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Research Article

An Assessment and Screening on Isolated Microbial Contaminants of Sugarcane Juice for Food Safety Sampling from Street Salesman at Guwahati City, Assam, India

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Abstract

Expected research based on newly implementation of harmful pathogens against road side juices. Respective doctors and Practitioner carry out their treatment against this pathogen along with antibiotic supplements. Here researcher conclusion is for to analyze and categorize the biochemical fermentation activities of pathogens with significant enzyme degrading analysis in sugarcane juice against gut acting probiotics, responsible for food toxicity and intestinal disorder.

Keywords: Sugarcane Juice; Food

Introduction

Sugarcane is a large perennial tropical grass belonging to the Tribe Andropogene of the Family Gramineae [1,4]. Sugarcane is an important agro industrial crop as worldwide manufacturing plant. Sugarcane (*Saccharum officinarum*) Juice is dark brown golden in color with medium flavor intensity contains caramel, butterscotch and green flavor and sucrose hydrolyzed to an equi molar mixture of glucose and fructose providing instant energy transported by fluid thermo dynamics.

Demography of sales corner in Gauhati city

Most of the villagers in Assam carry their serials and sugarcane by boat across the river Bramahputra later these are transported by pickup van from different cultivated places. The sells men buy this sugarcane as a bundle from different wholesale of markets in Gauhati city. Then the salesman along with his slicing machine (manual or generator operated) to slice and mash the sugarcane for preparing juice by extracted residues of sugarcane mixing with ice cube on street sides or unhygienic places of footpath.

Figure 1: Sugarcane juice and Sugarcane.

Figure 2: Graphical representation on demography of Sales corner in Gauhati city.

Sampling sites and Number of sampling

Ten sampling sites were visited in metropolitan area of Guahati to collect thirty samples.

Objectives

Considering all the aspects discussed previously, the present study was undertaken to perform the following objects

- To determine and compare the hygienic quality of collected samples.
- To identify the causative organism or the microbial viability are responsible for the spoilage of sugarcane juice.
- Isolation and purification of contaminants from samples to signify for public health assurance.

Epidemiology

Epidemiology is the study of out breaking sites (Endemic, Epidemic and Pandemic) of an infectious pathogen, its infection rate (virulence) on about a population (Sporadic) and establishment of measuring steps (Prevention and Control) against it [3].

Only few infectious bacterial and fungus particles are needed to cause infection that can be exist in sugercane juice as contaminents. This amount can readily be acquired through contact with contaminated hands and objects (Water, ice cubes, Dry suger cane, mechanical apparatus).

Red rots diseases of Sugarcane

- Vulnerable to fungal diseases named red rot diseaeses caused by *Collectottrichum falcatum.*
- This fungus attacks Sucrose accumulating parenchyma known as Cancer of Sugarcane [1].
- Controlled by using fertilizer named Atrazine 70% and Ametryn 30% (Herbicide).

Figure 3: Vulnerable to fungal diseases named RED ROT.

Flow chart of mechanisms

Figure 4

Figure 5: The salesman along with his slicing machine.

Figure 6: Flow chart of Sugarcane contaminants.

Methdology

Morphological and physiochemical characteristics of isolate

Isolates were characterized by considering the following features.

Morphology

Colony morphology: The effective isolate was grown on different agar media and 0.5% yeast extract agar medium. After 10 days at 20-22°C, under aerobic condition, cell shape and colony appearance of selected organisms were examined by phase contrast microscope to determine the cell morphology.

Cell morphology in liquid culture: Isolates were grown in GY medium containing 10% glucose at 300C and pH 5.00. After 1 day, culture were diluted and placed under phase contrast microscope.

Optimum Temperature for growth

Selected isolates were allowed to grow in 250ml flask containing 50ml GY medium containing 10% glucose with initial pH 5.0 at different temperature such as 20°C, 25°C, 30°C, 34°C and 37°C. Growths were measured by diluting culture and keeping the 496 to 500 nm.

Optimum pH for growth

Selected isolates were allowed to grow in 250ml flask containing 50ml GY medium containing 10% glucose at 300C with different initial pH such as 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0. Growths were measured by diluting culture and keeping the absorbance 496 to 500 nm.

Biochemical studies of the selected isolates

Biochemical characteristics are more important than morphological characteristics in the identification of microorganisms. For this reason, the following studies will guide to be made on the physiological activities of the selected microbes.

Catalase Reaction (Claus, 1995)

The enzyme catalase is capable of decomposing hydrogen peroxide into water and molecular oxygen.

Utilization of Citrate

The ability to utilize citrate as source of carbon and energy can be used to distinguish certain grain negative rods. For this test citrate medium can be inoculated with a very small amount of 48 to 72 hours old culture and incubated at 35^{0}C for 48 hours. Appearance of turbidity or growth in the medium will be indicated the utilization of citrate.

Deep Glucose Agar Test (Khan, 1962)

Microorganisms vary widely in their requirements for oxygen. With the help of this test, organisms can be classified with relation to free oxygen as strict aerobes, facultative anaerobes, micro-aerophilic and strict anaerobes.

Fermentation Test (SAB, 1957)

Fermentation test is of considerable significance in the identification and classification of bacteria. The microorganisms differ in their ability to ferment different carbohydrates. Some of them upon fermentation of carbohydrates produce both acid and gas, some produce only acid, no gas, some produce only alkali and still others that cannot ferment carbohydrates at all.

Gelatin Liquefaction (Collins and Lyne, 1984)

Gelatin in an incomplete protein, but its liquefaction helps to identify microorganisms. Many microorganisms possess the enzyme gelatinase, which is capable of hydrolyzing gelatin. This test indicates the presence or absence of the enzyme gelatinase in the tested organism.

Hydrolysis of Starch (Collins and Lyne, 1984)

Organisms capable of hydrolyzing starch to form monosaccharide or disaccharides possess the enzyme amylase. This test revealed the presence or the enzyme amylase in the organisms.

Proteolysis Test (SAB, 1957)

This is indicated the presence or absence of the enzyme proteinase in the organisms. This enzyme is capable of hydrolysis of protein.

Implements of cultured isolates in VITEK® 2 Compact system for analytical diagnosis

Culture of one loopful sample from MacConkey agar plates were used to transfer in The VITEK® 2 Compact system at SRL Diagnostic, Assam. The innovative VITEK 2 technology includes an expanded identification on Bacterial colonies in laboratory testing. After 24 hrs incubation of colonial growth from pure culture, colonies were needed for fast and accurate microbial identification by implemented database, for accurate result depending on the rapid outcomes with minimal training time.

Estimation of Enzymes produced by Microorganisms (Chettri and Tamang, 2014).

Another important reason to ferment foods is to coax microorganisms into producing enzymes that also provide very useful services. During food fermentation microorganisms produce enzymes to break down complex compounds to simple bio-molecules for several biological activities such as proteinase, amylase, mannase, cellulase, and catalase. So we have to determine the estimation of degraded enzymes for long storage of sugarcane juice.

Result

The main component of Sugarcane juice is sucrose or saccharose, C12H22O11, is a disaccharide composed of a molecule of Glucose connected via an α (1–2) glycosidic bond to a molecule of Fructose. After collections, samples were assembled for 10 fold di-

lutions and screened out to observe colony counting and reading out optical density.

Colony counting (McFarland., *et al.*): Clinical data confirming the effectiveness of probiotics in preventing tentative diagnosis (TD) is relatively limited. Nevertheless, prophylaxis of TD constitutes one of the largest markets for probiotics in Europe. McFarland et al. performed a meta-analysis of studies and found that isolates significantly prevented TD (RR ¼ 0.85, p o 0.001). No practice guidelines for the use of enzymatic degradation rate of estimated bacterial propagation in sugarcane juice as prophylaxis of TD are currently available (Avra pratim., *et al*. 2019).

Dilution Samples	Optical Density (496-500nm)	Colony counts In numbers.	Range of acceptable unit	
			Range (OD) 0.20 -0.85	Range (mcf) 0-1.8
GS1	1.70	4.02	Non acceptable	
GS2	0.26	3.24	Non Acceptable	
GS3	0.56	2.98	Non Acceptable	
MS4	0.50	2.44	Non Acceptable	
MS5	0.80	4.06	Non Acceptable	
MS6	0.30	1.08	Acceptable	
JS7	2.68	5.0	Non acceptable	
JS8	0.38	2.6	Non acceptable	
JS9	0.044	2.0	Non acceptable	
GMS10	0.12	3.6	Non acceptable	
GMS11	0.22	4.3	Non acceptable	
GMS12	0.14	5.6	Non acceptable	
DS13	1.8	4.8	Non acceptable	
DS14	1.25	5.3	Non acceptable	
DS15	0.40	1.4	Acceptable	
US16	0.11	4.96	Non acceptable	
US17	0.17	1.02	Non acceptable	
US18	0.12	2.6	Non acceptable	
PS19	1.79	3.88	Non acceptable	
PS20	1.37	6.1	Non acceptable	
PS 21	2.16	4.55	Non acceptable	
FS 22	1.92	6.4	Non acceptable	
FS23	0.61	4.2	Non Acceptable	
FS24	0.61	1.12	Acceptable	
KS25	1.11	2.4	Non acceptable	
KS26	1.56	2.7	Non Acceptable	
KS27	0.70	1.03	Acceptable	
SS28	2.92	4.7	Non acceptable	
SS29	1.1	3.23	Non acceptable	
SS30	1.33	4.5	Non acceptable	
Selected samples were four to clarify in Biochemical screening and further analysis for identifications.				

Table 1: Screening of samples in acceptable range.

Biochemical studies of the selected isolates

The physiological characters of all the isolates i.e. their growth at different temperature, pH, their salt (NaCl) tolerance and heat resistance were studied following the procedures described below:

Dilution Samples	Cultural characteristics				Biochemical test			Inference
	Nutrient agar (Colonies)	MacConkey Agar (Colonies)	PDA agar (Colonies)	BLOOD Agar (Hemo-Lysis)	Urease Test (+)	VP Test (+)	Baci-tracin Disc (-)	
MS6	+++	+++	+	-	+	+	-	GNB / GPC
DS15	+++	++	+	-	+	+	-	GNB / GPC
FS24	+++	++	+++	-	+	-	-	Fungal colonies
KS27	+++	++	-	+	-	-	-	GNB / GPC

Table 2: Screening of samples in cultural and biochemical characteristics.

This table indicated the summarized form of sample selections and their effect specified on customers health. However, there always remains the possibility that contaminants in juice for consumption can cause infection and that individuals will respond in different ways to a specific strain. The salesman, juice handler will need to carefully assess the safety and efficacy of all new species and strains of microbes before incorporating them into food products.

Sampling sites	Samples	Isolated reading from Vitec-2 and compared with Bergey's Manual of Determinative Bacteriology - 8th ed. (Buchanan and Gibbons, 1974) and 9th ed. (Holt *et al.,* 1984)	Culture from Saboured Dextrose Agar
		Bacteria	Fungus
Ganeshguri	Sample 1	Contaminants in Mixed culture	----------
	Sample 2	Contaminants in Mixed culture	----------
	Sample 3	Contaminants in Mixed culture	----------
Maligao	Sample 4	Contaminants in Mixed culture	----------
	Sample 5	Contaminants in Mixed culture	----------
	Sample 6	*E. coli*	----------
Jalukbari	Sample 7	Contaminants in Mixed culture	----------
	Sample 8	Contaminants in Mixed culture	----------
	Sample 9	Contaminants in Mixed culture	----------
GMC	Sample 10	Contaminants in Mixed culture	----------
	Sample 11	Contaminants in Mixed culture	----------
	Sample 12	Contaminants in Mixed culture	----------
Dispur	Sample 13	Contaminants in Mixed culture	----------
	Sample 14	Contaminants in Mixed culture	----------
	Sample 15	*Klebsiella pneumonia ssp pneumonie*	----------.
Ulubari	Sample 16	Contaminants in Mixed culture	----------
	Sample 17	Contaminants in Mixed culture	----------
	Sample 18	Contaminants in Mixed culture	----------
Panbazar	Sample 19	Contaminants in Mixed culture	----------
	Sample 20	Contaminants in Mixed culture	----------
	Sample 21	Contaminants in Mixed culture	----------
French bazar	Sample 22	Contaminants in Mixed culture	----------
	Sample 23	Contaminants in Mixed culture	----------
	Sample 24	Contaminants in Mixed culture	*Candida sp.*
Khanapara	Sample 25	Contaminants in Mixed culture	----------
	Sample 26	Contaminants in Mixed culture	----------
	Sample 27	*Staphylococcus aureus*	----------
Sixmile	Sample 28	Contaminants in Mixed culture	----------
	Sample 29	Contaminants in Mixed culture	----------
	Sample 30	Contaminants in Mixed culture	----------

Table 3: Tabular form of isolated findings.

Test Organisms: MS6				
Test Substrate	**Abbreviation**	**Well**	**Amount/well (mg)**	***Escherichia coli* McFarland:0.61**
Ala-Phe-Pro Anylamidase	APPA	2	0.0384	-
ADONITOL	ADO	3	0.1875	-
Alpha D Glucosidase	AGLU	41	0.036	-
Glutamyl Arylamidase pNA	AGLTp	12	0.0324	-
Alpha Galactosidase	AGAL	44	0.036	+
Beta D Glucopyranosidase	BGLU	17	0.036	-
Beta N Acetyle glucosaminidase	BNAG	11	0.0408	-
Beta D Xylosidase	BXYL	21	0.032	-
BETA -GALACTOSIDASE	BGAL	9	0.036	+

Beta Alanine arylamidase pNA	BAlap	22	0.0174	-
Beta Glucoronidase	BGUR	57	0.0378	+
Citrate Sodium	CIT	36	0.054	-
COUMARATE	CMT	56	0.126	+
D Maltose	d MAL	18	0.3	+
D TAGTOSE	d TAG	34	0.3	-
D Mannitol	d MAN	19	0.1875	+
D TREHALOSE	d TRE	35	0.3	+
D Glucose	d GLU	13	0.3	+
D Mannose	d MNE	20	0.3	+
D Sorbitol	d SOR	32	0.1875	+
D Cellulose	d CEL	7	0.3	-
Ellman	ELLM	62	0.3	+
Glycine Arylaminidase	Gly A	46	0.012	-
Glu-Gly-Arg- Arylamidase	GGAA	59	0.0576	-
Gama Glutamyl Transferase	GGT	14	0.0228	-
Hydrogen Sulphide	H_2S	10	0.0024	-
L Lactate alkalinisation	l LATK	40	0.15	-
L Malate Assimilation	l MLTa	61	0.042	-
L ARBITOL	l ARL	5	0.3	-
L Histidine assimilation	l HLSa	53	0.087	-
L Lactate assimilation	l LATa	64	0.186	-
Lysine Decarboxylase	LDC	48	0.15	+
Lipase	LIP	26	0.0192	-
Malonate	MNT	37	0.15	-
N-acetylgalactosaminidase	NAGA	43	0.0306	-
O/ 0129 Resistance	O129R	58	0.0105	-
Ornithine Decarboxylase	ODC	47	0.3	+
Fermentation of Glucose	OFF	15	0.45	+
Phosphates	PHOS	45	0.0504	-
Palatinose	PLE	27	0.3	-
L-Pyrrolydonyl Arylamidase	PyrA	4	0.018	-
L-Proline Arylamidase	ProA	23	0.0234	-
Sacharrose	SAC	33	0.3	-
Succinate	SUCT	42	0.15	-
Tyrsine Arylamidase	Tyr A	29	0.0276	-
Urease	URE	31	0.15	-
5 Keto D Gluconate	5KG	39	0.3	+
Test Organisms: DS15				
Test Substrate	Abbreviation	Well	Amount/well (mg)	*Klebsiella pneumonia ssp pneumonie* McFarland:0.61
Ala-Phe-Pro Anylamidase	APPA	2	0.0384	-
ADONITOL	ADO	3	0.1875	+
Alpha D Glucosidase	AGLU	41	0.036	-
Glutamyl Arylamidase pNA	AGLTp	12	0.0324	-

Alpha Galactosidase	AGAL	44	0.036	+
Beta D Glucopyranosidase	BGLU	17	0.036	+
Beta N Acetyle glucosaminidase	BNAG	11	0.0408	-
Beta D Xylosidase	BXYL	21	0.032	+
BETA -GALACTOSIDASE	BGAL	9	0.036	+
Beta Alanine arylamidase pNA	BAlap	22	0.0174	-
Beta Glucoronidase	BGUR	57	0.0378	-
Citrate Sodium	CIT	36	0.054	+
COUMARATE	CMT	56	0.126	-
D Maltose	d MAL	18	0.3	+
D TAGTOSE	d TAG	34	0.3	+
D Mannitol	d MAN	19	0.1875	+
D TREHALOSE	d TRE	35	0.3	+
D Glucose	d GLU	13	0.3	+
D Mannose	d MNE	20	0.3	+
D Sorbitol	d SOR	32	0.1875	+
D Cellulose	d CEL	7	0.3	+
Ellman	ELLM	62	0.3	-
Glycine Arylaminidase	Gly A	46	0.012	-
Glu-Gly-Arg- Arylamidase	GGAA	59	0.0576	-
Gama Glutamyl Transferase	GGT	14	0.0228	+
Hydrogen Sulphide	H_2S	10	0.0024	-
L Lactate alkalinisation	l LATK	40	0.15	+
L Malate Assimilation	l MLTa	61	0.042	-
L ARBITOL	l ARL	5	0.3	-
L Histidine assimilation	l HLSa	53	0.087	-
L Lactate assimilation	l LATa	64	0.186	-
Lysine Decarboxylase	LDC	48	0.15	+
Lipase	LIP	26	0.0192	-
Malonate	MNT	37	0.15	-
N-acetylgalactosaminidase	NAGA	43	0.0306	-
O/ 0129 Resistance	O129R	58	0.0105	+
Ornithine Decarboxylase	ODC	47	0.3	-
Fermentation of Glucose	OFF	15	0.45	+
Phosphates	PHOS	45	0.0504	+
Palatinose	PLE	27	0.3	+
L-Pyrrolydonyl Arylamidase	PyrA	4	0.018	+
L-Proline Arylamidase	ProA	23	0.0234	-
Sacharrose	SAC	33	0.3	+
Succinate	SUCT	42	0.15	+
Tyrsine Arylamidase	Tyr A	29	0.0276	+
Urease	URE	31	0.15	+
5 Keto D Gluconate	5KG	39	0.3	-

Test Organisms: KS27				
Test Substrate	Abbreviation	Well	Amount/well (mg)	*Staphylococcus aureus* McFarland:0.62
Ala-Phe-Pro Anylamidase	APPA	2	0.0384	-
ADONITOL	ADO	3	0.1875	NA
Alpha D Glucosidase	AGLU	41	0.036	+
Glutamyl Arylamidase pNA	AGLTp	12	0.0324	NA
Alpha Galactosidase	AGAL	44	0.036	-
Beta D Glucopyranosidase	BGLU	17	0.036	NA
Beta N Acetyle glucosaminidase	BNAG	11	0.0408	NA
Beta D Xylosidase	BXYL	21	0.032	NA
BETA -GALACTOSIDASE	BGAL	9	0.036	-
Beta Alanine arylamidase pNA	BAlap	22	0.0174	NA
Beta Glucoronidase	BGUR	57	0.0378	-
Citrate Sodium	CIT	36	0.054	NA
COUMARATE	CMT	56	0.126	NA
D Maltose	d MAL	18	0.3	+
D TAGTOSE	d TAG	34	0.3	NA
D Mannitol	d MAN	19	0.1875	+
D TREHALOSE	d TRE	35	0.3	+
D Glucose	d GLU	13	0.3	NA
D Mannose	d MNE	20	0.3	+
D Sorbitol	d SOR	32	0.1875	-
D Cellulose	d CEL	7	0.3	NA
Ellman	ELLM	62	0.3	NA
Glycine Arylaminidase	Gly A	46	0.012	NA
Glu-Gly-Arg- Arylamidase	GGAA	59	0.0576	NA
Gama Glutamyl Transferase	GGT	14	0.0228	NA
Hydrogen Sulphide	H_2S	10	0.0024	NA
L Lactate alkalinisation	l LATK	40	0.15	+
L Malate Assimilation	l MLTa	61	0.042	NA
L ARBITOL	l ARL	5	0.3	NA
L Histidine assimilation	l HLSa	53	0.087	NA
L Lactate assimilation	l LATa	64	0.186	NA
Lysine Decarboxylase	LDC	48	0.15	NA
Lipase	LIP	26	0.0192	NA
Malonate	MNT	37	0.15	NA
N-acetylgalactosaminidase	NAGA	43	0.0306	-
O/ 0129 Resistance	O129R	58	0.0105	+
Ornithine Decarboxylase	ODC	47	0.3	NA
Fermentation of Glucose	OFF	15	0.45	NA
Phosphates	PHOS	45	0.0504	+
Palatinose	PLE	27	0.3	NA
L-Pyrrolydonyl Arylamidase	PyrA	4	0.018	+
L-Proline Arylamidase	ProA	23	0.0234	-
Sacharrose	SAC	33	0.3	+
Succinate	SUCT	42	0.15	NA
Tyrsine Arylamidase	Tyr A	29	0.0276	-
Urease	URE	31	0.15	-
5 Keto D Gluconate	5KG	39	0.3	NA

Table 4: Biochemical analysis on Microbial Enzymatic Reaction values.

Isolation and identification of *Candida sp*

Plate No	Form	Color	Elevation	Margin	Appearance	Opacity	Texture	No of Colony
1	Circular	Black with white border	Slightly raised	Two halves	Dull	Opaque	Velvety	5
2	Circular	Bluish green with white border	Raised	One third	Shiny	Opaque	Velvety	8
3	Irregular	Cream	Slightly raised	Entire	Shiny	Opaque	smooth	6
4	Circular	Cream	Slightly raised	Entire	Dull	Opaque	Smooth	1

Table 5

Figure 7: Isolation bacterial colonies.

Figure 8: VITEK 2, SRL Diagnostic.

Figure 9: Identified gram negative *bacilli* and fungal spores from sugarcane juice.

Figure 10: Identified gram negative *bacilli* and gram positive *cocci* from sugarcane juice.

Discussion

Hand-washing and sanitation does not work due to the resistant nature of the contaminants. Hospitalisation rates in countries with high in food poisoning, dehydration and flatulent gastric pain which may occur due to poor sanitation of prepearing juice in manual on

road. Handwashing can reduce the spread of the infection, but this is an ineffective means of eliminating it. A study was reviewed that after 24-48 hours incubation period [Table 2, 3 and 4], these infectious pathogens were followed by an abrupt onset of vomiting and diarrhoea. Later on these could be migrated for urinary tract infections; a low grade fever may also be present due to secondary infection [Figure 9 and 10] [5-14].

Prevention and control

1. Sales man should to work with gloves.
2. Only selected piece should be placed on machine where as others should be kept in unsliced.
3. Juice processing should be done in hygienic places.
4. Hygienic ice cubes and water should be processed in plastic glass using for one time.
5. Machine van should be covered by glass for processing.

Chemical process to neutralize bacterial contaminants:

- One spoon black salts.
- Addition of lemon juice.
- Washing of machinery apparatus with 1% acetic acid. (Vinegar) at least five times.

Nearly every scientific paper concerning the human health effects associated with unhygienic juice from road sides but it should be concerned about regarding microbial infection among consumers. But this particular research was highlighted on simple featuring to put an emphasis on the effect of the drinking sugarcane juice from street salesman in metropolitan area respective to human health analyzed on the isolation of different bacterial and fungal component. According to my study different colonies of fungi and bacteria were isolated and characterize from the sample. Samples consist of more bacterial contaminants and mixed colonies then beverage or manufactured. No beverage or manufactured sugar cane juice was found in local market of Assam. On the contrarily isolates from road side juice were Candida spp., somehow several samples were mixed with bacterial colonies (*Staphylococcus aureus and Streptococcus sp*.). Sugarcane juice extraction easily manipulated by contaminants from aerosol that contain endotoxin, peptidoglycan and various fungal constituents.

Conclusions

The prevalence on consumption of sugarcane juice from street sales man is seen to be more among people of rural areas with very little information available about concentration of toxic agents present in them. This knowledge may help to explain the high prevalence of gastrointestinal disorders such as diarrhea and food poisoning since these symptoms are also commonly associated with exposure to bio aerosols. Public awareness that sugarcane juice from unhygienic place contains high concentration of bacterial and fungal constituents may contribute to injurious for health.

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