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DISSERTATION II

**FORMULATION OF A SINGLE CELL PROTEIN BASED HIGH
PROTEIN ENERGY DRINK**

Submitted by:

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Under the guidance of

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DECLARATION

I hereby declare that the project entitled“Formulation of a single cell protein based high protein energy drink” is an authentic record of my own work carried out at School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, for the partial fulfilment of the award of M.Sc. Biotechnology degree under the guidance of Dr.Jibanananda Mishra. This work is my original work and has not been submitted for any degree in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

Place: Phagwara, Punjab
Date: 29 Nov. 2017

Shiwani
Regd. No.: 11613578

CERTIFICATE

This is certify that Shiwani Regd. No.: 11613578) have completed the project entitled **“Formulation of a single cell protein based high protein energy drink”** under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study. No part of the report has ever been submitted for any other degree at any University. The report is fit for submission and the partial fulfilment of the conditions for the award of M.Sc Biotechnology .

Place: Phagwara, Punjab
Date:

Jibanananda Mishra, MS, MPhil., PhD.
Associate Professor

INTRODUCTION

Malnutrition due to dietary protein deficiency is a major problem in most of the developing countries, which is affecting the human health and causing serious diseases (Miller, O and Krawinkel et al 2005). (Therefore, it is necessary to supplement dietary protein in the food and beverages, which are being used by human on an everyday basis. In this way it is extremely important to expand protein content in nourishment generation and furthermore helpfully accessible to populace by using all the modest and simple technique and ways. Marasmus and Kwashiorkor are a standout amongst the most genuine protein vitality insufficiency ailments found all through the world generally in youngsters at a little age. Marasmus is portrayed by being under weight, loss of parcel of solid mass and subcutaneous fat, skin and hair wind up noticeably dry and fragile, it causing incessant, loose bowels, respiratory disease, scholarly incapacity, hindered development. Kwashiorkor has the accompanying qualities, for example, impeded development and advancement, extended liver, anorexia, modification of shading and surface of hair and skin. This happens in youthful youngsters because of low admission of proteins, sugars, fats. Ladies experiencing lack of iron, number of dietary items influence the assimilation of non-haem press. It is additionally ok for pregnant and bosom bolstering ladies. Aged lactic corrosive sustenance can enhance non-haem press assimilation. Caffeine is a stimulant which is available in the majority of the beverages, give readiness to purchaser for the most part produce to enhance focus and memory.

SCP creation has ended up being an exceptionally helpful innovation for quick generation of proteins from modest sources. New shabby wellsprings of proteins for the most part microorganisms, yeast, parasites and green growth were utilized to create biomass are known as single cell proteins (SCP) this term coin via Tune L wilson in 1966. It can be developed from natural waste items as a substrate, for example, orange peel buildup, sugarcane deposits, paper process squander rice husk, wheat rice husk, wheat straw buildup, and sugarcane beet

mash (Nesseri et al; 2011). It is ecofriendly, can develop on squander, substrates for the most part utilized, for example, starch, molasses, vegetable waste, organic products. The least

expensive and best hotspot for substrate is banana skin, cucumber squander with orange peels. It contains high protein content as amino corrosive (fundamental amino acids), low fat, and rich in protein sugar. The most noteworthy protein got from *Saccharomycetes cerevisiae* utilized as an eating routine supplement, *Lactobacillus plantarum* gives valuable wellbeing impacts utilized as a, yogurts, drain, organic product beverages and plant based items *Lactobacillus acidophilus* create vitamin k and in addition lactase which separate lactose.

Kwashiorkor and Marasmus are a standout amongst the most genuine protein inadequate maladies found in young people and ladies. So interest for vitality protein drinks expanding step by step. There is likewise an expansion in cognizance of significance of caffeinated drink welfare. Most mainstream caffeinated drinks Red Bull, Power Stallion, Buffalo, and Blast are devour by the people groups for enhancing the mental quality. These caffeinated beverages may contains caffeine and taurine and when these are devoured by blending with liquor may cause intense renal disappointment. Many investigations done to recognizing the potential and advantages or impacts. Many investigations reveales that Seizures is the a standout amongst the most well-known hazard reports which is related with

Caffeine is a stimulant which is available in the greater part of the beverages, give sharpness to shopper primarily make to enhance fixation and memory. Numerous probiotic microorganisms are utilized wordwidely to improve creature and human health(Guarner and Malagelada, 2003; Mercenier et al., 2003; Lee et al., 2008). LAB (Lactic corrosive microbes) are delivering lactic corrosive and broadly utilized as a part of numerous enterprises. LAB are perceived as protected GRAS microorganisms. The most contemplated probotic strains, includes the species *Lactobacillus acidophilus*, *L. fermentum*, *L. plantarum*, *L. casei*, *L. brevis* (De Vries et al., 2006; Galdeano et al., 2007; Randheere et al ., 2010). LAB are occupants of the human gastro intestinal tract (GIT) (Marco et al., 2006). Determination of good nature of probiotic includes the wellbeing for creatures and human consumption and survelty in GIT (Gastrointestinal tract) (Hyronimus et al., 2000).

utilization of caffeinated drink, and some more typical hazard are cerebral pain, I nsomnia, tachycardia, apprehension. Single cell proteins are the microorganisms like green growth, organisms , microbes and yeast which deliver biomass, protein (amino acids) and these protein utilized as a supplements in human nourishment

SCOPE OF STUDY

1. Energy protein drink contains high measure of protein content and additionally high vitality mixes which can take care of the issue of vitality lack and protein inadequacy.
2. We plan a protein caffeinated drink that would give high protein content fundamental amino acids and in addition vitality parts.
3. It would be least expensive item every destitute individuals can purchase this item
4. It is human agreeable can take care of the issue of protein insufficiency and in addition vitality inadequacy all the world.

Following would be the undertaking result

1. Formulation of protein caffeinated drink is exceptionally helpful for the general population of each age like games men, recuperating patients, youngsters and more seasoned individuals.
2. It would be least expensive and high wholesome esteems in this manner it is helpful to entire country.

OBJECTIVES OF STUDY

1. Select the specific strain which is reasonable for the generation of single cell protein and effectively accessible at low drift.
2. SCP necessities substrate for the generation of caffeinated drink like molasses. It is a crude material as sustenance for microorganism.
3. High fundamental amino acids amino acids content present.
4. Glucose use for giving vitality since it contains vitality rich starches and modest wellspring of vitality.
5. Caffeine and Taurine are utilized to give quality and vitality to the body and brain.
6. To increment the yield focus including centrifugation, coagulation, and filtration.
7. For expelling nucleic content after treatment improved the situation SCP'.
8. Treatment of cells with 10% Nacl.
9. Lactobacillus MRS broth and MacConkey agar are the prerequisites.

REVIEW OF LITERATURE

Sources of protein:

Proteins are basic supplement that contains amino corrosive for individual which helps in building tissues, cells and muscles and in addition essential for making hormones and immunity. Protein allow before pregnancy is assume an imperative part in newborn children conceived with ordinary body weight.

Vegetable protein sources	Animal protein sources
Whey, casein, and soy, fruits, grains, and legumes	: Egg, milk, meat, fish and poultry provides highest amount or proteins

TABLE: 1 Types of protein source

For athletes: After exercise proteins is a critical supplement to recuperate and develop muscles. For instance: egg, drain, yogurt, angle, fish, soya, pork, pistachio nuts, beans, beats, lentil, chick pea and milk which contain calcium. Translucent liquid part of milk is known as Whey that remaining part of the process during cheese manufacturing. It is one of the major protein source groups of bovine milk, in which 20% is milk and 80% is remainder that is rich in vitamin.

As whey protein rich in purest protein source (90%) is a very attractive supplement for theathlete.

SCP microorganisms

MICROBES	COMPOSITION (%)		
	PROTEIN (%)	FAT (%)	NUCLEIC ACID (%)
ALGAE	40-60	7-20	3-8
FUNGI	30-45	2-8	7-10
BACTERIA	50-65	1-3	8-12
YEAST	45-55	2-6	6-12

TABLE 2: Average protein composition (%) in different main groups of Microorganism

Miller *et al*; 1976

Single cell protein from algae

Algae contain good quality proteins and that are equivalent to vegetable proteins. Healthful generation from green growth is the essential concentration of microalgae biotechnology. It confines and used as well as spreads the entire algal biomass. A perfect biomass depends on starches, proteins, vitamins, lipids and minerals. It is fit for integrating every single amino corrosive, traditional sustenance things and reference example of an all around adjusted protein is prescribed by WHO/FAO (suman et al 2015). Larger part of microalgae biotechnology items are advertised as wellbeing sustenance. Spirulina and chlorella are most generally utilized green growth in biotech enterprises for the generation of nourishment supplements. The economically overwhelmed four strains are Spirulina, chlorella, d.salina. Contain more nutritious substance as contrast with other living life forms. Clinical examinations demonstrated that DHA utilization has been appeared to profit patients with constant conditions, for example, hypertension, coronary heart ailments, gloom and diabetes.

The aggregate sum of oil and fat substance runs around 1% to 70% of dry weight, lipids of microalgae and unsaturated fats are of C14 to C22.

SCP items are widely alright for wellbeing in light of the fact that amid pre-promoting stage last items experience thorough testing. Harmful mixes, substantial metals and polycyclic hydrocarbons must be evacuated. It is imperative that SCP items must be protected and cheap.

The SCP can be unacceptable for shoppers because of quality of outside proteins and may prompt skin responses, sensitivities or GI responses bringing about sickness and retching. Some may convey cancer-causing agents which got from substrates utilized. So sterilization of the item is required before utilizing as a nourishment source.

Limitations

Limitation for human consumption is algal cell made up of cellulose. It cannot be digested by human because they lack cellulose enzymes. Algal production done outdoors and favorable conditions are very important like sunlight, plenty of water, CO₂ for synthesis of starch. Elaborate methods and preparations required to remove contamination.

Single cell protein from yeast:

A very large proportion of yeast biomass was produced by aerobic fermentation and contains ammonium as nitrogen source. Specialists' apply advanced food processing technologies to improve the wide variety of food flavours' and aromas products are produced from the processing of yeast. A company which started producing myco-protein and fungal protein based products under commercial trademark were derived for human consumption in 1964 from batch cultivation of *Fusarium venenatum* strain A3/5 on starch and with waste products. Yeast is another source of SCP, *Candida utilis* produced and used in soups and sausages. In food industry is a major outlet of microbial biomass (Ugalde et al; 2002). Many of them

converts waste into valuable product and at same time prevent from environmental pollution. For example: *Candidalipolytica*, *Saccharomycescerevisiae* and many other. Food and fodder yeast also manufactures from sawdust, strain, corn cobs, and other agriculture waste and assimilates the carbon source also.

Molasses can be used as substrates, then sulphide remove by pretreatment and precipitate the protein by heating. It is deficient in some amino acids and supplements so ammonium salts and may be added as additional nitrogen sources and Ph should be adjust 4 to 4.4.

Limitations:

The yeast remove most of the paraffinic portion, leave behind aromatic fractions hence these are difficult to separate out completely because of carcinogenic activity. Protein content is very low as compared with bacteria. (Ugalde *et al*; 2002)

Yeast	Substrate
Candida utilis	Ethanol in USA Sulphite liquor in USA, Russia
Saccharomyces cerevisiae	Molasses in most countries
C. intermedia	Whey in Vienna
C. lipolytica	n-alkanes and ammonia in Russia
Kluyveromyces fragilis	Whey

TABLE 3: substrate used by yeast

Single cell protein from fungi:

Fungi grow on complex organic compounds and high amount of fungal biomass is produced because of their non toxic nature. So are hazardous to human such as *Fusarium graminearum*, *Aspergillus niger*, *Aspergillus fumigatus*. It has advantages as well as disadvantages

ADVANTAGES	DISADVANTAGES
<ol style="list-style-type: none">1. Easily converts into various texture foods.2. Protein content high and less nucleic acid contamination as compared to yeast and bacteria.3. Good digestibility as compare to algae SCP.4. Increases economy as compared to single celled organism.5. Biomass production is further used because it provides carbohydrates, lipids, minerals, vitamins and protein.	<ol style="list-style-type: none">1. Moderate development rate as contrast with microbes and yeast.2. High odds of Tainting.3. A few strains create mycotoxins.4. Bacterial pollution not happens underneath pH 5-6 since it is alluring for the vast majority of the growths species.

TABLE 4: Advantages and disadvantages of SCP fungi

Single cell protein from bacteria

Bacteria only microorganism can fix atmospheric nitrogen. They are decomposers; decompose dead plants and animals also used for bioremediation process of toxic substances.

The cost of producing SCP is relatively high because of small size and low density, it cannot harvest from fermentation easily. High nucleic content as compare to yeast and fungi, therefore additional method used to remove these contaminations.

It grows best in slightly acidic medium ranges from 5-7 pH. Temperature ranges from 35° to 45°. Many bacteria are able to convert carbohydrates to organic acids with high yield. For example *Lactobacillus spp.* Produce lactic acid. *Acetobacter spp.* Produces acetic acid. Many are used for production of alcoholic beverages, gums, amino acids, acetic acids, organic acids, vitamins, oils and fats.

ADVANTAGES	DISADVANTAGES
<ol style="list-style-type: none"> 1. High rate of multiplications as compare to yeast and fungi hence rapid succession of generation (0.5-2hrs). 2. They can easily modified genetically by varying amino acid composition. 3. Yield high protein content about 43-85%. 4. Not more land required and is also increases economy. 5. carbon sources and waste products use as raw material. 6. Produce easily and profitably. 	<ol style="list-style-type: none"> 1. Some toxic substances produce by some type of bacteria to human and animals. 2. Some may leads to indigestion and allergic reactions in humans. 3. High nucleic acid and carcinogenic content may leads to serious health problems by some types of microbes. 4. It needs high level of sterility control during production in laboratory.

TABLE 5: Advantages and disadvantages of bacteria

SUBSTRATE USED FOR SCP PRODUCTION:

The most commonly used substrate for SCP production is those in which the carbon and energy source is derived from carbohydrates. Because of their building blocks and renewable resource it considers as natural microbial substrates. Molasses is one of the cheapest natural by-products of sugar manufacturing. It requires suitable nitrogen source like ammonium salts and phosphorus.

Organic waste and residues are the renewable sources of energy. Waste may be inorganic, organic or mixed types. Dairy industries release wastes containing milk whey, butter milk, unused skim milk and traces of detergents. The dilute solution or suspension containing lactose, protein, fats and minerals. Therefore dairy wastes are food substrate for production of single cell protein, lactic acid, vitamin and alcoholic beverages. (Garg *et al*, 1985). Molasses is the cellulosic material of sugarcane obtained after the extraction of sugary juice. It used for the production of single cell protein.

Review of Literature

S.NO	WORK DONE	REFERENCES
1.	Study of effects of dietary <i>lactobacillus plantarum</i> on the growth performance and immune gene expression of white shrimp by different. Study under normal condition and stress acute low salinity and RT.PCR analysis of expression of shrimp immune gene. The result shows that the lysozyme acts as an integral component of antibacterial defense mechanism.	Zhenget <i>et al</i> ; 2017
2.	Advantages and applications of single cell protein. Production of SCP by cheap substrates by fermentation process. Studies the effects of Nitrogen, Phosphorus and glucose sources on SCP.	Srividya <i>et al</i> ; 2014
3.	Single cell protein study that the microbes as a marvelous source and their properties. SCP production from petroleum fraction such as ethanol, kerosene by using <i>Candida</i> species.	Nangul and Bhatia; 2013
4.	Study of energy drinks psychological effects and impacts on human beings. The result is that people these days consuming drink by mixing with alcohol which is dangerous for health.	Ishak <i>et al</i> ;2012
5.	Effects and effectiveness of soft drinks. significantly studied by currently level among the teens and adults. Results that drinks effects more on performance and mood	Dorota ; 2014

	of consumers.	
6.	Study of single cell protein microorganisms and their production, cultivation methods and fermentation. They show that different types of substrates are used by microorganisms for the cheap production of product.	Gour <i>et al</i> ; 2015
7.	Study of <i>Lactobacillus plantarum</i> surface protein area, its functions and applications. They studies that carbohydrates as the binding site for S-layer protein cell wall	Ulla <i>et al</i> ; 2013
8.	Study of single cell production, process and substrates by fermentation. They Study that glucose is compound for provide energy.	Nasseri <i>et al</i> ; 2014
9.	Potential of Probiotic <i>Lactobacillus plantarum</i> 2621 for the Management of Infertility. <i>L. plantarum</i> interfered to different extents with the adherence of <i>E. coli</i> . <i>L. plantarum</i> 2621 decreased the adhesion by displacement and competition in a significant level.	Bhandari <i>et al</i> ;2014
11.	Development of a minimal growth medium for <i>Lactobacillus plantarum</i> . A medium with minimal requirements for growth of <i>Lactobacillus plantarum</i> WCFS was developed. The minimal medium composition compared with a genome-scale metabolic model of <i>L.plantarum</i> . By omission experiment repetition. Two minimal media developed. P MM5 and PMM7. The specific growth rate of <i>L. plantarum</i> growth is 50% to 60% which compared to growth on established growth media	Wegkamp <i>et al</i> ;2010
12.	The effect of new probiotic strand <i>lactobacillus plantarum</i> on coliform counts of <i>Lactobacilli</i> and bacterial enzyme activities in rats. They evaluate the effects of strain <i>L.plantarum</i> on chemically induced carcinogens in rats.	Cokasova <i>et al</i> ; 2012
13.	Antagonistic effects of <i>Lactobacillus</i> strains against gas producing coliform isolated from Colicky infants. The causes of abdominal discomfits in infants suffering from colic. The beneficial supplements recently reported. The	

	study evaluated interaction between <i>Lactobacillus spp.</i> strains and gas forming coliform isolated from stools of colicky infants.	Savino <i>et al</i> ; 2011
14.	Evaluation of profertility effects of probiotic <i>Lactobacillus plantarum</i> 2621 in a murine model. They study to determine the capability of <i>L. plantarum</i> 2621 strain with probiotic properties, to prevent the vaginal colonization of <i>E. coli</i> causing agglutination of sperms and to evaluate its profertility effect in a murine model. methods: Screened mice were divided into five groups i.e. control group, <i>E. coli</i> group, <i>Lactobacillus</i> group, prophylactic and therapeutic groups. The control group was infused with 20 µl PBS, <i>E.coli</i> group was administered with 10 ⁶ cfu/20 µl <i>E. coli</i> , and probiotic group was administered with <i>Lactobacillus</i> (10 ⁸ cfu/20 µl) for 10 consecutive days.	Bhandari <i>et al</i> ;2015
15.	Sensory and microbiological quality yogurt drinks with probiotic and probiotics. The study assess the sensory profile of drinkable yogurts made with probiotics and to determine viability of probiotic in yogurt drink. A control sample of prebiotic and probiotic was included in the experimental design.	Allgeyer <i>et al</i> ;2010
16.	Production of probiotic milk drink containing <i>Lactobacillus acidophilus</i> , <i>Bifidobacterium animalis subsp. Lactis</i> and <i>Lactobacillus Cacei</i> . fermented milks were a valuated for their physical, chemical properties, rheological properties, microbiological properties and sensory analysis. They study that fermented milk containing three probiotic cultures.	Yerlikaya <i>et al</i> ; 2013
17.	Determining odor and flavour characteristics of probiotic, Health promoting ingredients' and effects of repeated exposure and consumer Acceptance. Results that exposure to probiotic orange juices enhance consumer acceptance, significantly increases show in probiotic and shows that 4	Luckow <i>et al</i> ; 2005

	juices had significantly different sensory profiles.	
18	The study of intake energy drinks in association with alcoholic beverages in group of students of the school of Medicine of university of Messina.	Oteri <i>et al</i> ; 2007
19	They study the effects of energy drinks on health of children, adolescents and young adults.	Seifert <i>et al</i> ; 2011
20	The study of ingredients and effects of energy drinks.	Grosz <i>et al</i> ; 2008
21	They study the effect on mood and cognitive performance on human being of drink constituents: caffeine, glucose and carbonation.	Smit <i>et al</i> ; 2004
22	They study the effects of energy drink on mood , cognition and cortisol levels.	Sunram <i>et al</i> ; 2012
23	They study the effect of low dose caffeine and self-reported mood effects on normal volunteers.	Silverman <i>et al</i> ; 1992
24	They study the effect on human (addictive behavior) Sociodemographic correlates of energy drink consumption with alcohol and without alcohol by community survey.	Berger <i>et al</i> ; 2011
25	They study the effects of energy drink with and without alcohol on neuropsychological mechanism.	Curry <i>et al</i> ; 2009
26	They study the effect of caffeine on mentally and psychiatric disorders on human being.	Lara <i>et al</i> ; 2010
27	They study the effects of energy drinks by mixing with alcohol on behavioural control and risk of consuming cocktails by college students.	Marczinski <i>et al</i> ; 2011
28	They study the kinetics and Modelling of Lactic Acid Production by <i>Lactobacillusplantarum</i>	Frederico <i>et al</i> ; 1994

METHODOLOGY

Culture: *L. plantarum* MTCC NO: 2621

1. Dissolve the 14g of Lactobacillus MRS Broth in 250ml of double distilled water.
2. Mixing it altogether at that point warmth to heating up the medium sterilizing via autoclaving at 15lbs pressure at 121 c° for 15 minutes and avoid from overheating.
3. If we not utilizing around then store that in fridge(-4 c°)
4. If we doing in the meantime include *L.plantarum* strain that is in the powder frame precisely under aseptic condition.
5. Incubate for 24 hours in incubator a 37 c°

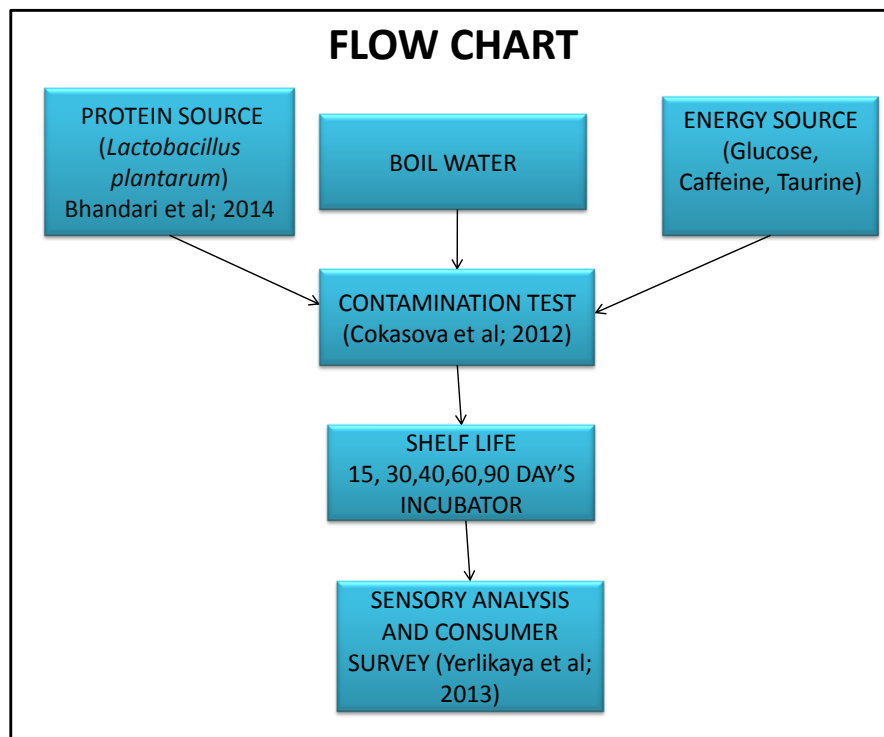
Glycerol stock:

1. Take cryotube of 2ml. cover each of them with silver foil, place them in a beaker with proper cover and autoclave it .
2. Take five cryotubes , include a 150ul of glycerol in each tube and autoclave them .
3. Add 850ul of culture in the autoclaved glycerol containing tubes.
4. Make aggregate volume 1000ul (1ml).
5. This is the 15% glycerol stock we arranged and put in - 80c°/ - 20c°.
6. Remaining the 1ml for the development of growth curve of *L.plantarum*.

Media need for growth of bacteria is providing a mixture of nutrients, moisture and other chemicals. Lactobacillus MRS Broth is an improved medium for lactobacilli and support good growth of microbes. Peptone and dextrose provides the carbon, nitrogen and other

nutrients which are necessary for growth. Some precautions should be follow, do not ingest, inhale, or not allow to in contact with skin.

It is recommended that these are treated as potentially infectious. Incubate Broth at 3 degree Celsius for 5 days in aerobic conditions. Biochemical and immunological, molecular testing or spectrometry testing performed on colonies from pure culture for complete identification commercially prepared culture perform quality control testing check for sign of contamination and deterioration, perform quality control testing. Lactobacillus MRS Broth should be appearing clear with very slight opalescence and dark amber in colour. Water should be check, copper ions, conductivity and pH because it inhibits the growth of microbes. Sterilize in autoclave at 121 degree Celsius. Lactobacillus plantarum has ability to produce extracellular glucansucrase. It prevent digestion problem, Colic in babies, crohn's disease, inflammation and gut problems also used for skin disorders

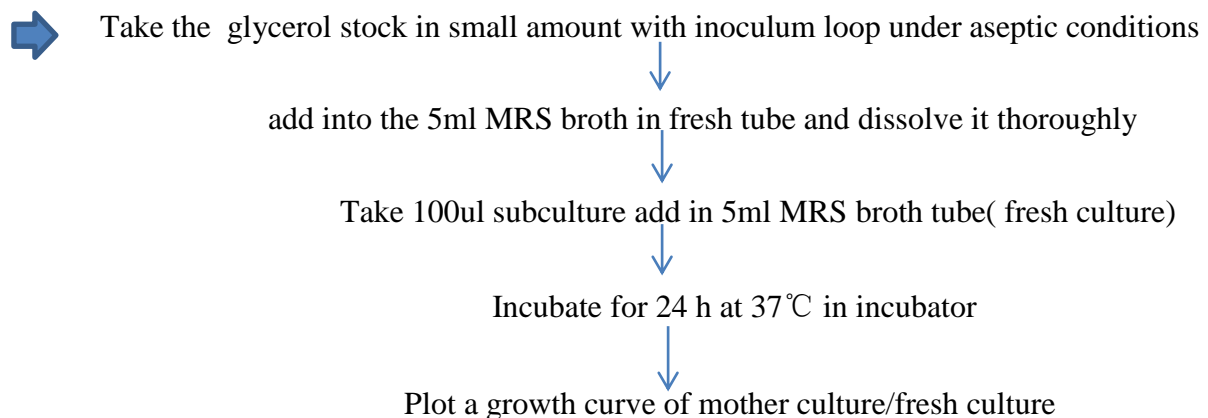
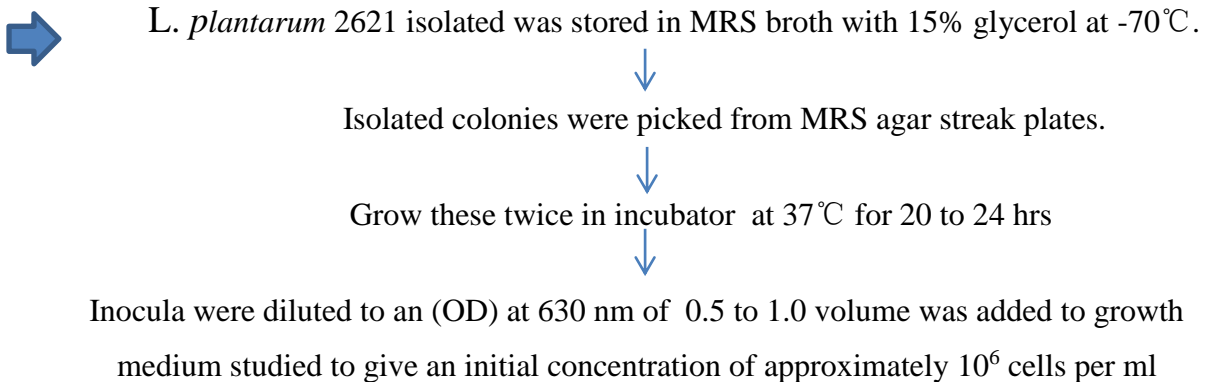


RESULT AND DISCUSSION

Energy drinks end up noticeably mainstream refreshments everywhere throughout the world. Add up to bacterial result, coliform, *Escherichia coli* and *staphylococcus aureus* were incorporated into this investigation. In Saudi Arabia, board of pastors boycott caffeinated toast purchasers of all age gatherings and restricts offer of caffeinated drinks in eateries, don clubs, government bottles. The data of utilizing caffeinated drink among understudies is obscure and misdirecting; many individuals expend doubtful, conceivably destructive and even restricted caffeinated drinks. Red Bull caffeinated drink is carbonated taurine drink contains caffeine that vitalize the body and brain. One of a kind vitality mixes for the most part contains caffeine and taurine at different focus which isn't useful for well being and psyche. Introduced other sugar substitutes like notably aspartame, or Nutra-sweet, are widely used throughout the world in 1990s for production of soft drink. In Monster energy drink not any changes can take place in which no effect cause on cardiovascular (Dentzer *et al*, 2009) function and no significant changes seen in electrocardiograms. It increases heart rate and systolic blood pressure

Caffeinated drink are a gathering of beverages utilized by consumers to give an additional mind upgrade. for example red bull is a standout amongst the most mainstream energy drink was presented in Austria in 1987 and to joined States in 1997. energy beverages for the most part contains caffeine, taurine , 1-Carnitine Sugars, Glucuronolactone, vitamins and other herbal supplements.

METHODOLOGY



Protein extraction

Take 2ml culture in eppendorf centrifuge aseptically at 10,000 rpm for 15 minutes



Take the pellet and discard supernatant



Washing the pellet twice with 0.9% NaCl (normal saline)

for washing

Take two falcon tube of 15ml ,add 5ml normal saline in each tube



disperse it thoroughly



Centrifuge at 10,000 rpm for 5 minute



Take cell pellet and discard supernatant



Again add 5ml normal saline centrifuge it at same rpm



Take pellet and discard supernatant



Add 1ml normal saline into pellet and disperse it thoroughly



Take 100ml from it ,add 900ul of water in eppendorf (mix)



This becomes 1/10th dilution (water should be taken as blank)



Measure OD by spectrophotometer at 600 nm



Compare the OD with growth curve (no. of cells in the particular OD)

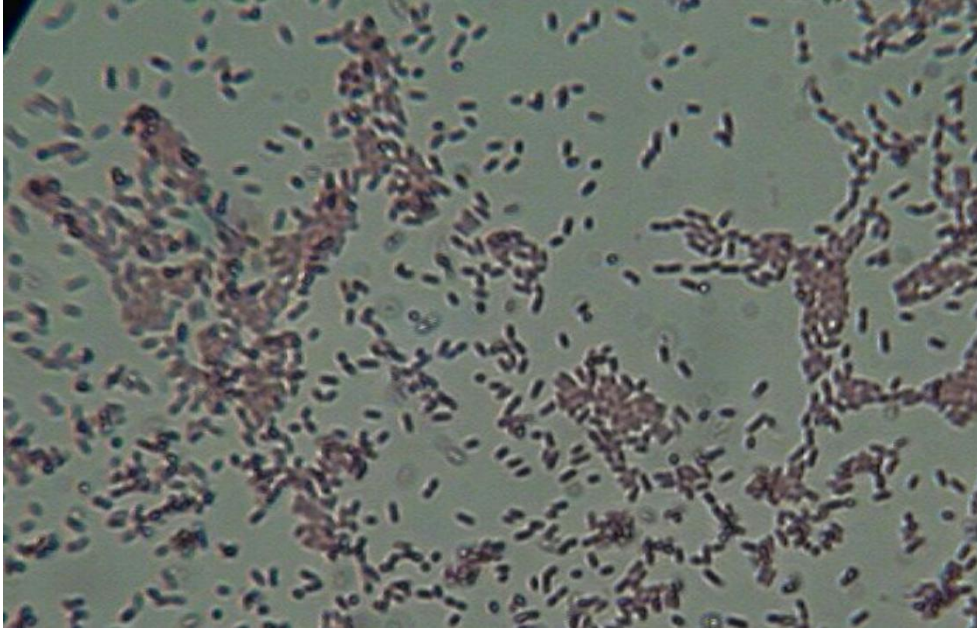


IMAGE 1: *Lactobacillus plantarum* under light microscope at 100 X



IMAGE 2: *Lactobacillus plantarum* colonies

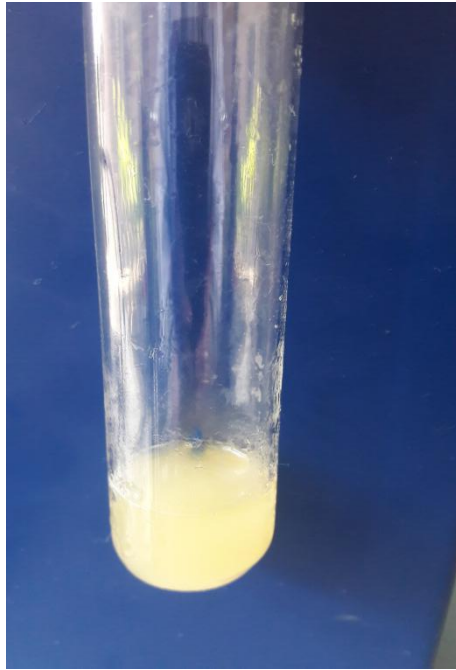
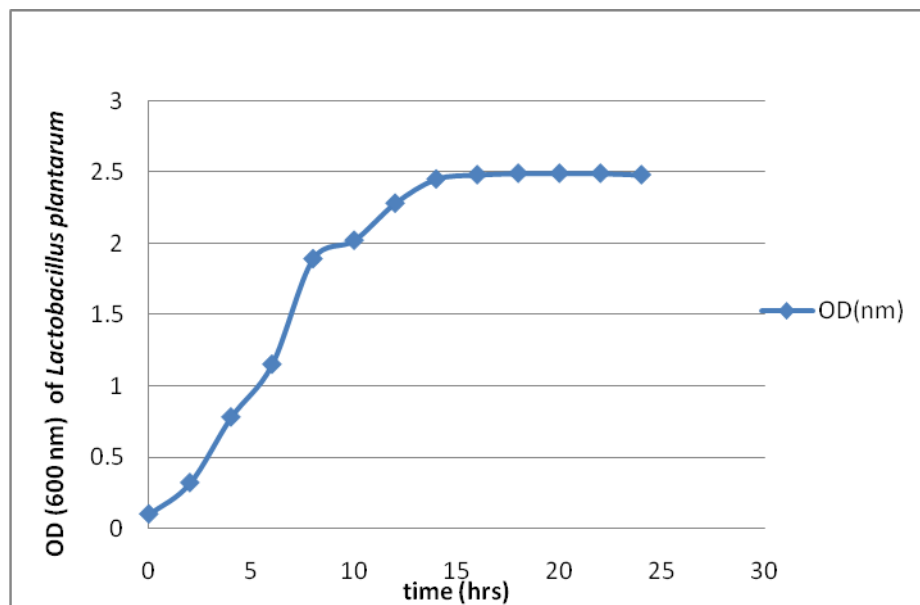


IMAGE 3: *Lactobacillus plantarum* in Lactobacillus MRS broth



GRAPH 1: graph plot between OD (600 nm) of *Lactobacillus plantarum* and time(hrs)

TIME (hrs)	OD (nm)
0	0.10
2	0.32
4	0.78
6	1.15
8	1.89
10	2.02
12	2.28
14	2.45
16	2.48
18	2.49
20	2.49
22	2.49
24	2.48

TABLE 5: OD (600 nm) changes with change in time

CONCLUSION

Single cell generation alludes to the microorganisms utilized as makers develop as single as opposed to as intricate multicellular living being. The vast majority of the lactobacillus species are appropriate to deliver single cell protein. Microorganisms have shorter age time, change is simple, can use squander crude materials.. The cell yield fluctuates as indicated by the substrate and the sort of microorganisms used. The caffeinated drink builds vitality, and alertness, to expand vitality of body. Devouring caffeinated drink with liquor is more risky to human. Caffeinated drink have blended mental and prosperity impacts, It give an additional lift in vitality, advance attentiveness, look after readiness. A large portion of the caffeinated drink contains caffeine goes about as stimulant. Taurine is another fixing; communicate contrarily with caffeine and liquor. The customer devours caffeinated drink-liquor blend with taurine and caffeine and vodka to get vitality that may brings about intense renal disappointment. Accordingly segments of vitality in drink are critical to progress. Evacuation of nucleic substance of SCP is accomplished by including NaOH or NaCl (0.9%).

Energy drinks become popular beverages all over the world. Total bacterial count, coliform, Escherichia coli and staphylococcus aureus were included in this analysis. In Saudi Arabia, council of ministers ban energy drink to consumers of all age groups and prohibits sale of energy drinks in restaurants, sport clubs, government canteens. The information of using energy drink among students is vague and misleading; many people consume unproven, potentially harmful and even banned energy drinks. Red Bull energy drink is carbonated taurine drink contains caffeine that vitalize the body and mind. Unique energy blends mainly contains caffeine and taurine at varying concentration which is not good for health and mind.

Sugar is widely used in energy drink because it provides energy. But many brands have sugar free options also. It is the second main ingredient for production of soft drinks and also balances flavors and acids. It turned to high-intensity sweeteners, saccharin that is phased out and declared a potential carcinogen. Mixing alcohol with energy drink has been controversial issue among students. Consumption of energy drink and occurrence of cardiovascular events has recently recognized and questioned but supporting documents are limited. The psychological and performance enhancing effect of caffeine reported. It increases physical and mental stress and improve alertness, concentration. All test energy drink showed lethal effect on lactobacillus and bifidobacterial strains as the bacterial count decreased to below 2 log CFU/ml. Caffeine raised many health concerns over the past decade. It increases energy levels, alertness after mood and sleep patterns and at high doses.

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APPENDIX

1. *Lactobacillus plantarum* strain MTCC NO. 2621
2. Lactobacillus MRS broth
3. Agar agar
4. Normal saline(0.9%)
5. 15% Glycerol



TOPIC APPROVAL PERFORMA

School of Bio Engineering and Bio Sciences

Program : P260-H::M.Sc. (Hons.) (Biotechnology)

COURSE CODE : BTY797

REGULAR/BACKLOG : Regular

GROUP NUMBER : BSRGD0178

Supervisor Name : Dr. Jibanananda Mishra

UID : 21039

Designation : Associate Professor

Qualification : _____

Research Experience : _____

SR.NO.	NAME OF STUDENT	REGISTRATION NO	BATCH	SECTION	CONTACT NUMBER
1	Shiwani	11613578	2016	B1617	9736354424

SPECIALIZATION AREA : Zoology

Supervisor Signature: _____

PROPOSED TOPIC : Formulation of a single cell protein based high protein energy drink

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	Parameter	Rating (out of 10)
1	Project Novelty: Potential of the project to create new knowledge	7.50
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.50
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	6.50
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	7.50
5	Social Applicability: Project work intends to solve a practical problem.	7.00
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	7.50

PAC Committee Members		
PAC Member 1 Name: Dr. Ashish Vyas	UID: 12386	Recommended (Y/N): Yes
PAC Member 2 Name: Himanshu Singh	UID: 11691	Recommended (Y/N): NA
PAC Member 3 Name: Dr. Joydeep Dutta	UID: 14336	Recommended (Y/N): NA
PAC Member 4 Name: Dr. Umesh Goutam	UID: 14691	Recommended (Y/N): NA
DAA Nominee Name: Mamta Sharma	UID: 18431	Recommended (Y/N): Yes

Final Topic Approved by PAC: Formulation of a single cell protein based high protein energy drink

Overall Remarks: Approved

PAC CHAIRPERSON Name: 11840::Dr. Neeta Raj Sharma

Approval Date: 07 Mar 2017

