EFFECTS OF SILVER NANOPARTICLES ON THE GROWTH OF *L. casei*

A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree of

Bachelor of Technology

in

Biomedical Engineering

by

ANKIT ANITOSH DHIR

111BM0542

Under the supervision of

Dr. SUBHANKAR PAUL

Department of Biotechnology & Medical Engineering

National Institute of Technology

Rourkela, Odisha-769008

MAY 2015

Department of Biotechnology and Medical Engineering
National Institute of Technology, Rourkela,
Odisha 769008, India

CERTIFICATE

This is to certify that the report entitled **"Effects of Silver Nanoparticles on the Growth of *L. casei*"** submitted by **Ankit Anitosh Dhir (111BM0542)** towards the partial fulfillment of the requirement for the degree of Bachelors of Technology in Biomedical Engineering at Department of Biotechnology & Medical Engineering, NIT Rourkela is a record of bonafide work carried out by him under my guidance and supervision**.**

Rourkela

Date

Dr. S. Paul

Dept. of Biotechnology and Medical Engineering

National Institute of Technology, Rourkela

ACKNOWLEDGEMENT

I feel immense pleasure and privilege in expressing my deep gratitude, indebtedness and thankfulness towards all the people who have helped, inspired and encouraged me during the preparation of this report.

I would like to thank **Dr. S. Paul**, who provided me this opportunity to highlight the key aspects of an upcoming technology and guided me during the project work preparation. I place on record my sincere gratitude to **Prof. Krishna Pramanik**, Head of Department, Department of Biotechnology and Medical Engineering, NIT Rourkela for her constant encouragement.

I would like to thank **Mr. Sailendra Mahanta**, **Mr. Deependra K. Ban**, Ph.D. Scholars, Department of Biotechnology and Medical Engineering, NIT Rourkela and **Ms. Usha Pandey** for their regular support, help and motivation.

Last but not the least, I would like to thank whole heartedly my parents and family members whose love and unconditional support, both on academic and personal front, enabled me to see the light of this day.

Ankit Anitosh Dhir

111BM0542

CONTENTS

TABLE OF CONTENTS	*PAGE NO*
ABBREVIATIONS	6
LIST OF FIGURES	7
LIST OF TABLES	8
ABSTRACT	9
CHAPTER 1	**10**
1.0 INTRODUCTION	11
1.1 Overview	12
1.2 Silver NP	12
1.3 *L. casei*	13
1.4 Objective	13
CHAPTER 2	**14**
2.0 LITERATURE REVIEW	16
2.1 Nanoparticles	16
2.2 Properties of Cell wall	17
2.3 Effect of NP type and surface	18
2.4 Effect of growth rate	18
2.5 Effect of biofilm formation	19
2.6 Toxicity mechanism of NPs	19
2.7 Defense Mechanisms	20
2.8 NP against environment	20
2.9 *Lactobacillus casei*	21
2.10 Effect of AgNP on *L. casei*	21

CHAPTER 3 **22**

3.0 MATERIALS AND METHODS 24

3.1 Materials used 24

3.2 Instruments used 24

3.3 Methodology 24

3.3.1 Preparation of *L. casei* 24

3.3.2 Preparation of Silver nanoparticles 25

3.3.3 Addition of silver into *L. casei* 26

CHAPTER 4 **28**

4.0 RESULTS AND DISCUSSIONS 30

4.1 Analysis of growth profile curve of *L. casei* 30

4.2 Effects of AgNPs on *L. casei* 32

CHAPTER 5 **38**

5.0 CONCLUSION 40

5.1 FUTURE WORKS 41

REFERENCES **42**

ABBREVIATIONS

WHO	World Health Organization
AgNPs	Ag Nanoparticles
L. casei	Lactobacillus casei
OD	Optical Density
Conc	Concentration
Ag	Silver
gm	gram
mg	milligram
ml	milliliter
hr	Hour
TB	Tuberculosis

LIST OF FIGURES

Sl. No	Fig. Title	Description	Page No.
1.	Figure 1	Bacterial Structure.	17
2.	Figure 2	ROS Formation.	19
3.	Figure 3	Preparation of *L. casei* culture.	25
4.	Figure 4	Silver nanoparticles.	25
5.	Figure 5	5 test tubes with MRS media.	27
6.	Figure 6	Growth of *L. casei* in media.	30
7.	Figure 7	Growth profile curve of *L. casei*.	31
8.	Figure 8	Effect of AgNPs on *L. casei* growth	33
9.	Figure 9	Effect of AgNPs on *L. casei* growth	34
10.	Figure 10	Effect of AgNPs on *L. casei* growth	35
11.	Figure 11	Effect of AgNPs on *L. casei* growth	36

LIST OF TABLES

Sl No.	Table Title	Description	Page No.
1.	Table 1	Analysis of Growth profile curve.	30
2.	Table 2	Different OD values of the culture 1	32
3.	Table 3	Different OD values of the culture 2	33
4.	Table 4	Different OD values of the culture 3	35
5.	Table 5	Different Od values of the culture 4	36

ABSTRACT

The current study presents the investigation of the effect of silver nanoparticles (AgNP) on the growth of probiotic bacteria *Lactobacillus casei*. The study was aimed to find out the growth behavior of *L. casei* in the presence of different AgNPs concentrations. It was observed from our investigation that at low concentrations of AgNP (concentration < 0.3 mg/ml), AgNP exhibited no antibacterial effect on *L .casei*, moreover, it enhanced the growth of *L .casei*. However, when AgNP was applied at a concentration of 0.3 mg/ml and above, it exhibited significant antibacterial effect on the bacteria by inhibiting its growth. Since, *L. casei* is a probiotic and helps in reducing cholesterol and keeping the heart healthy, the fact that low concentration of AgNP can accelerate *L. casei* growth can be exploited to tackle the level of cholesterol and other heart related problems in humans.

Keywords: Silver Nanoparticles, *Lactobacillus casei*, Antibacterial effect, Spectrophotometer

Chapter 1

Introduction

1. INTRODUCTION:

1.1 Overview:

Lactobacillus casei, generates lactic acid that is used for several applications in biotechnology related fields as they have various useful effects such increase in the immune system, reduced chance for bladder tumor and cancer, and most importantly decrease in cholesterol quantity. Silver nanoparticles on the other hand are one of the most widely used nanoparticles used for killing bacteria and other pathogens (Conway et al., 1987). However the antibacterial effects of AgNPs and its mechanism are not clearly known yet. Ag- based antiseptics are widely in use to provide bacterial resistance these days over antibiotics (Catauro et al., 2004).

1.2 Silver Nanoparticles:

The antibacterial properties of silver ions are widely renowned, however the properties of AgNPs on microbes and antimicrobial effects are not understood completely yet. The results of various research suggest that AgNPs can be used for inhibiting the growth of several microbes, making them a widely used NP for biomedical applications. At the moment AgNPs are widely used in controlling various bacterial growths in several biomedical fields that include dental, catheters, and in wounds that are related to burning. The fact that Ag based ions and particles and highly toxic and deadly to several microbe species is quiet well known and hence silver is widely used in different biomedical sectors for its antibacterial properties. There are many fields where AgNPs have proved to be very useful and effective against bacteria and other microbes. One possible way to use AgNPs is as a catalyst. Ag NPs can also be used as real time optical sensors. They also have the property of inhibiting bacterial cell growth and this is the characteristics that we are going to use in study. The Ag can be categorized and divided into two types as per their bactericidal effects and they are: Ag ions and Ag NPs (Huh and Kwon, 2011). While Ag ions are simply positively charged ions,

AgNPs are different being simply single crystals. However the purpose of the study is to find out the effects of AgNPs on the *L. casei* cells. While Ag has shown antibacterial effects against many known microbes, our study focuses to show that AgNPs can also aid in the growth of probiotic bacteria such as *L. casei* (Huh and Kwon, 2011).

1.3 *Lactobacillus casei*:

L. casei is one of the species found in the genus of *Lactobacillus* (Banks and Williams, 2004). It's very commonly available in the parts like intestine and mouths of the human beings. It has a very wide temperature and pH range. *L. casei* is a probiotic bacteria that is pretty much safe for consumption. One of the most common use of *L. casei* is in dairy industires. Several *L. casei* strains can be used as probiotic, and can be very useful against gastrointestinal diseases. As per WHO (Adams and Marteau, 1994), these characteristics of *L. casei* have been properly studied, demonstrated and are completely valid. Also studies have shown that L. casei can be used to recover from diarrhea at a very fast rate especially in children.

1.4 Objectives:

The objectives of this work are as follows: -

1. Studying the pattern of growth profile of the bacteria *L. casei*.
2. Studying the effects of silver nanoparticles on the *L. casei* liquid culture under various concentration of nanoparticles.

Chapter 2

Literature Review

2.0 Literature Review

2.1 Nanoparticles:

Antimicrobial mediators are of great importance in fabric industries, water decontamination, medicine, and food industries. Organic mixtures that are used in decontamination have lots of drawbacks that include harmfulness to humans, consequently, the attention in inorganic decontaminators similar to metal oxide nanoparticles (NPs) has hence increased. Such enhanced antimicrobial mediators locally terminate the microbes, without being harmful to the neighboring cells and tissues (von Nussbaum et al., 2006).

Antimicrobial action is associated with the mixtures that locally destroy the cell wall of the bacteria and kill them or inhibit their growth rates, without causing any harm to the neighboring tissues and cells. Recently the antibacterial compounds are chemically revised natural matters, like β-lactams, cephalosporins or carbapenems. Likewise, unadulterated natural compunds, like aminoglycosides can also be used for the cause (Oldenburg, 2004). These substances can be categorized as either bactericidal, that completely kills the bacteria, or bacteriostatic, that slows down or inhibits the bacterial growth. They can be used to fight against infectious diseases. Though, common and repetitive use bacteria started developing resistance to them which has become a huge problem (Rakow and Suslick, 2000). Superbugs is the term given to such bacterial strains that develop resistance and can survive against the antibacterial agents for years causing disease. Tuberculosis (TB) is a common example of such superbug that has developed resistance to previous antibacterial treatments. Along with that, disadvantages for conventional antibacterial compounds are not only the multiple drug resistance bacteria that are developed, but they also exhibit certain side effects. Since for drug resistance bacteria very high dose of antibiotics are used, they often generate high toxicity levels that cannot be tolerated by the human body and hence they harm the surrounding cells and tissues. Hence these factors have led to introduction of other tactics that can produce

antibacterial effects. One of them is the development of nano scale materials in treating bacterial disease. Especially several groups of nanoparticles like Silver, Zinc etc. have shown excellent antibacterial effects and are not as toxic to the surrounding cells and tissues in contrary to their ion counterparts. Why nanoparticles? The reasons can be stated as they have very high surface area to volume ratio enhancing the mechanical, chemical and electrical properties of their bulk counterparts. Before commenting any further on nanoparticles we first understand different bacterial properties that need to be dealt with in order to eliminate the bacteria (Witte, 2006).

2.2 Property of the cell wall:

The figure below has been adapted from TRENDS in Biotechnology journal.

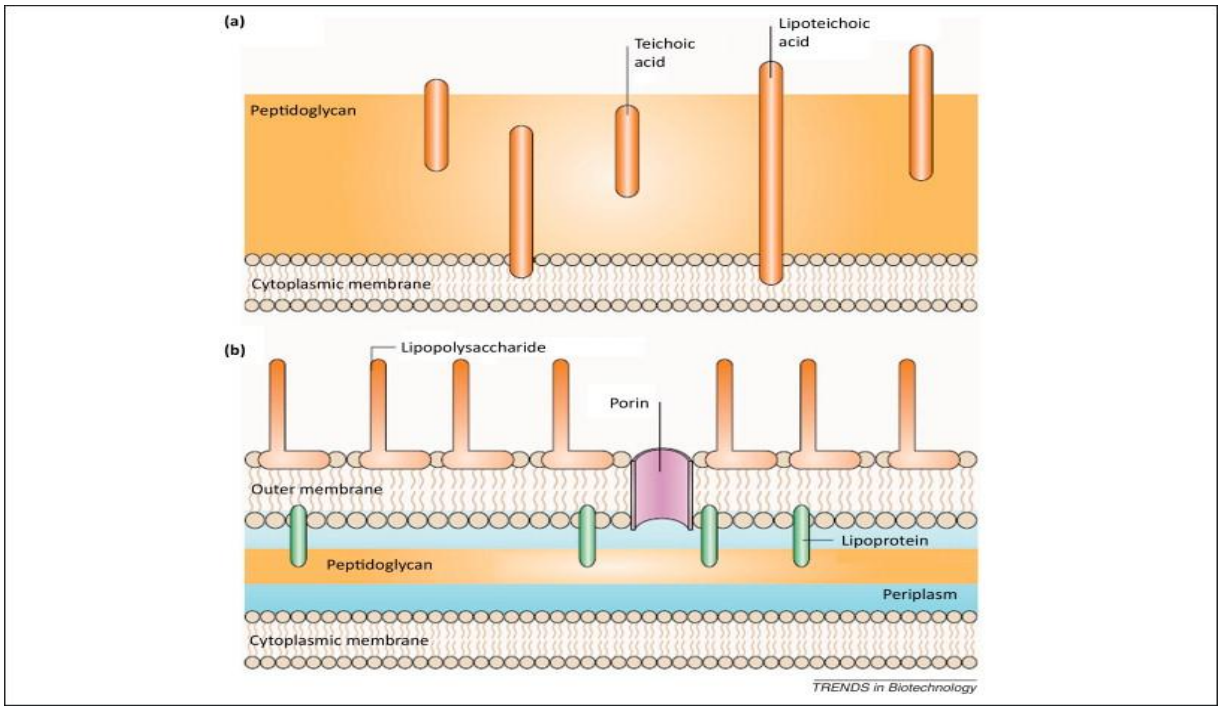


Figure 1. Bacterial Structure. (a) Gram Positive (b) Gram Negative.

The cell wall of the bacteria has been developed or designed to give the cell shape, structure and provide it with required mechanical strength to avoid rupture from pressure. As per the cell wall's component, structure and use the bacteria can be classified as: Gram positive (+ve) and Gram negative (–ve). The cell wall of the +ve has a very thick layer (i.e., 20–50 nm) of peptidoglycan (PG) (Scott and Barnett, 2006). While the –ve cell walls are more complex in nature. They are more complex both chemically and structurally. The Gram negative type has a thin PG layer and also contrary to the +ve type they have an outer membrane.

2.3 Effect of the NP type and surface:

Class sensitivity is not only depend on the type of cell wall. Various other reasons can affect the vulnerability or tolerance of the microbe to NPs. As an example, the susceptibility of *E. coli* is much higher than *Staphylococcus aureus* (+ve) and *Bacillus subtilis* (+ve) to the CuO NPs (Baek, 2011; Whitesides, 2005; Scott and Barnett, 2006).

2.4 Effect of growth rate:

One more factor that can affect the antibacterial properties of the NPs and antibiotics is the growth rate of the bacteria. It depends a lot on whether the bacteria has a very fast growth rate or has a very slow growth rate. Fast growing bacteria are more susceptible to the NPs (Lu et al., 2009).

2.5 Effect of biofilm formation:

A very big disadvantage of the NPs and other antibacterial drugs is their failure against bacteria that can develop biofilm. Biofilms are a multifaceted bacterial community that are formed by bond to a solid superficial and by emission of a medium (Huh and Kwon, 2011) (proteins, DNA, and extra-polysaccharide), this protects the bacterial community. The biofilms are a major problem since they protect the pathogenic microbes against the NPs and can cause chronic diseases (Lu et al., 2009).

2.6 The Toxicity Mechanism of NPs against bacteria:

The accurate and exact principle behind the antibacterial effect of the NPs against bacteria has yet to be fully understood. However one thing is certain that the NPs manage to get attached to the cell membrane of the bacteria via electrostatic interactions and rupture the cell wall or disrupt its functioning (Baker-Austin et al., 2006). The main reason behind it can be explained by the formation of ROS (Reactive Oxygen Species), they are induced when NPs are administered (Roberts, 1996). The figure below has been adapted from the journal TRENDS in Biotechnology.

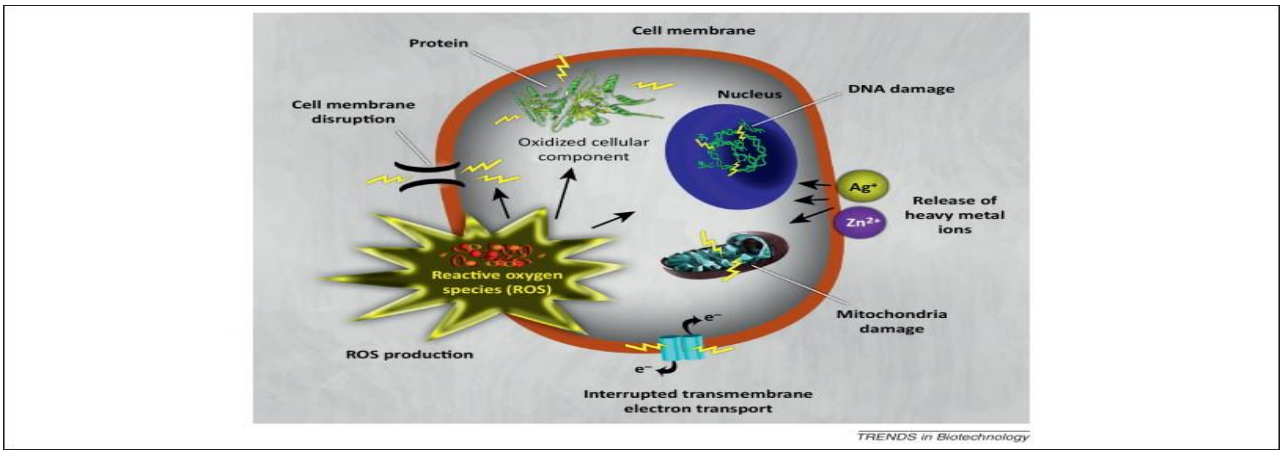


Figure 2. Reactive Oxygen Species (ROS) formation in bacterial cells.

The NPs in their ion forms can induce a reactive oxygen species in the bacterial cell that damage the bacterial cell components like mitochondria, DNA and nucleus and kill the cell.

2.7 Defense mechanisms developed by bacteria against NPs:

Bacteria have adapted various natural techniques to tolerate against the toxins or NPs added to them (Witte, 2004). For example presence of EDTA can hamper the toxicity of Cu based NPs. The bacteria can produce extracellular polymeric substances (EPS) and develop resistance against the NPs. Some can alter the fatty acid levels in order to avoid toxicity by NPs. Others may induce the expression of genes that are responsible for repairing of the DNA and alter the metal homeostasis in the presence of the NPs (von Nussbaum et al., 2006; Baker-Austin et al., 2006).

.

2.8 NPs against environment and ecosystems:

Repetitive use of NPs in various places can lead to leakage of the NPs to the environment that is land (soil) and water (Xia, 2008). In the environment there are many useful and beneficial bacteria present and the released NPs may kill them causing damage to the environment. The leakage of NPs into the environment has been one of the most serious threats to the beneficial bacteria and can hamper and affect the public health as well (Whitesides, 2005).

To summarize things up, the antibacterial effects of the NPs depend upon: (i) physicochemical characteristics of the particles (ii) Nature of the microbe (Huh and Kwon, 2011).

2.9 *Lactobacillus casei*:

Lactobacillus casei is mesophilic in nature and is a +ve bacteria (Scott and Barnett, 2006), its rod shaped in nature, nonsporing, anaerobic, and contains no cytochromes. *L. casei* is available in several environments like dairy products, intestines and reproductive systems of both animals and human beings. It can also be found in plant products that are fresh and fermented.5.5 is the optimum pH for *L. casei*. It produces lactic acid that can be used in making products such as cheeses and yogurts, it also decreases the cholesterol levels, improves the immune response, helps in fast recovery from diarrhea (Conway et al., 1987), improves the lactose tolerance in lactose intolerant humans, inhibits the growth of several pathogens found in the intestinal tract, and hence serves as a probiotic. They are beneficial for the body (Singleton, 2004).

2.10 Effect of Silver NPs on *Lactobacillus casei*:

In studies involving effects of Silver nanoparticles on lactic acid bacteria found in the intestinal tracts it has been stated that Silver NPs indeed do not affect the Lactic acid bacteria much. It can be seen from the research carried out by several researches for example in the midterm research report of 2010 of the chwalibog, it has been observed that the control and the silver added lactic acid bacteria have almost same ODs emphasizing that silver doesn't the inhibit the growth of the lactic acid bacteria. In another research carried out by Lane Pineda it also states that silver nanoparticles do not influence the microbial population found in the digestive tract which includes *L. casei* (Pineda et al., 2010a).

Chapter 3

Materials and methods

3.1 Materials used:

1. *L. casei* strain
2. AgNPs
3. Distilled Water
4. Lactobacillus MRS broth
5. Test tubes
6. Flasks
7. Pipette

3.2 Instruments used:

1. UV – Vis Spectroscopy (Spectrophotometer)
2. Laminar Flow chamber
3. Autoclave
4. Incubator

3.3 Methodology:

3.3.1 Preparation of *L. casei* culture:

The *L. casei* strain was provided in the laboratory. 5.515 gm of Lactobacillus MRS Powder was weighed in the electronic weighing balance and it was added to a flask containing 100 ml of distilled water. Then it was thoroughly mixed by shaking. Finally after it was properly mixed, the media was taken to be autoclaved for 15-20 min at 121°C at 15 lbs. pressure. After it was autoclaved, the media was allowed to cool down in room temperature. Once the media was cooled down, it was taken into the laminar flow chamber where, the *L. casei* strain was added to it. Then the media was stored in the incubator at 37 degree Centigrade at 100

rpm. The OD of the broth was constantly measured for plotting the growth profile curve of the *L. casei*.

Figure 3. Preparation of the L. casei culture in the laminar flow chamber.

3.3.2 Preparation of Silver Nanoparticles (AgNP):

The AgNPs were prepared by Mr. Deependra K. Ban, Ph.D student in the Laboratory of Structural Biology and Nanomedicine. The concentration of AgNP was 10 mg/ml and average size of the particles was 10-15 nm. They were prepared by Citrate reduction method.

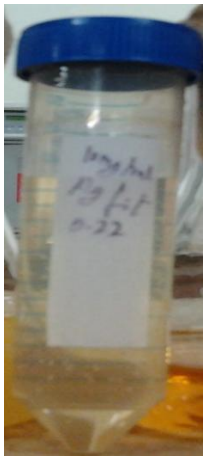


Figure 4. Silver Nanoparticles extracted from Citrate Reduction method.

Citrate reduction method is one of the most commonly used bulk synthesis method of nanoparticles of metals that uses the chemical reduction of metal salts.

Sodium citrate was obtained from Alfa Aiser. Silver colloids were prepared by the renowned Turkevich method (Turkevich et al., 1951). Silver Nitrate solution was heated in deionized water until it was boiled. As soon as the boiling started, the Sodium Citrate solution was added to the boiling solution of Silver Nitrate drop by drop. Eventually as time passed the color of the solution changed into grayish yellow which indicated that there is reduction of Silver ions. Heating was done for more 15 minutes and then was cooled to room temperature.

3.3.3 Addition of Silver Nanoparticles into *L. casei* broths:

This was carried out in 4 sets of experiments with different silver concentrations in the broth. The *L. casei* stock and the silver nanoparticles stock were taken. The L. casei stock was the previously prepared culture of *L. casei* that was used for calculation of the growth profile curve of the *L. casei*. The Silver nanoparticles as mentioned earlier were prepared by citrate ion reduction method and were provided in the laboratory. The general steps involved preparation of 50 ml media that were poured into 5 test tubes (9 ml each) and another 50 ml media was prepared for blank which would be used to measure OD as the reference media. 5 test tubes were taken and named A, B, C, D and E. The image below shows the five test tubes A, B, C, D and E with 9 ml each of the Lactobacillus MRS broth media.

Figure 5. Five test tubes with 9 ml Lactobacillus MRS Media were taken.

100 micro liter of *L. case*i from the previously made culture was added to the 5 test tubes. Then AgNP at different concentrations were added to the test tubes. In test tube A, no silver nanoparticles were added and every time A was the control. Rest were added with silver nanoparticles and the concentration of silver in the broth ranged from 0.02 mg/ml to 1 mg/ml. Then the test tubes were incubated in the incubator at 37 degree Centigrade at 100 rpm. The OD readings were taken at 16, 20, 24, 28, 36 hours respectively to perform the analysis of effects of AgNP on the different *L. casei* culture.

Chapter 4

Results and Discussions

4. Results and Discussions:

4.1 Analysis of growth profile curve of *Lactobacillus casei*:

It was observed that the media had dense growth of *L. casei* in it as shown in the figure below.

Figure 6. Image of MRS media on left and of *L. casei* growth in the media on right.

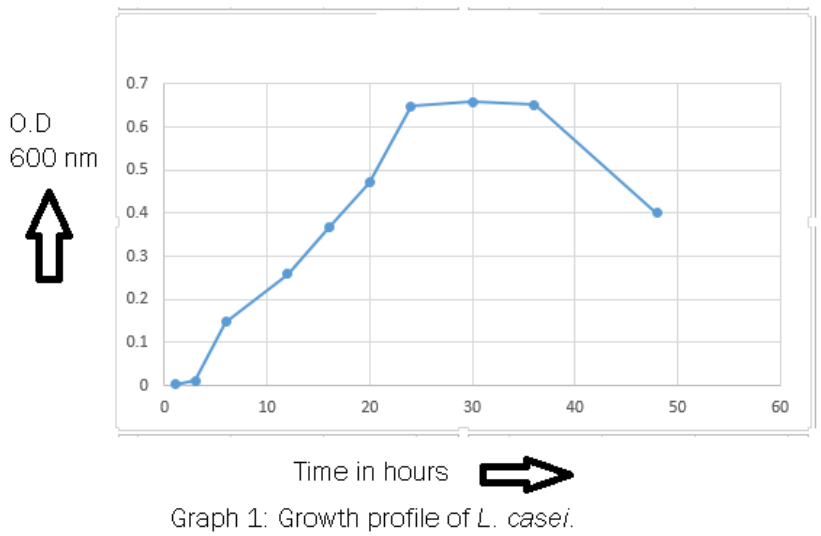
The growth profile curve was plotted as OD vs time in hours. The spectrophotometer reading were taken as:

Table 1. Analysis of growth profile of *L. casei*.

Time in hours	**Optical Density**
1	0.003
3	0.109
6	0.1475
12	0.2596
16	0.3672
20	0.4720

24	0.6490
30	0.6582
36	0.6582
48	0.4008

The growth profile curve was plotted taking OD on Y- axis and time along the X- axis.



Time in h	O.D
1	0.003
3	0.0109
6	0.1475
12	0.2596
16	0.3672
20	0.472
24	0.649
30	0.6582
36	0.652
48	0.4008

Figure 7. Graph showing the growth profile curve of *L. casei* over 48 h.

It's observed and analyzed from the growth profile curve of *L. casei* that it remains in lag phase for first 3 h. Next it enters the log phase and stays in log phase for 20 h. From 24 to 36 h it maintains a stationary phase and then it's seen that at 48th hour, it enters its death phase.

4.2 Effect of different concentration of AgNPs on *L. casei*:

4 sets of reading were taken to study the effects of AgNPs on *L. casei*. In the first set of reading the concentration of silver in the broth were:

A- Nil – control

B- 0.02 mg/ml

C- 0.03 mg/ml

D- 0.04 mg/ml

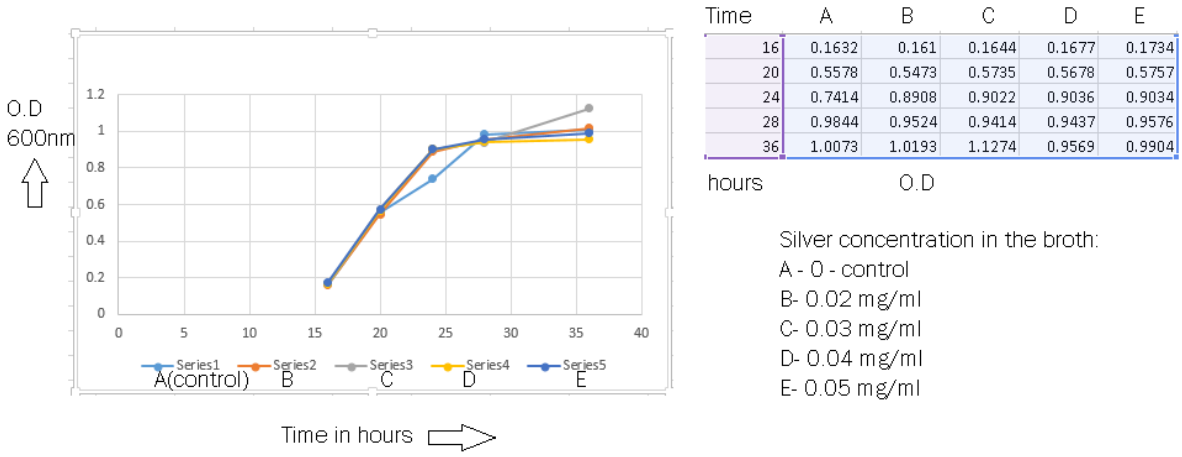
E- 0.05 mg/ml

The respective ODs were noted as:

Table 2. Different OD values for different AgNP concentration 1.

Time in hrs	OD (A)	OD (B)	OD (C)	OD (D)	OD (E)
16	0.1632	0.1610	0.1644	0.1677	0.1734
20	0.5578	0.5473	0.5735	0.5678	0.5757
24	0.7414	0.8908	0.9022	0.9036	0.9034
28	0.9844	0.9524	0.9414	0.9437	0.9576
36	1.0073	1.0193	1.1274	0.9569	0.9904

The graph of the above table can be plotted as:



Graph2: Effect of AGNPs on *L. casei* 1.

Figure 8. Graph showing effect of AgNPs on *L. casei* 1.

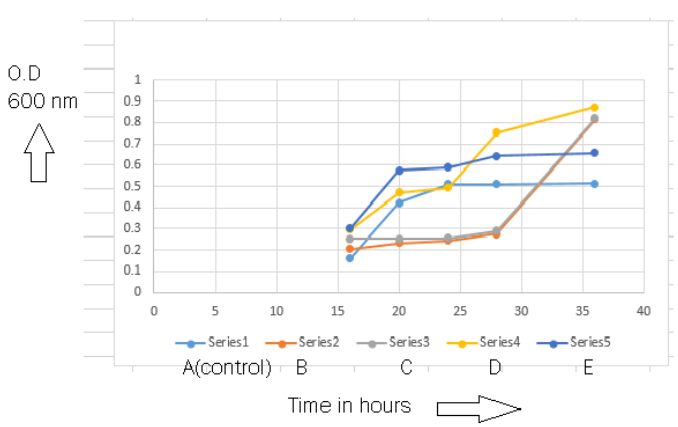
It's observed that AgNPs at low concentrations of 0.02 mg/ml – 0.05 mg/ml doesn't inhibit the growth of *L. casei* rather it aids the growth as a catalyst. This can be clearly seen from the fact that some broths have better ODs than the control.

The results of the next set of readings were found out as follows:

Table 3. Different OD values for different AgNP concentration 2.

Time in hrs	OD (A)	OD (B)	OD (C)	OD (D)	OD (E)
16	0.1602	0.2054	0.2522	0.2958	0.3001
20	0.4232	0.2319	0.2534	0.4710	0.5744
24	0.5087	0.2455	0.2567	0.4920	0.5896
28	0.5099	0.2746	0.2899	0.7540	0.6436
36	0.5136	0.8143	0.8193	0.8740	0.6573

The graph of the above table was plotted and analyzed as:



Time	A	B	C	D	E
16	0.1602	0.2054	0.2522	0.2958	0.3001
20	0.4232	0.2319	0.2534	0.471	0.5744
24	0.5087	0.2455	0.2567	0.492	0.5896
28	0.5099	0.2746	0.2899	0.754	0.6436
36	0.5136	0.8143	0.8193	0.874	0.6573

hours O.D

Silver concentration in the broth:
A- 0- control
B- 0.06 mg/ml
C- 0.12 mg/ml
D- 0.18 mg/ml
E- 0.24 mg/ml

Graph3: Effect of AGNP on *L. casei* 2.

Figure 9. Graph showing effect of AgNPs on *L. casei* 2.

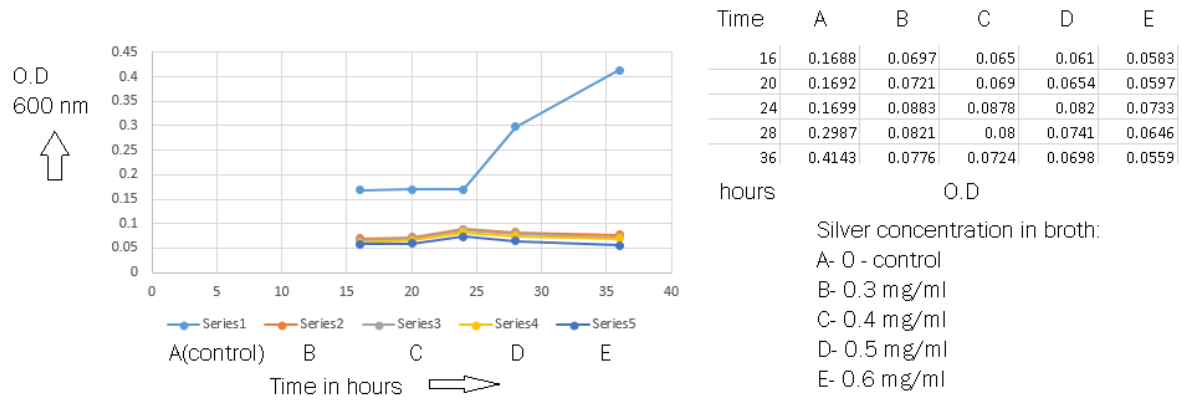
Again it's observed that AgNPs do not show much of antibacterial effects at the lower concentration. However it is analyzed that at concentration of 0.18 mg/ml AgNPs have acted as a catalyst for rapid growth of the bacteria. At concentrations of 0.06 – 0.12 mg/ml, It can be seen initially the broths have lowed OD than the control but by 36th hour they have higher ODs showing more growth than the control. In the broth E where AgNPs concentration was 0.24 mg/ml, the initial OD value was highest but with time its OD value failed to remain highest and became the lowest. This is the only concentration where certain antibacterial effect was observed at later hours.

The results of the next set of readings are as follows:

Table 4. Different OD values for different AgNP concentration 3.

Time in hrs	OD (A)	OD (B)	OD (C)	OD (D)	OD (E)
16	0.1688	0.0697	0.0650	0.0610	0.0583
20	0.1692	0.0721	0.0690	0.0654	0.0597
24	0.1699	0.0883	0.0878	0.0820	0.0733
28	0.2987	0.0821	0.0800	0.0741	0.0646
36	0.4143	0.0776	0.0724	0.0698	0.0559

The graph can plotted as:



Graph4: Effect of AGNP on *L. casei* 3.

Figure 10. Graph showing effect of AgNPs on *L. casei* 3.

From the OD values of the different broths, it is very well observed that the OD values of the broth A which is the control, is the highest. Other broths having silver of concentration 0.3 mg/ml – 0.6 mg/ml have very low OD value in comparison with the control A. This clearly

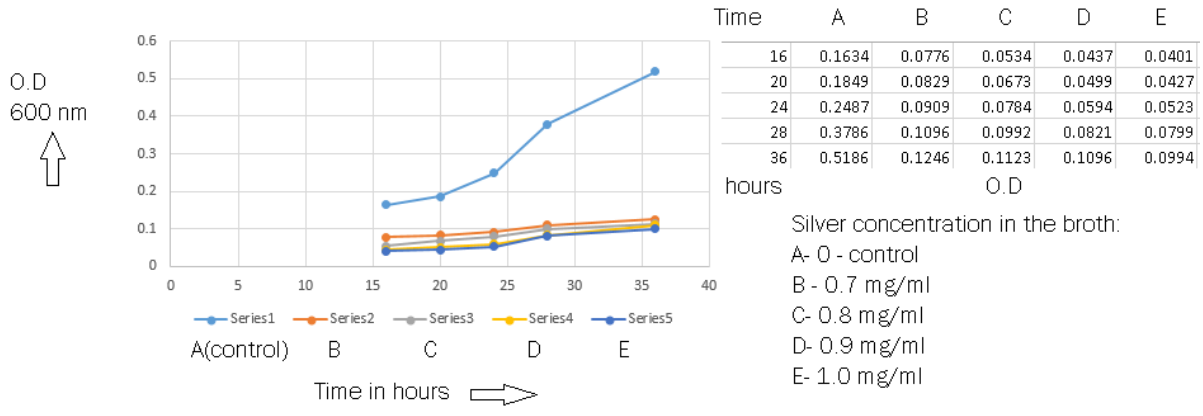
shows that the AgNPs are inhibiting the growth of *L. casei* at concentrations 0.3 mg/ml and higher and thus show antibacterial effects.

The final set of readings were taken with silver concentrations of 0.7 mg/ml – 1.0 mg.ml and it was observed as follows:

Table 5. Different OD values for different silver AgNP 4.

Time in hrs	OD (A)	OD(B)	OD (C)	OD (D)	OD (E)
16	0.1634	0.0776	0.0534	0.0437	0.0401
20	0.1849	0.0829	0.0673	0.0499	0.0427
24	0.2487	0.0909	0.0784	0.0594	0.0523
28	0.3786	0.1096	0.0992	0.0821	0.0799
36	0.5186	0.1246	0.1123	0.1096	0.0994

The graph of the above table can be plotted as:



Graph5: Effect of AGNP on *L. casei* 4.

Figure 11. Graph showing effect if AgNPs on *L. casei* 4.

It can be clearly observed from the graph that the OD values of the control A is the highest while the OD values of other broth having AgNPs concentrations of 0.7 mg/ml – 1.0 mg/ml are much lower and they keep on decreasing as the concentration of silver nanoparticles is kept on increasing. This shows that silver nanoparticles at higher concentrations of 0.7 mg/ml and above clearly inhibits *L. casei* growth and shows clear antibacterial effects.

It's clear from the above sets of readings that at lower concentrations of silver nanoparticles that is less than 0.3 mg/ml, silver doesn't inhibit the growth of *L. casei* and in some case it acts as a catalyst in *L. casei* growth which is clear from the fact that some of the broths having silver nanoparticles in them grew faster and had higher OD values than the control broths. Hence it can be analyzed that at lower concentrations silver can act as a catalyst in the *L. casei* growth. However as the concentrations of silver nanoparticles is kept on increasing from 0.3 mg/ml to 1 mg/ml the OD values keep on decreasing and its observed that silver inhibits the growth of *L. casei* at higher concentrations showing clear antibacterial effects.

Chapter 5

Conclusion

5.1 Conclusion:

From the study, it was concluded that at low concentrations of AgNP i.e. less than 0.3 mg/ml it accelerated the *L. casei* growth. This implies that AgNP at a low concentration enhanced the growth of *L. casei* which indeed can be considered to be a practical strategy to produce the probiotic in higher amount. However, at higher concentration i.e. 0.3 mg/ml to 1 mg/ml AgNP showed significant antibacterial effect on *L. casei* culture, implying that the AgNP can also be used as growth inhibitor. Hence, we can conclude that AgNP can play as a growth activator of *L. casei* at lower concentrations, but it also acts as growth inhibitor at higher concentration. Therefore, such fact can be exploited in different biomedical research area and can be used to develop agents to enhance *L. casei'*s probiotic effects.

5.2 Future Work:

Present work shows the analysis of the effects of Silver nanoparticles on the probiotic *Lactobacillus casei*. It is understood from the study that at very low concentrations of AgNP works as a catalyst in the growth of *L. casei*. However it shows clear antibacterial effects at higher concentrations. But since at lower concentrations AgNP enhances *L. casei's* growth, this fact can be exploited for biomedical applications. *L. casei* help in reducing cholesterol level that can reduce the heart disease conditions as well. Even it helps in many gastrointestinal diseases and also helps in fast recovery from diarrhea. So, for future work other probiotic bacteria can be used to treat with our AgNP for more information.

References

- A.A. Ashkarran, et al. (2012), Bacterial effects and protein corona evaluations: crucial ignored factors for prediction of bio-efficacy of various forms of silver nanoparticles Chem. Res. Toxicol., 25, pp. 1231–1242
- A.J. Huh, Y.J. Kwon (2011), "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era J. Control. Release, pp. 128–145
- A.L. Oldenburg, et al. (2004), Magnetic contrast agents for optical coherence tomography Proc. of SPIE, pp. 91–98
- Adams MR, Marteau P (1995). "On the safety of lactic acid bacteria. Int J Food Micro", 27: 263-264.
- Banks JM, Williams AG (2004). "The role of the nonstarter lactic acid bacteria in Cheddar cheese ripening". International Journal of Dairy Technology 57 (2–3): pp. 145–152.
- C. Baker-Austin, et al. (2006), Co-selection of antibiotic and metal resistance Trends Microbiol., pp. 176–182
- C. Lu, et al.(2009), Slow growth induces heat-shock resistance in normal and respiratory-deficient yeast Mol. Biol. Cell, 20, pp. 891–903
- Conway PL, Gorbach SL, Goldin BR (1987). "Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J Dairy Sci", 70: pp. 1-12.
- F. von Nussbaum, et al. (2006), Antibacterial natural products in medicinal chemistry – exodus or reviva Angew. Chem. Int. Ed., pp. 5072–5129
- G.M. Whitesides (2005) Nanoscience, nanotechnology, and chemistry Small, 1, pp. 172–179
- I.S. Roberts (1996), The biochemistry and genetics of capsular polysaccharide production in bacteria Annu. Rev. Microbiol., pp. 285–315
- J.R. Scott, T.C. Barnett (2006), Surface proteins of gram-positive bacteria and how they get there Annu. Rev. Microbiol., pp. 397–423
- M. Catauro, M.G. Raucci, F.D. De Gaetano, A. Marotta (2004), Antibacterial and bioactive silver-containing Na2O × CaO × 2SiO2 glass prepared by sol-gel method J Mater Sci Mater Med, 15 (7) , pp. 831–837

- N.A. Rakow, K.S. Suslick (2000), A colorimetric sensor array for odor visualization Nature, 406, pp. 710–713
- P. Singleton (2004), Bacteria Biology, Biotechnology and Medicine (6th ed.)John Wiley & Sons Ltd, West Sussex, England, pp. 570-575
- Pineda, L., Chwalibog, A., Sawosz, E., Hotowy, A., Elnif, J., Sawosz, F., Niemiec, T, Ali, A. (2010a) Effect of nanoparticles of silver and gold on gas exchange and heat production of chicken embryos. Energy and protein metabolism and nutrition. 3re EAAP International Symposium on Energy and Protein Metabolism and Nutrition, ed G. M. Crovetto, Parma, Italy, EAAP publication 127, pp. 213-214.
- Turkevich, T., et al. (1951). "A Study of the Nucleation and Growth Processes in the Synthesis of Colloidal Gold." Discussions of the Faraday Society, Vol. 11, pp. 55-75.
- W. Witte (2004), International dissemination of antibiotic resistant strains of bacterial pathogens Infect. Genet. Evol, pp. 187–191
- Y. Xia (2008), Nanomaterials at work in biomedical research Nat. Mater., pp. 758–760
- Y.W. Baek, Y.J. (2011), An Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb2O3) to Escherichia coli, Bacillus subtilis, and Streptococcus aureus Sci. Total Environ., 409, pp. 1603–1608