Available online at http://jddtonline.info

RESEARCH ARTICLE

STUDIES ON MICROBIOLOGICAL QUALITY OF SPROUTS OF MUNG BEAN (*VIGNA RADIATE* L.)

Shilpi Chauhan[1], Ankit Saini[2], Devendra Pratap Singh*[3], Umesh Dhaked[3], Poonam Gupta[1]

[1]Krishna College of Science and Technology, Bijnor, India

[2]Krishna College of Pharmacy, Bijnor, India

[3]Bhagwant Institute of Pharmacy, Muzaffarnagar, India

Corresponding Author's Email: mdps1341619@gmail.com

ABSRACT

Sprouts are now well known for their high nutritive value and digestibility. They are rich in enzymes, bioavailable vitamins, minerals, amino acids and fibers. Sprouts are low in fat and calories, being rich in nutrients and known to promote health, sprouts now days are available in most of the grocery stalls. Survey of sprouted seeds available at retail venders has shown the presence of pathogenic bacteria like *E. coli* 0157, *Salmonella* and *Listeria monocytogens*, which is of concern for health conscious public. In the present study an attempt has been made to identify and examine the microorganisms present in seed sprouts of mung bean.

Key-Words: Mung Bean Sprouts, Morphological Identification, Biochemical Characteristics.

INTRODUCTION

Sprouting is the practice of germinating seeds to be eaten either raw or cooked. They are a convenient way to have fresh vegetables for salads, in any season and can be germinated at home or produced industrially. Sprouts are believed to be highly nutritious and rich in enzymes which promote good health. Sprouts, including mung beans and alfalfa sprouts, have become a common food item in grocery stores, salad bars around the world. Sprouts are low in calories and fat and provide substantial amounts of key nutrients, such as vitamins, minerals, proteins, enzymes, folate, and fiber.[1,2,3]

Chavan and Kadam (1989) have described importance of sprouts as, "The desirable nutritional changes that occur during sprouting are mainly due to the breakdown of complex compounds into a more simple form, transformation into essential constituents and breakdown of nutritionally undesirable constituents that are easy to digest."[4] A large number of food borne disease outbreaks reported world over have been found to be linked to sprouts. In most instances, the illnesses were caused by either *Escherichia coli* O157:H7 or *Salmonella* bacteria. Anyone who eats raw sprouts or lightly cooked mung bean sprouts is at risk for exposure to *Escherichia coli* O157:H7 or *Salmonella* bacteria.[5]

The consumption of raw sprouts has become the focus of increasing concern during the past decade. Despite increased awareness and guidelines set forth by the Food and Drug Administration (FDA), food borne disease outbreaks linked to sprouts have continued. [6,7,8] Contamination of sprouts is generally thought to arise from contaminated seed.[6,8,9,10] The one method is disinfecting the seeds that kill bacteria but that still allows the seeds to germinate to produce sprouts. Further some of the consumers do not want chemicals to be used to disinfect the seeds. Thus the only alternate available is to either control the contamination of seeds meant for consumption as sprouts or restrict the growth of pathogens during sprouting. Therefore the present study was undertaken to determine the microbiological quality of sprouts prepared in the laboratory.

OBJECTIVES

The primary objectives of the work presented here were

(i) To determine the number of microorganisms associated with mung bean sprouts prepared under laboratory conditions

(ii) To characterize and identify the microorganisms associated with sprouts.

MATERIAL AND METHODS

The present study entitled "Studies on microbiological quality of sprouts of Mung Bean (*Vigna radiata*) was carried out in Department of Microbiology, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun.

Collection of Sample

Two seed samples of mung bean were purchased from local market from two different retail shops.

Seed Sprouting

Each type of seeds (5.0g) were placed in a sterile petri plate containing Whatmann No. 113 (qualitative wet strengthen) filter paper saturated with sterilized water or tap water. The seeds were incubated at room temperature (25^{O}C).

Microbial Analysis of Seed Sprouts

Sprouts after 24, 48, 72, 96 h of sprouting from 5.0 g of seeds were suspended in 95 ml sterile water blank. The

flasks were placed on a rotary shaker for 15 minute and ten fold serial dilutions were prepared. Aliquots of 0.1 ml of appropriate dilutions were spread on plate containing Mac Conkey Agar and Brilliant Green Agar and plates were incubated at 37^{O} C for 24 to 48 hrs.

The plates were observed after incubation for growth and colony Characteristics. Number of different colonies appearing on the plates was counted and number of bacteria present on each sprout was calculated as follows

$$\text{No. of Bacteria per Sprout} = \frac{\text{Number of colonies} \times \text{dilution factor} \times 0.1}{\text{Number of seeds}}$$

Cultural Characterization

The colonies were observed for their color, texture, outline, opacity, pigmentation etc and different type of colonies appearing on all the three media were counted.

Morphological Characterization

The colonies were picked and processed for Gram staining technique to differentiate between Gram positive and Gram negative bacteria, shape and arrangement of cells.

Media Composition

Different types of media used in the study were Mac Conkey Agar, Brilliant Green Agar, Baired Parker's Agar etc., which were sterilized by autoclaving at121^{O}C for 20 minute at 15 lbs psi.

Mac Conkey Agar Medium (pH- 7.4)[11]

Ingredients	Amount (g/ l)
Peptone	5.0
Lactose	10.0
Bile salt	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	15.0

Brilliant Green Agar Medium (pH- 6.9)[12]

Ingredients	Amount (g/l)
Lactose	10.0
Sucrose	10.0
Peptone	5.0
Sodium chloride	5.0
Phenol red	0.08
Brilliant green	0.0125
Agar	20.0

Baired Parker's Medium (pH-7.0) [13]

Ingredients	Amount (g/ l)
Casein enzyme hydrolysate	10.0
Beef extract	5.0
Yeast extract	1.0
Glycine	12.0
Sodium pyruvate	10.0
Lithium chloride	5.0
Agar	20.0

Dissolve all the components in 950 ml distilled water sterilize by autoclaving and cool at 50^{O}C.

MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION

Some of the colonies from different sample and media were picked and purified by streak plate method and ensured by microscopic examination that all the isolates were pure. The bacterial isolates were identified based on the following biochemical tests:

Staphylococcus aureus: Catalase, Coagulase, Sugar fermentation, Salt tolerance, Phosphatase tests.

Salmonella, *Klebsiella*, *E.coli*: IMViC, TSI, Urease, Sugar fermentation.

IMViC (Inddole, Methyl red, Vogues-Proskauer, Citrate Test)

The IMViC tests are designed to differentiate gram negative intestinal bacilli (family enterobacteriaceae) particularly *E .coli*, *Enterobacter*, *Klebsiella* group on the basis of their biochemical properties and enzymatic reactions in the presence of specific substrate. These tests were carried out as follows:

a) Idole Test

Tryptophan an essential amino acid is oxidized by some bacteria by the enzyme tryptophanase resulting in the formation of indole, pyruvic acid and ammonia. The reaction is detected by adding Kovac's reagent which produce a layer of cherry red colour.

b) Methyl Red Test

The methyl red test is quantitative test for acid production requiring positive organism to produce strong acid from glucose through the mixed acid fermentation pathway. The pH indicator dye methyl red changes the colour of the inoculated Methyl Red & Vogues-Proskauer broth with test organism.

c) Voges – Proskauer Test

The purpose of Voges – Proskauer is to determine whether an organism can produce acetyl methyl carbinol (acetoin) from fermentation of glucose. In the presence of atmospheric oxygen and alkali (4o% potassium hydroxide), the small amount of acetyl methyl carbinol present in the medium is converted to diacetyl which react with the peptone of the broth to produce a red colour.

d) Citrate Utilization Test

The citrate utilization test is to differentiate among enteric bacteria on the basis of their ability to utilize

/ferment citrate as the sole carbon source. The utilization of citrate depends on the presence of citrase that breaks citrate to oxaloacetic acid and acetic acid. These are later converted into pyruvic acid and carbon dioxide. The CO_2 combines with sodium and water to form sodium carbonate an alkaline product which changes the colour of Simmon Citrate Agar slants from green to blue after incubation for 24-48 h at 37^OC.

TSI Agar Test

Triple sugar iron agar medium is composed of three sugars i.e. lactose, sucrose and small amount of glucose, iron (ferrous sulphate) and phenol red as indicator. The indicator is employed for the detection of fermentation of sugars indicated by the change in colour of the medium due to the production of organic acid and hydrogen sulphide gas. If an organism ferments any of three sugars or any combination of them, the medium will become yellow due to acid production. The tubes containing TSI agar slants were inoculated with test culture and incubated at 37^OC. The tubes were observed the growth and change in colour after 24- 48 hrs.

Urease Test

The urease a hydrolytic enzyme excreted by some microorganisms attack the carbon and nitrogen bond of amide compounds with the liberation of ammonia. It is useful diagnostic test for identifying bacteria especially to distinguish *Proteus* from the gram negative pathogens. The presence of urease shows red colour and for no urease it is yellow colour.

Catalase Tests

Catalase degrades hydrogen peroxide in the bacterial cell before it can do any damage to the bacterial cell. Production of the enzyme catalase can be demonstrated by adding hydrogen peroxide to colonies of bacteria. If catalase is present it is indicated by the presence of free gas bubbles. If catalase is absent no gas bubbles will be seen.

Cogulase Test

The enzyme cogulase produced by *S. aureus* is a key feature of pathogenic *S. aureus*. The enzyme causes coagulation of blood allowing the organism to wall its infection off from the host's protective mechanism rather effectively. Coagulase is a protein having a prothrombin like activity capable of converting fibrinogen into fimbrin which results in the formation of visible clot.

a) Slide Test

- The colonies of test organism were picked and emulsified with the drops of saline on a glass slide.
- A drop of serum was added to the bacterial suspension and observed for the clumping of the bacterial suspension.

b) Tube Test

- An aliquote of 0.5 ml of serum in a tube and approximately 5.0 drops of overnight broth culture grown in a test tube were added.
- The tubes were incubated at 37^OC for 24 hrs and observed the clot formation by gently tilting the tube.

Phosphatase Test

Staphylococcus aureus produces an enzyme which can split phosphate with the help of enzyme called as phosphatase. Cogulase positive *S.aureus* give positive phosphatase test. While cogulase negative Staphylococci and Micrococci are usually phosphatase negative.

Glucose Fermentation Test

Staphylococcus aureus are fermentative capable for producing acid from glucose and mannitol anaerobically. However *S. epidermidis* is mannitol negative Micrcocci differ from Staphylococci in the fact that they can not ferment glucose and mannitol anaerobically.

Procedure

- Inoculated the Baired parker carbohydrate agar slant containing glucose and mannitol as sugars (separately) and bromocresol purple as indicator with the test culture.
- The slants were incubated under anaerobic condition at 37^OC for 24 to 48 hrs.
- Observe the slants after incubation for the colour change from purple to yellow.

RESULT AND DISCUSSION

In the present study an attempt has been made to examine the microorganisms present in seed sprouts of mung bean. The observations of the experiments conducted are discussed below:

Number of Total Coliforms Present in Mung Bean on Mac Conkey Agar

Sprouts of mung bean were prepared under normal and aseptic conditions as per details given in material and methods and total number of coliforms were determined by spreading appropriate dilution of each type of sprout on plates containing Mac Conkey Agar medium. The plates were observed for type and number of colonies present after 24 h of interval of incubation for 4 days.

In mung bean sprouts one type of colonies were observed for three days under aseptic condition in sample A, while in sample B one type of colonies were present till 2 days. There after two distinct types of colonies were found on Mac Conkey Agar in both the samples. However two type of colonies appeared after three two day in sprouts prepared under normal conditions in sample A and B respectively.

Total number of cfu/sprout increased from 1.4×10^3 to 6.7×10^6 in different samples and treatments (Table1). For example total cfu/sprouts in sample A under aseptic conditions were 1.4×10^3 which increased to $2.6\text{-}5.2\times10^5$ in both the samples. Total cfu/sprouts prepared under normal conditions in sample B, however, were found to be higher i.e 6.7×10^6.

Table 1: Number of Coliforms Present in Mung Bean Sprouts on Mac Conkey Agar Medium (cfu/ sprout)

Sample	Days	Sterilized water			Unsterilized water		
		Types of colonies		Total count	Types of colonies		Total count
		Dark pink with centred, flat, small, circular	Light pink with centred, gummy, dome shaped, circular, small		Dark pink with centred, flat small , circular	Light pink with centred, gummy, dome shaped, small, circular	
A	0	1.0×10^{1}	_	1.0×10^{1}	2.5×10^{2}	_	2.5×10^{2}
	1	1.4×10^{3}	_	1.4×10^{3}	3.5×10^{4}	_	3.5×10^{4}
	2	2.0×10^{4}	_	2.0×10^{4}	6.8×10^{4}	_	6.8×10^{4}
	3	3.6×10^{4}	_	3.6×10^{4}	2.7×10^{5}	1.3×10^{3}	2.7×10^{5}
	4	2.6×10^{5}	6.5×10^{3}	2.6×10^{5}	3.9×10^{6}	6.9×10^{4}	3.9×10^{6}
B	0	2.3×10^{1}	_	2.3×10^{1}	2.9×10^{2}	_	2.9×10^{2}
	1	8.1×10^{3}	_	8.1×10^{3}	3.2×10^{4}	_	3.2×10^{4}
	2	2.2×10^{4}	_	2.2×10^{4}	1.4×10^{4}	1.6×10^{3}	1.5×10^{4}
	3	3.1×10^{4}	3.2×10^{4}	6.3×10^{4}	4.9×10^{5}	2.2×10^{5}	7.1×10^{5}
	4	3.6×10^{5}	1.6×10^{5}	5.2×10^{5}	6.5×10^{6}	2.4×10^{5}	6.7×10^{6}

Types and Number of Bacteria present in Mung Bean Sprouts on Brilliant Green Agar Medium

Number of *Salmonella* and *Shigella* spp. present on sprouted seeds was determined by spreading 100µl of appropriate dilution of sprouted seeds on plates containing Brilliant Green Agar medium for four days at an interval of 24 h.

Two distinct creamish, small, gummy and yellow large dome shaped colonies were observed in both the samples of mung bean sprouts prepared under normal as well as aseptic conditions (Table 2). However yellow, large, dome shaped colonies were observed after 48-72 hour of incubation under aseptic conditions.

This type of colonies appeared on Brilliant Green Agar after 24 and 48 h under normal conditions. The number of cfu/ml in sprouted mung beans were found to be 7.9×10^{2} and 3.9×10^{3} after 24 hour of incubation of sprouts under normal conditions which increased to 7.0×10^{5} cfu/ ml and 7.3×10^{6} in sample A and B respectively after 96 h. However in sprouts prepared under aseptic conditions, the number of bacterial count after 96 hours of incubation observed was 5.9×10^{4} and 1.3×10^{5} cfu/ml in sample A and B respectively.

Table 2: Number of Bacteria Present in Mung Bean Sprouts on Brilliant Green Agar Medium (cfu/ sprout)

Sample	Days	Sterilized water			Unsterilized water		
		Types of colonies		Total count	Types of colonies		Total count
		Cream, small , circular, without centred, slightly gummy raised	Yellow, large, gummy, dome shaped, circular		Cream, small , circular, without centred, slightly gummy raised	Yellow, large, gummy, dome shaped, circular	
A	0	7.1×10^{1}	_	7.1×10^{1}	7.1×10^{1}	7.3×10^{0}	7.8×10^{1}
	1	7.9×10^{2}	_	7.9×10^{2}	1.1×10^{3}	4.8×10^{3}	5.9×10^{3}
	2	6.5×10^{3}	2.4×10^{3}	8.9×10^{3}	3.9×10^{4}	1.0×10^{4}	4.9×10^{4}
	3	1.5×10^{3}	1.0×10^{4}	1.1×10^{4}	8.1×10^{4}	2.5×10^{3}	8.3×10^{4}
	4	4.8×10^{4}	1.1×10^{4}	5.9×10^{4}	6.5×10^{3}	9.8×10^{4}	7.0×10^{5}
B	0	2.5×10^{1}	_	2.5×10^{1}	6.3×10^{1}	_	6.3×10^{1}
	1	4.3×10^{2}	_	4.3×10^{2}	2.5×10^{3}	_	2.5×10^{3}
	2	8.4×10^{3}	_	8.4×10^{3}	3.8×10^{4}	1.2×10^{4}	5.0×10^{4}
	3	2.5×10^{4}	3.9×10^{3}	2.8×10^{4}	1.3×10^{4}	6.5×10^{4}	7.8×10^{4}
	4	1.1×10^{5}	2.4×10^{4}	1.3×10^{5}	9.1×10^{4}	7.3×10^{6}	7.3×10^{6}

Types and Number of Bacteria Present in Mung bean Sprouts on Baired Parker's Medium

Number of *Staphylococcus aureus present* on sprouted seeds was determined by spreading 100µl of appropriate dilution of sprouted seeds on plates containing Baired Parker's Medium for 4 days at an interval of 24 hours.

In mung bean sprouts dark black, small, circular, flat colonies were observed under normal as well as aseptic condition. Total number of cfu/sprout increased from 1.8×10^{1} to 2.0×10^{3} in different sample and treatment (Table 3). For example total cfu/ sprouts in sample A under aseptic conditions were 1.8×10^{1} which increased to 4.5×10^{3} while total cfu/sprout under normal condition in sample B was found to be high i.e 2.0×10^{3}

.

Table 3: Number of Bacteria Present in Mung Bean Sprouts on Baired Parker's Medium (cfu/ sprout)

Sample	Days	Sterilized water			Unsterilized water		
		Types of colonies		Total count	Types of colonies		Total count
		Dark black , small, circular, flat	Light grey, large, gummy, circular		Dark black, small, circular, flat	Light grey, large, gummy, circular	
A	0	_	_	_	1.0×10^1	_	1.0×10^1
	1	1.8×10^1	_	1.8×10^1	9.0×10^1	_	9.0×10^1
	2	3.1×10^2	_	3.1×10^2	7.2×10^2	_	7.2×10^2
	3	2.4×10^3	_	2.4×10^3	6.5×10^3	_	6.5×10^3
	4	4.5×10^3	_	4.5×10^3	9.1×10^3	_	9.1×10^3
B	0	1.0×10^1	_	1.0×10^1	1.8×10^1	_	1.8×10^1
	1	1.1×10^2	_	1.1×10^2	2.0×10^2	_	2.0×10^2
	2	4.0×10^2	_	4.0×10^2	9.8×10^2	_	9.8×10^2
	3	7.3×10^2	_	7.3×10^2	1.0×10^3	_	1.0×10^3
	4	1.3×10^3	_	1.3×10^3	2.0×10^3	_	2.0×10^3

Morphological and Biochemical Characteristics of Selected Isolates

List of isolates from different samples of mung bean sprouts picked at different time interval is given in table 4.

Table 4: List of isolates from mung bean sprouts

Sample	Media	Colony characteristics	Code no.
A	Mac Conkey Agar	Dark pink, flat ,small ,circular	I –A1 ,I-A2 ,I-A3 ,I-A4
		Light pink with centred, gummy, dome shaped, circular	II –A1 ,II-A2 ,II-A3 ,II-A4
	Brilliant Green Agar	Cream, small, circular raised	III –A1 ,III-A2 , III-A3, III-A4
		Yellow, gummy, large Dome shaped, circular	IV –A1 ,IV-A2 , IV-A3 ,IV-A4
	Baired Parker's Agar	Dark black, small, circular	V–A1 ,V-A2 , V -A3, V -A4
B	Mac Conkey Agar	Dark pink, flat, small, circular	I –B1 ,I-B2, I-B3, I-B4
		Light pink with centred, gummy, dome shaped, circular	II –B1 ,II-B2, II-B3, II-B4
	Brilliant Green Agar	Cream, small, circular raised	III –B1 ,III-B2, III-B3, III-B4
		Yellow, gummy, large Dome shaped, circular	IV –B1 ,IV-B2, IV-B3, IV-B4
	Baired Parker's Agar	Dark black, small, circular	V –B1 ,V-B2, V-B3, V-B4

All these cultures were transferred to nutrient agar slants and stored for further use. Some of the morphological and biochemical characteristic of these isolates were examined. Twelve isolates from Mac Conkey agar plates, four isolates from Brilliant Green Agar medium plates and seven isolates from Baired Parker's medium were picked and examined for morphological and biochemical characteristics as given in table 5, 6, 7.

Table 5: Morphological and Biochemical Characteristics of Selected Isolates on Mac Conkey Agar

Sample no.	Morphological Characterstics				Biochemical Characteristics								Suspected Miroorga-nism
					IMViC				TSI	H_2S	Glucose ferment-ation	Urease	
	Cell shape arrangement	Gram reaction	Spore formation	Motility	Indole	MR	VP	Citrae					
I-A3	Rod shaped	_	_	+	+	+	_	_	A/A	_	+	_	*E.coli*
I-A4	Rod shaped	_	_	+	+	+	_	_	A/A	_	+	_	*E.coli*
I-B2	Rod shaped	_	_	+	+	+	_	_	A/A	_	+	_	*E.coli*
I-B3	Rod shaped	_	_	+	+	+	_	_	A/A	_	+	_	*E.coli*
II-A1	Rod shaped	_	_	_	_	_	_	_	A/A	_	+	+	*Klebsiella*
II-A2	Rod shaped	_	_	_	_	_	+	+	A/A	_	+	+	*Klebsiella*
II-A3	Rod shaped	_	_	_	_	_	+	+	A/A	_	+	+	*Klebsiella*
II-A4	Rod shaped	_	_	_	_	_	+	+	A/A	_	+	+	*Klebsiella*
II-B1	Rod shaped	_	_	_	_	_	+	+	A/A	_	+	+	*Klebsiella*
II-B2	Rod shaped	_	_	_	_	_	+	+	A/A	_	+	+	*Klebsiella*
II-B3	Rod shaped	_	_	_	_	_	+	+	A/A	_	+	+	*Klebsiella*
II-B4	Rod shaped	_	_	_	_	_	+	+	A/A	_	+	+	*Klebsiella*

Alk/A – alkalineslant/acid butt , A/A - acid slant/acid butt, TSI – Triple Sugar Iron

Table 6: Marphological and Biochemical Characteristics of Selected Isolates on Brilliant Green Agar Medium

Sample no.	Morhologcal Characteristics				Biochemical Characteristics								Suspected Micro-organism
	Cell shape arrangement	Gram reaction	Spore formation	Motility	IMIViC				TSI	H_2S	Glucose fermentation	Urease	
					Indole	MR	VP	Citrate					
III-A1	Rod shaped	–	–	+	–	+	–	+	Alk/A	+	+	–	*Salmonella*
III-A4	Rod shaped	–	–	+	–	+	–	+	Alk/A	+	+	–	*Salmonella*
III-B3	Rod shaped	–	–	+	–	+	–	+	Alk/A	+	+	–	*Salmonella*
III-B2	Rod shaped	–	–	–	–	+	–	–	Alk/A	–	–	–	*Shigella*

Table 7: Marphological and Biochemical Characteristics of Selected Isolates on Baired Parker's Medium

Sample no.	Morphological Characteristics				Biochemical Characteristics					Suspected Microorganism
	Cell shape arrangement	Gram reaction	Spore formation	Motility	Catalase test	Coagulase test	Salt tolerance test	Phosphatase Test	Glucose fermentation test	
VA-1	Cocci in bunches	+	–	–	+	+	+	+	+	*S.aureus*
VA-2	Cocci in bunches	+	–	–	+	+	+	+	+	*S.aureus*
VA-3	Cocci in bunches	+	–	–	+	+	+	+	+	*S.aureus*
VA-4	Cocci in bunches	+	–	–	+	+	+	+	+	*S.aureus*
VB-1	Cocci in bunches	+	–	–	+	+	+	+	+	*S.aureus*
VB-2	Cocci in bunches	+	–	–	+	+	+	+	+	*S.aureus*
VB-4	Cocci in bunches	+	–	–	+	+	+	+	+	*S.aureus*

SUMMERY AND CONCLUSION

The present study was conducted to examine the microorganisms present on the surface of mung bean sprouts prepared under laboratory conditions using sterilized and unsterilized water.

- The number of microorganisms present on the surface of sprouts was determined by suspending the sprouts in sterilized distilled water and spreading the appropriate dilution on three different types of media ie Mc Conkey Agar, Brilliant Green Agar and Baired Parker's medium.
- The initial number of coliforms present on seed surface was low but it increased from 1.0×10^1 cfu/sprout to 6.7×10^6 cfu /sprout after 4 days of incubation on Mc Conkey Agar medium.
- The number of microorganisms was found to be varying from 5.9×10^4 to 7.3×10^6 cfu/sprout on Brilliant Green Agar after 4 days of incubation whereas on Baired Parker's medium the number of microorganisms varied from 1.3×10^3 to 9.1×10^3 cfu/sprout.
- Twenty three number of isolates were picked from different samples on three different types of media and purified for morphological and biochemical characterization. They were found to belong to *Escherichia coli*, *Klebsiella*, *Salmonella*, *Shigella* and *Staphylococcus aureus.*
- Thus, care should be taken in the consumption of the raw sprouts irrespective of their source i.e. whether prepared at home or procured from the retail market.

ACKNOWLEDGEMENT

The authors are very thankful to the Head, Department of Microbiology, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun for providing permission and necessary help during research work.

REFERENCE

1. Augustin, J. and B. P. Klein 1983. Nutrient content of sprouted wheat and selected legumes. *Cereal Foods World*, 28: 58–361.
2. Fordham, J. R., C. E. Wells, and L. H. Chen 1975. Sprouting of seeds and nutrient composition of seeds and sprouts. *J. Food Sci.*, 40: 552–556.
3. Kylen, A. M and R. M. McCready 1975. Nutrients in seeds and sprouts of alfalfa, lentils, mung beans and soybeans. *J. Food Sci.* 40: 1008–1009.
4. Chavan, J. K. and S. S. Kadam 1989. Nutritional improvement of cereals by sprouting *CRC Crit. Rev. Food Sci. Nutr*. 28:401-437.
5. http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2003/Outbreak_Linelist_Final. (Accessed on July, 2011).
6. Buck. P., K. Grimsrud, J. Waters, R. Cardinal, J. Talbot and C. Anand 1998. Would you like a little *Salmonella* with your sandwich? *In* Proceedings and abstracts of the 47th Annual Epidemic Intelligence Service Conference, Atlanta, GA, April p13–17.
7. Harb, J., S. Isaacs and M. Fyfe 2003. Outbreak of *Salmonella enteritidis* phage type 11B in the providences of Alberta and Saskatchewan. *Epidemiol. Infect.*, 128: 523–527.
8. Proctor, M.E., Marge Hamacher, Mary Lou Tortorello, John R. Archer, and Jeffrey P. Davis, 2001. Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. *J. Clin. Micorbiol*. 39: 3461–3465.
9. Bari, M. L., D. Nei, K. Enomoto, S. Todoriki and S. Kawamoto 2009. Combination treatments for killing *Escherichia coli* O157:H7 on alfalfa, radish, broccoli, and mung bean seeds. *J. Food Prot*. 72: 631-636.
10. Himathongkham, S., S. Nuanualsuwan, H. Riemann and D. O. Cliver, 2001. Reproduction of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in artificially contaminated alfalfa seeds and mung beans by fumigation with ammonia. *J. Food Prot.* 64: 1817–1819.
11. www.himedialabs.com/TD/M081B.pdf (Accessed on February, 2012).
12. www.himedialabs.com/TD/M016.pdf (Accessed on February, 2012).
13. www.himedialabs.com/TD/M043.pdf (Accessed on February, 2012).

 ISSN: 2250-1177 CODEN (USA): JDDTAO