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A Comparative Study of Bacterial and Fungal Flora in Glucose Fermentation

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Abstract— Microbes can be used as efficient live factories to produce beneficial products. They are cheap resources that consist of numerous enzymes which can convert complex chemical structure into simple digestible molecules with a high efficiency. The diversity of fermentation products produced by the microbes is attributed to the rich diversity of microbes which have a diverse metabolism that can yield various types of fermentation products. Bacterial flora is a major part of the gut flora that performs a wide variety of functions and plays a very important role in fermentation. Many fermented foods are fermented predominantly by bacteria. Some are fermented largely by fungi and a few are fermented by both (a double ferment). Various studies have shown the production of Korean wine or *Makgeolli* using both bacterial flora and fungal flora. The current study is a preliminary effort to compare the rate of fermentation of glucose using bacterial and fungal flora.

Keywords— Microbes, Fermentation, Bacterial flora, Fungal flora, Glucose

1. Introduction

Ultrasonic Fermentation is a anaerobic (without oxygen) chemical breakdown of a substance by microbes (bacteria, yeast etc..,) typically involving effervescence and giving off heat.

Ethanol fermentation is a biological process in which sugars such as glucose, fructose and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products *(Thomas Edward,1922)*

$$\underset{Sugar}{C_6H_{12}O_6} \rightarrow \underset{Alcohol}{2C_2H_5OH} + \underset{carbon\ dioxide}{2CO_2}$$

Yeasts are eukaryotic microorganisms classified as members of the fungus kingdom. Yeasts are mostly unicellular, although some species may also develop multicellular characteristics. *Saccharomyces cerevisiae* is an best known yeast which converts carbohydrates to carbon dioxide and alcohols. For thousands of years it is used in the baking and brewing industries *(Feldmann, Horst,2010).*

Escherichia coli (also known as *E.coli* (Escherichia coli)) is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus Escherichia that is commonly found in the lower intestine of endotherms *(Singleton P et al, 1999).* Most *E.coli* strains are harmless, the harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 *(Bentley R, Meganathan R, 1982)* and preventing colonization of the intestine with pathogenic bacteria.

These *E.coli* strains ie., gut flora or more appropriately, gut microbiota, consists of a complex community of microorganism species that live in the digestive tracts of animals and is the largest reservoir of microorganisms mutual to humans. Gut flora benefits the host by gleaning the energy from the fermentation of undigested carbohydrates and the subsequent absorption of short-chain fatty acids. The most important of these fatty acids are butyrates, metabolised by the colonic epithelium; propionates by the liver ; and acetates by the muscle tissue. The human body carries about 100 trillion microorganisms in its intestines, a number ten times greater than the total number of human cells in the body *(Björkstéin et al, 2001).* This comparative study proceeds to estimate the rate of fermentation and their capability in standard temperature and pressure because they are said to be masters in fermentation in different ambience. The estimation of fermentation can be done in two criteria ie., by quantitative estimation of the alcohol released during fermentation and by quantitative estimation of carbon dioxide released during fermentation. Here our approach is using the quantitative estimation of carbon dioxide released during fermentation because the commonest by-product produced in both fermentation processes is carbon dioxide.

The common fermenting substrates that have been used is Glucose. Glucose is the most widely used aldohexose in living organisms. Glucose is a ubiquitous fuel in biology. It is used as an energy source in most organisms, from bacteria to humans, through aerobic respiration, anaerobic respiration, or Fermentation *(Rome: Food and Agriculture Organization, 2003).* Glucose is also fermented by yeast. Yeast species are largely used in the production of wine and

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alcoholic products such as whiskey, rum and beer etc. Yeast is also used in bread making processes. These products are commonly and commercially used food and beverage products around the globe. Glucose fermentation by yeast is done widely in bakery as well as brewery industries *(United states Department of Agriculture, July 2006)* and *(Ribéreau-Gayon et al, 1965).*

2. Methods

2.1 Yeast

Yeast is used from the commercially available bakers yeast powder.

A. Isolation of E.coli

E.coli was isolated from human faecal matter. The human stool was taken and streaked over the EMB and Nutrient Agar. The petri plates were incubated at 37°C for 24 hours.

B. Gram staining

Gram staining was done to confirm the E.coli culture. A small portion of the culture was taken and heat fixed on a clean grease free slide. Crystal violet stain was added and kept for few minutes. Later it was washed off. Iodine was added to the culture, which act as a mordant. Decolourization was done using 95% ethanol. Gram negative cells can be rendered visible with a suitable counter stain, which is usually positively charged safranin, which stains them pink. Gram negative rod shaped E.coli was observed under microscope. Various other biochemical tests were done to confirm the presence of E.coli.

Confirmatory test for carbon dioxide release during fermentation by chalky white precipitate method *(A.Siedell, W.F. Linke et al, 1953)*

Equal amount of the microorganisms ie., yeast and *E.coli* are inoculated in the 100ml of glucose substrates and tightly closed with cotton plug and paraffin wax. A delivery tube is dipped in a measuring cylinder containing limewater. As the fermentation occurs the released CO_2 is passed through the delivery tube and reacts with the lime water present in the measuring cylinder. The reaction as follows:-

$$Ca(OH)_{2\,(aq)} + CO_{2\,(g)} \rightarrow CaCo_{3\,(s)} + H_2O_{(l)}$$

Lime water — *carbon dioxide* — *calcium carbonate (chalk powder)* — *water*

The reaction leads to the production of calcium carbonate (chalk powder or chalky white precipitate) this confirms and proves the fermentation of the substrate has occurred. Turbidity of the substrate shows the presence of chalky white precipitate. Turbidity of the substrate is measured using spectrometry. Spectrometry analysis is done for substrates at 640nm (since it is a colourless substrate). The spectrometric analysis of the substrates is shown in table1 and figure1 showing the graphical representation of spectrometry analysis of the substrates.

2.2 Chemical confirmatory test for the ethanol production during fermentation process or qualitative test for ethanol

Chemical test for ethanol confirms the fermentation has occurred. Ethanol is the by-product of glucose fermentation. Hence, this proves the fermentation of glucose has occurred. Chemical test includes colour indication by the reaction with potassium permanganate and potassium dichromate.

A. Chromic acid oxidation*(J.M. Grill et al, 2006)*

Acidified potassium dichromate is used for the chromic acid formation which oxidize a primary alcohol to an aldehyde and then to a carboxylic acid. This test is known as chromic acid oxidation.

$$CH_3CH_2OH + K_2Cr_2O_7 + H_2SO_4 \dashrightarrow CH_3CHO + Cr_2(SO_4)_3$$

ethanol — *potassium dichromate* — *acid* — *aldehyde* + *Blue-green colour*

The same type of colour change is shown in the figure 2.

B. Ritter test*(Gabriel Tojo et al, 2006)*

This test is similar to the chromic acid oxidation and provides the same information. It is the oxidation of primaryalcohol to carboxylic acid using acidified potassium permanganate($KMnO_4$).

$$CH_3CH_2OH + KMnO_4 + H_2SO_4 \dashrightarrow CH_3COOH + MnO_2$$

ethanol — *potassium permangnate* — *acid* — *carboxylic acid* — *brown colour*

The same type of colour change is shown in the figure 3.

2.3 Estimation of carbon dioxide released during fermentation using *balloon bulging method* or quantities analysis of carbon dioxide

Use round balloons for this experiment. Then wrap a string around the widest part of the ballon. Lay down the string and measure the length (this is done after incubation of the substrate for 24 hours) shown in Figure 4

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and 5. Calculate the radius to the centre of the circle using the equation:-

$$C/2\pi = r$$

Calculating the radius, we can figure out the volume of the balloon using the equation:-

$$V = 4/3\pi r^3$$

The balloon contains pure CO_2, and considering the STP (Standard Temperature and Pressure), we can calculate the moles of CO_2 generated during the fermentation process by using *Ideal gas law.*

2.4 The Ideal Gas Law

P is pressure, V is volume, n is number of moles, T is temperature, and R is gas constant

$$PV = nRT$$

to get the moles of CO_2 released, need to modify the equation:

$$n = PV/RT$$

by this equation the no of moles of CO_2 can be calculated. The calculated moles of CO_2 are show in **Table.2.** A graphical representation is done for the number of CO_2 released during fermentation process in different substrates shown in **Fig.6.**

3. Observation

The chalky white precipitate is observed in the substrates. The spectrometric value at 640nm is taken.

SUBSTRATES	VALUES
BLANK(GLUCOSE)	0.0
T_1-*E.coli*	0.12
T_2-yeast	0.40
T_3-both(*E.coli & saccharomyces cerevisiae*)	0.82

Table 1. The spectrometric values of the substrates

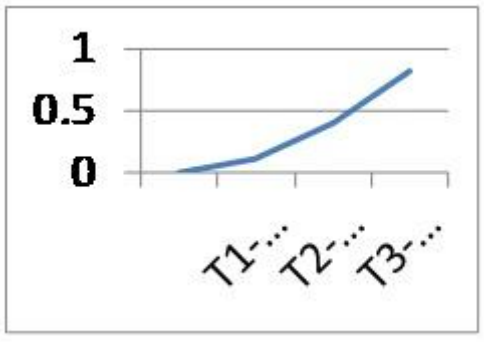

Fig.1: Showing the graphical representation of spectrometry analysis of the substrates

3.1 Chemical test observation

Blue-green colour gradient is observed in the chromic acid oxidation test done for the substrates using potassium dichromate.

- P-positive(ETHANOL)
- N-negative(GLUCOSE)
- T_1-*E.coli*
- T_2-yeast
- T_3-both(*E.coli & saccharomyces cerevisiae*)

Fig.2: Shows the colour gradient in substrates

Brownish gradient is observed in the chromic acid oxidation test done for the substrates using potassium permanganate.

- P-positive(ETHANOL)
- N-negative(GLUCOSE)
- T_1-*E.coli*
- T_2-yeast
- T_3-both(*E.coli & saccharomyces cerevisiae*)

Fig.3: Shows the colour gradient in different substrates

The above confirmatory tests confirms the release of CO_2 and ethanol which are the By-products of fermentation reaction. The presence of ethanol in the substrates and the release of CO_2 confirms the fermentation process has occurred in the substrates with the help of fermenting flora.

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3.2 CO_2 Quantitative test's observation

The balloon bulging is observed after 24 hours inoculation of fermenting flora.

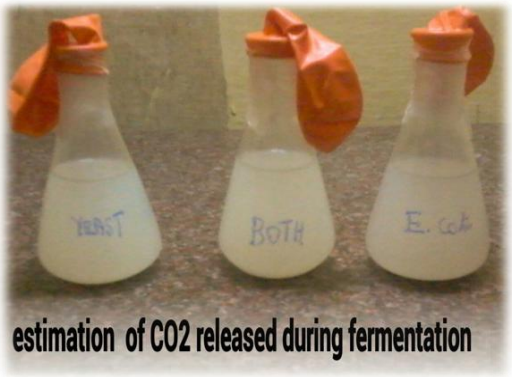

Fig.4: showing the initial time of the balloon tied to the conical flask

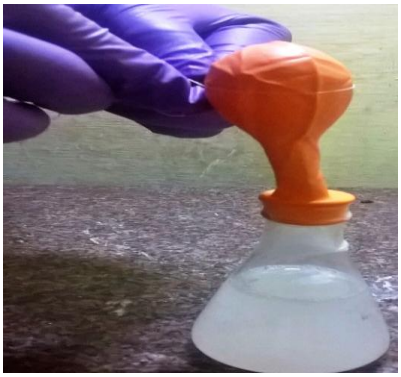

Fig.5: showing the bulged balloon 24 hours after inoculating the fermenting flora. The circumference of the balloon is measured with the help of a thread

Table 2. Shows the number of moles of CO_2 released during fermentation from different substrates.

Substra-tes	**Circumf-erence(b-alloon reading) In cm's**	**Radius (r) [C/2π=r] In cm's**	**Volume [V=4/3 πr^3] In cm^3**	**No.of moles of co_2 released(n) [by ideal gas law]**
Both yeast & *E.coli*	11.8	1.87	2.74	$2.2806x10^{-4}$
yeast	10.7	1.70	2.06	$1.7146x10^{-4}$
E.coli	6.62	1.05	0.48	$0.3992x10^{-4}$

4. Result and discussion

The number of moles of CO_2 released is varies in the substrates. Table 2 shows the number of moles of CO_2 released during fermentation from different substrates.

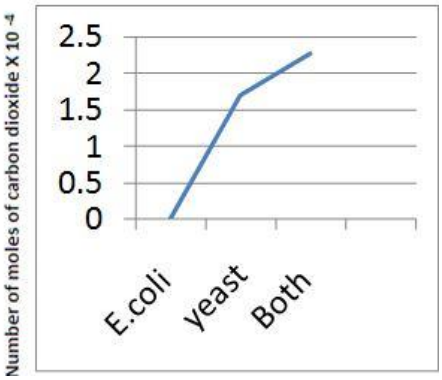

Fig.6: A graphical representation is done for the number of CO_2 released during fermentation process in different substrates

The above results shows the fermentation ability of the organisms ie., both combined *E.coli* and yeast have great fermentation ability comparing them individually.

5. Conclusion

The result shows the fermenting ability of both flora which are said to be masters in fermentation in different criteria. The above comparative study also shows that the both flora together have an extraordinary fermenting capability than their individual fermentation.

Acknowledgement

The project was carried to study the comparison of fermenting flora on their fermenting ability. The further study will be made to understand the fermentation in detail and also about the fermenting flora. We are grateful towards God for making the project in an successive path. We express our sincere gratitude to Dr. Christy Selvamangai, HOD, Department of Biotechnology, Alpha Arts and Science College. We also thank the faculty members of Biotechnology department, who were encouraging and supportful by leading us in a successive path. We specially thank our friend Mantu Kumar. M for his support. Last but not the least; we thank our families for their support and financial assistance.

References

[1] Balch R.T,(2013) sugarhouse product, Bureau of chemistryand soils, Washington DC "Measurement of turbidity using spectrometry".
[2] Bentley R, Meganathan R (September 1982). "Biosynthesis of vitamin K (menaquinone) in bacteria".
[3] Bjorkstéin et al, 2001 "The human body carries about 100 trillion microorganisms in its intestines, a number ten times greater than the total number of human cells in the body".
[4] Coxter,H.S.M.:regular polytopes (1948)"Volume of sphere"
[5] Clapeyron,E(1834) Journal de I'Ecole polytechnique (in french). "Tdeal gas law".

ISSN 2394-7187(Online)
ISSN 2394 - 7179 (Print)

[6] Clausius.R,(1857) Annalen der physic und chemie(in German) "Tdeal gas law".
[7] Feldmann/, Horst(2010)"baking & brewing industries".
[8] Gabriel Tojo et al, (2006) "Ritter test"(oxidation of primaryalcohol to carboxylic acid using acidified potassium permanganate).
[9] Grill J.M. et al,(2006) "chromic acid oxidation"(Acidified potassium dichromate is used for the chromic acid formation which oxidize a primary alcohol to an aldehyde and then to a carboxylic acid).
[10] Jen Kershaw(2013) ,flex books"radius of a circle given circumference".
[11] Ribéreau-Gayon et al,(1965)."Glucose fermentation by yeast are done widely in bakery as well as brewery industries".
[12] Rorres, Chris 'Tomb of Archimedes sources',"Volume of sphere".
[13] Rome: Food and Agriculture organization (2003)" Glucose is a ubiquitous fuel in biology. It is used as an energy source in most organisms, from bacteria to humans, through either aerobic respiration, anaerobic respiration , or Fermentation".
[14] San diego state university(2004)"Perimeter,Area and Circumference".
[15] Siedell.A, W.F. Linke et al, (1953) "Confirmatory test for carbon dioxide release during fermentation by chalky white precipitate method"
[16] Singleton P (1999). "Bacteria in Biology, Biotechnology and Medicine".
[17] Thomas Edward ,(1922)" fermentation products Ethanol and Carbon-dioxide".
[18] United states Department of agriculture(July 2006) "Glucose fermentation by yeast are done widely in bakery as well as brewery industries".
[19] Wikibooks: https;//en.m.wikibooks.org/wiki/biology_answering _the_big_questions_of_life_fermentation. "Balloon bulging method".
[20] X.Asbin Mary, T.Mathuran, "Biosynthesis and Characterization of Silver nanoparticles from the marine seaweed Sargassumplagiophyllum", Engineering and Scientific International Journal, Volume 2, Issue 3, July – September 2015, PP.95-98.

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