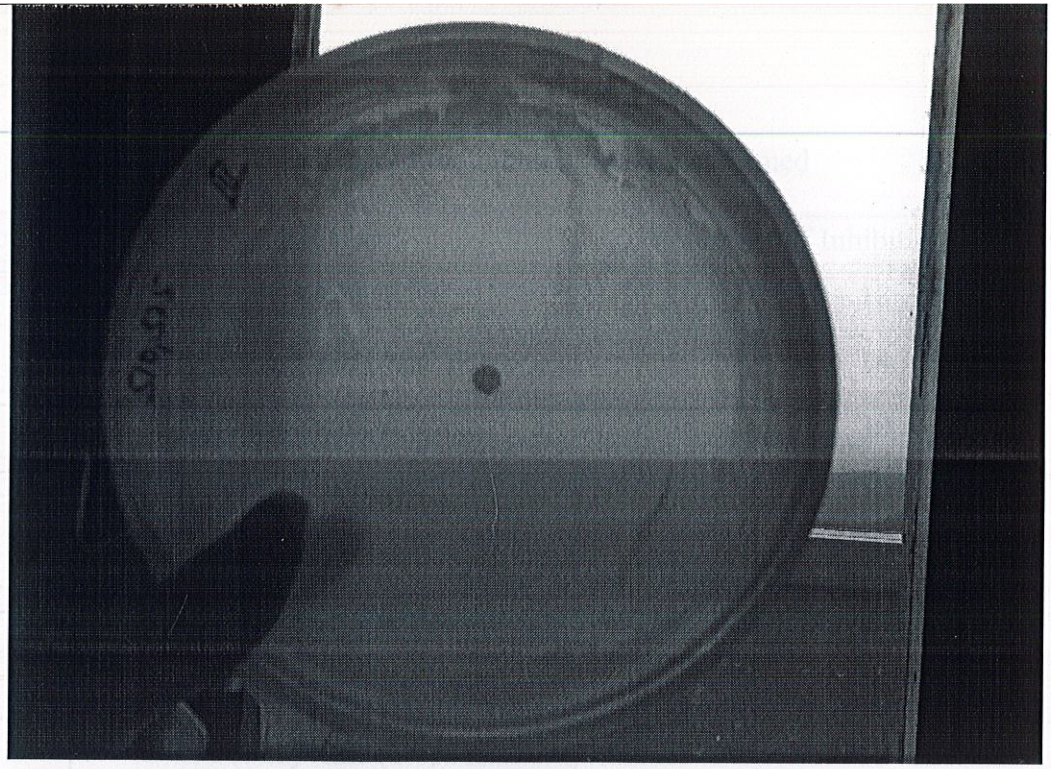


Fig 3.19
Colony III

Table 3.3
Diameters of the Inhinbition Zones Formed

S.No.	Disc Used	Diameter Of Inhibition Zone (mm)
1	Kanamycin (K^{30})	18
2	Amoxycillin (Am^{30})	14
3	Chloramphenicol (C^{30})	30
4	Streptomycin (S^{10})	19
5	Rifampicin (R^{30})	40
6	Ofloxacin (Of^{30})	36
7	Erythromycin (E^{15})	28
8	Tetracycline (T^{30})	30
9	Colony I (C.F.S)	-
10	Colony II (C.F.S)	10
11	Colony III (C.F.S)	6

As shown by the diameters, Rifampicin has the maximum antibacterial effect on growth of *E. coli* and Amoxyillin has the least antibacterial effect.

It is evident from the results that Colony I has no antibacterial effect, at least on E. coli. On the other hand Colonies II and III have an antibacterial effect on E. coli but are not as effective as the antibiotics used as references.

CHAPTER IV

PROSPECTIVE APPLICATIONS

- The microbe isolated may help in understanding specific metabolic pathways and effect of high temperatures on their activity.
- The gene responsible for heat resistance and sulphur tolerance can be identified and can be used to develop recombinant strains.
- Inorganic sulphur removal from coal is achieved by sulphur oxidizing microbes, thus can be used for desulphurization of fossil fuels.
- Microbes regulate the rate of $CaCO_3$ precipitation, hence controlling the shape of $CaCO_3$ crystals. This property can be used for dating sedimentary rocks.
- Can be useful in cleaning the high temperature areas in industries like furnaces.
- Medicinal value – for treatment of skin allergies, joint pain, etc.

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