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Sugar Cane Industry – Source for Microbial Diversity

Seema Paroha[1], Shubham Awadhiya[2], D. Chandra[3]
[1, 2, 3]National Sugar Institute, Kanpur, U.P. India
Email address: seemaparoha @ rediffmail.com

Abstract— *For the present study, the microbial diversity in samples from entire sugar house was carried out. The abundant variation in the types of microorganisms was observed. Depending on the processing condition and nature of processing material the microbial load varies greatly. In sugar manufacturing process microbial population is higher at the juice extraction. It increase in mixed juice but suddenly decreases drastically during the clarification and filtration due to the action of chemical and heat. After sulphitation the activity of most of the organism stopped but the thermo resistance and spore farming organisms can survive and retain their activity to destruct the sugar. Bacterial isolates observed were Leuconastoc mesenteroids, Leuconostoc dextranium E.coli, Bacillus.subtilis, Straphylococcus.aureus, Salmonella spp, Pseudomonas Putida were present in various samples. Fungi species recorded in various samples were Alternaria gaisen, Aspergillus flavus, Aspergillus candidus, Aspergillus niger, Aspergillus nidulans, Peniciilium pinophillum, Trichoderma reesei, Trichoderma viride along with Pichia spp,. Hansenula spp, Pleocyta sacchari, Saccharomyces spp, and Candida tropcalis.*

Keywords— *Bacteria, fungi, sugar industries, Bagasse, molasses, Aspergillus niger*.

I. INTRODUCTION

Microbial action in sugarcane processing has been commonly associated with undetermined losses of sucrose and the presence of dextran, among other effects. When microorganisms are present, total recoverable and fermentable sugars present in sugarcane decrease, the quality of materials is affected and the overall recovery efficiency in sugar and ethanol production in plants decreases significantly. Knowing the destination of sugars metabolized by microorganisms could help, however to mitigate microbial impact. The accurate identification of microbes is essential for scientists involved in many areas of applied research and industry. In nature there are various fungi, bacteria and microorganisms that are constantly at work to break down organic compounds. In this sense, several authors have expressed that metabolite quantification is a better indicator of microorganism action in sugar production factories [1,2,3]. In sugar-production factories, in addition to Leuconostoc mesenteroides, other bacterial species, mainly of the genera Bacillus, Lactobacillus and Enterococcus, were found, these being associated with sucrose consumption and conversion to polysaccharides (35–41% of sucrose consumed), mannitol (15–34%), lactic acid (20–23%), and acetic acid (2–15%). [4]. Studies were carried out to identify the main contaminating microbial strains in samples from entire sugar house. To study microbiological populations and their concentrations throughout sugarcane processing can be a long, expensive, and highly demanding process and this studies may help explain the performance of physicochemical variables in unit operations.

II. MATERIALS AND METHOD

2.1 Collection of Water Sample

The samples from entire sugar house were collected from sugar cane to the final sugar crystals obtained after processing. For the collection of sample sterilized polythene bags, collection bottle and cotton swab were used. Following samples were collected from the sugar Factory –

- Standing sugarcane (Small piece of sugarcane)
- Microbes in prepared cane in the mill. (Small piece of sugarcane)
- Samples in the milling train (some swab from milling area)
- Mixed juice,
- Process juice
- press mud
- 1st body condensate to 4th body condensate
- Stored Dry Bagasse.
- Molasses
- Final sugar.

Fig. 1. Samples from sugar factory

2.2 Isolation of Bacteria

Samples like Juices, Molasses and condensates water after being serially diluted in sterile distilled water were plated on nutrient agar plates and then incubated for 48 hrs at 30°C. Other samples like dry Bagasse, Sugar cane, Sugar crystal and

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press mud for these samples direct sampling method were used. Some swabs from milling area were directly streaked on the plate.

Discrete bacterial colonies that grew on agar plates were initially grouped on the basis of gram staining and different morphological characteristics such as pigmentation motility and colony forms. Bacterial isolates were then picked, sub-cultured and subjected to further biochemical tests for identification according to Bergey's manual of Determinative Bacteriology[5].

2.3 Physiological and Biochemical Tests

The physiological and biochemical tests were conducted following the methods of Somasegaran and Hoben [6] and Josey et al., [7] respectively, as described by Cappuccino and Sherman [8] to identify the bacteria and also confirmed with the help of PIB computer kit. Bryant.

2.4 Isolation of Fungi

1ml of the wet samples were taken in a 250ml conical flask containing 90ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and transferring serially 10ml of the water suspension to 90ml of sterile distilled water made different dilution viz., $10^{-1,}$ 10^{-2} and 10^{-3}. 1 ml 10^{-3} dilution was and plated in petridishes containing Potato Dextrose Agar medium (PDA). The pH of the medium was adjusted to 5.6. Streptomycin sulphate (100 ml) was added to the medium to prevent the bacterial growth. The plates were incubated at 25 + 2^0C for five days and fungi appearing on the medium were mounted over a clean slide, stained with lacto phenol cotton blue and observed under the microscope. Dry Samples like dry Bagasse, mill Bagasse, Sugar cane, Sugar crystal and press mud were directly sprinkled on potato dextrose agar medium containing Petri dish. After inoculation of sample plate were incubated at 28^{0}C for 48 hours for fungal appearing on the medium

The fungi were identified by using standard manuals, such as Manual of soil fungi Gillman, [9]

III. Results and Discussion

For the present study, the microbial diversity in samples from entire sugar house was carried out. The abundant variation in the types of microorganisms and their distinctive morphological and anatomical features visible to the naked eye enable us to identify the different genera and species and, therefore we can easily assess the extent of their diversity. Hence, the present study was undertaken to know the bacterial and fungal in different samples collected from sugar factory. For the present investigation samples were collected from sugar factory from sugar cane to the final sugar crystals obtained after processing. For the collection of samples sterilized polythene bags, collection bottles and cotton swab were used. Following samples were collected - Small piece from standing and prepared cane, samples in the milling train (some swab from milling area), dry stored bagasse sample, mixed juice, Process juice, , press mud, condensate from first to four bodies, molasses and final sugar .

3.1 Visual Observation-

Samples after being serially diluted in sterile distilled water were plated on nutrient agar plates and then incubated for 48 hrs at 30^{o}C.The results of investigation revealed that the all samples collected from sugar factory contained microscopic and macroscopic elements, whereas samples of all the vapour condensate (1 to 4 Bodies) exhibited negligible growth of the same. This indicates that vapour condensates are most promising and can be converted into fresh water after required processing. On an average the macroscopic characters exhibited off white colour, round shape, flat elevation and rough/slimy appearance in all collected samples whereas microscopic characters of these samples showed rod /cocci shape and both gram positive and grams negative bacteria (Table 1).

TABLE 1. Macroscopic and Microscopic visual characters of selected samples collected from sugar house.

S.NO	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	Standing sugarcane (Small piece of sugarcane)	Off White	Lawn	Flat	Slimy	Rods	G-ve
2.	Microbes in prepared cane in the mill. (Small piece of sugarcane)	Off White	Lawn	Flat	Slimy	Cocci/ Rods	G-ve/G+ve
3.	Samples in the milling train (1-4th mill Bagasse samples)	Off White	Lawn	Flat	Slimy	Cocci/rod	G-ve/G+ve
4.	Mixed juice	Off White	Lawn	Flat	Slimy	Cocci/rod	G-ve/G+ve
5.	Process juice,	Off White	Lawn	Flat	Slimy	Long rod	G-ve/G+ve
6.	Press mud	Off White	Lawn	Flat	Rough	Cocci	G-ve
7.	Crystals from pan	Off White	Lawn	Flat/rough	Slimy	Cocci	G-ve
8.	Some crystals from centrifuge process	Off White	Lawn	Flat	Slimy	Cocci	G-ve
9.	1st body condensate to 4th body condensate	Negligible Growth					
10.	Stored Dry Bagasse.	Off White	Lawn	Flat	Rough	Rods	G-ve
11.	molasses	Patches	Elevated	Flat	Slimy	Cocci	G-ve
12.	Final sugar.	Off White	Lawn	Flat	Slimy	Cocci	G-ve

Bacterial flora in the collected samples

Bacteria were isolated from the collected samples by serial dilution techniques. Identification of Bacterial Strains was done based on Biochemical Test evaluation. Depending on the processing condition and nature of processing material the microbial load varies greatly. Hygiene in factory also plays a major role. The Biochemical Test investigation of obtained

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bacterial population revealed that following bacterial strains were present in collected samples.

Microbes in harvested sugar cane

Harvested cane brought from the field was highly infected and many bacteria were found. May be because more the time between the harvested cane and till loaded on mill more will be the chances of infection. *Aerobacter* generally gram negative rod shaped were seen predominantly. Also *Pseudomonas sp along with Brevibacterium imperiale*, was also recorded in cane. Forty nine isolates of endophytic bacteria and three isolates of rhizobacteria were isolated from different sugarcane varieties, clones of *Saccharum spontaneum* and *Erianthus* sp. [10]

Microbes in prepared cane in the mill

All sample of the prepared cane taken after shredding and hammer milling are found to be extremely heavily contaminated with the organism found on the chopped cane billets. As reported contamination is comparatively less in hand cut cane with only 2 cuts on the either side. Our result obtained in prepared cane contained *Pseudomonas sp, Aerobacter, E.coli, Salmonella spp* and *leuconostoc*. It is also reported that organism usually some bacteria, *leuconostoc*, fungi and the extremely heat tolerant *thermomactinomyces sthalpophilus capable* of growing at 75^0C, are present all able to utilize the common sugars, glucose and fructose with rapidity to produce various organic acids.

Micro-organisms in the milling train

Sample taken from the milling trains by mean of swabs showed a population which was predominant *leuconostoc.* The same is also reported by others and it grows equally well at the both ends of the milling trails despite the difference in temperatures these Cocci have been found to be flourishing remarkably well on sugar well on sugar deposits adhering to the last mills, temperature high as 70-75^0C, but prefer to grow in the cooler environment provided at the side of the trains where the hot juice come in contact with the bacterial mat. Organism producing organic acids especially the sporing aerobes are found at the hot end and it is possible that some sugar is removed by the action of these organisms.

Microbes in mixed juice

The organism found in mixed juice are, naturally enough, characteristic of the organism coming into the mix juice on cane, but because certain *leuconostoc* are able to with stand relatively higher temperature the species found were *Leuconastoc mesenteroids & Leuconostoc dextranium* then most of the other contaminants, when *leuconostoc* infection occur in mix juice tanks, they may form toped like grains which on examination are found to be composed of their organism, these are *leuconostoc* and a small rod shaped organisms. The other type of organism tend to predominate in the mix juice observed were *Lactobacills fermentum, Lactobacillus Cellubioscus, Bacillus spp.* Fungi were predominantly present in mixed juice.Some other organism as reported by others found in the mixed juice are able to metabolize starch, in particular *Brevibacterium lipolyticum, B .imperiable.* Some of heat resistant thermomactinomyces, which are isolated in mixed juice tank.

Microbes in process juice

Thermophilic actinomycetes, organisms resembling *leuconostoc* in appearance and biochemical characteristic, and numerous thick bacilli are found in process liquor after heating and liming as this organism can adapt to temperature of 80-100^0C. Large rod shaped sporing organism have been found in clarified juice.

Typically, the sugarcane is crushed in the mill to obtain the juice. The juice is further heated and neutralized by adding lime.

Microbes in 1st body condensate to 4th body condensate

Samples of all the vapour condensate (1 to 4 Bodies) exhibited negligible growth of the micro-flora.

Microbes in Press mud

The residue formed is separated by filtration and is known as Press mud. *Bacillus spp,* are some of the species that were present in Pressmud. Fungi were predominant in press mud

Microbes in dry stored Bagasse

Bagasse, the byproduct of sugar industry, is one of the largest cellulose wastes being produced in our country by sugar factories. It is mainly utilized for the production of steam by feeding it into boilers. Many heat resistant bacteria and fungi are present in Bagasse *Thermoactinomyces sacchari* and *Thermoactinomyces vulgaris* that may cause allergy.

The identification of bacteria species that were isolated from stored bagasse were *Bacillus subtlis, Bacillus pumilus* and *Escherichia coli*. In Bagasse also fungi were predominant.

Microbes in Molasses

The Biochemical Test investigation of obtained bacterial population revealed diverse bacterial strain viz *E.coli , Leuconostoc, P.putida, Bacillus.subtilis, Straphylococcus.aureus, Pseudomonas putida , Salmonella spp. and B.cereus etc* were bacteria identified in molasses. [11] suggested the polluted habitats found mostly *Pseudomonas* because it is having ability to degrade various pollutants from water samples. Most studies on the metabolism of organic contaminants have been performed with bacteria especially in the context of bioremediation [12] Bacteria generally are easier to culture and they grow more quickly than fungi. They are more amenable to molecular genetic manipulations. In Molasses also varieties of fungi were predominant.

Microbes in Sugar Crystal

Moisture play major role in crystal getting contaminated with microbes. In present study bacterial strain *Pseudomonas sp and E.coli* were seen.

3.2 Fungal Flora in the Collected Samples

The Fungal evaluation and identification was done in the selected samples. It was observed those fungal colonies were

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seen mainly in Juices, press mud, bagasse and molasses. For identification of fungus culture was mounted on clean slides and stained with lacto phenol cotton blue. The slides were observed under the microscope. The fungus strains were identified based on colony characteristics and staining methods. The mycofloristic composition of above samples of sugar house varied significantly. Maximum numbers of fungal species identified in Mixed juice samples were *Pichia spp,. Hansenula spp, Pleocyta sacchari, Saccharomyces spp,* fungi especially, *Aspergillus Niger*, *Candida tropcalis* and *Trichoderma reesei*.

Most of isolated fungi in stored bagasse sample were especially, *Aspergillus Niger, Candida tropcalis* and *Trichoderma reesei these* are hemi-and cellulolytic fungi. Cellulase, Endogalactanase, Endo- 1, 4 –Mannasase are the high specific activity enzymes produced by *Aspergillus Niger* that showed hydrolytic effect on cellulose and hemicellulose.

Maximum numbers of fungal species were Fungal strain *Aspergillus flavus, Aspergillus niger, Aspergillus terrus, Peniciilium , Fusarium and Rhizopus.* were identified in the molasses sample. Same findings were found by [13] Among the genus *Aspergillus* was recorded as dominant genus with 3 species such as *A.niger, A.flavus and A.terrus*. The remaining genus such as *Penicillium, Fusarium,* and *Rhizopus* were recorded single species each. In other samples like cane, prepared cane and press mud *Alternaria gaisen, Aspergillus flavus, Aspergillus candidus, Aspergillus niger, Aspergillus nidulans, Peniciilium pinophillum and Trichoderma viride* were also seen. The genera *Aspergillus* was frequently found in sugar industrial effluent of different sugar factories. Table 2. Some potential fungal strains such as *Penicillium pinophilum*, *Alternaria gaisen*, *Fusarium monolifome Aspergillus flavus,, Aspergillus phoeniciss, Aspergillus candidus, A.niger, Cladosporium sp.*were also isolated and reported from sugarcane industrial effluent [14] A total number of 15 species belonging to 9 genera of fungi were isolated during our investigation in various sugarcane industries of Madhya Pradesh by [15]. Diverse fungal cultures have been investigated recently for bioremediation process [16] and [17]. By virtue of their aggressive growth, greater biomass production and extensive hyphal reach in the environment, fungi have been seen to perform better than bacteria. The high surface –to – cell ratio of filamentous fungi makes them better degraders under certain niches [18].The fungus have capability to purify the effluent by consumption of organic substances, thus, reducing its COD and BOD, and at the same time to obtain some valuable product, such as fungal biomass for protein rich animal feed or some specific fungal metabolite.. Maximum numbers of fungal species were recorded compare to bacteria.

TABLE 2. Macroscopic and Microscopic characters of fungal strains

S.No	Macroscopic Characters	Microscopic Characters	Fungi
1	-Pale white color, Texture deeply cottony; -White becoming gray-brown on surface, -Very rapid growth.	-Hyphae broad, not or scarcely septate; - Rhizoids and stolons present; - Sporangiophores brown, sporangiophores ovoid, sporangia rather round,.	*Rhizopus*
2	Colonies are green , black or grey in color.	-Conidia are club shaped, -Spores are single or form long chains.	*Fusarium*
3	Colonies are green , black or grey in color.	-Conidia are club shaped, -Spores are single or form long chains.	*Alternaria gaisen*
4	-Green in color with yellow or white color margin. -Wrinkled growth. -Become black in color after ageing.	-Dense mats of mycelium with conidia . -Conidial heads are radiate. -Conidia are globose to subglobose. -Pale green in color.	*Aspergillus flavus*
5	-Produced white, yellow, green and brown color colonies. -Cottony in texture. -Slightly elevated.	-Conidial heads are short columnar. -Conidiophores are usually short , brownish and smooth walled. -Conidia are globosend rough walled.	*Aspergillus terrus.*
6	-White cottony growth. -Sometime it gives pale yellow color, black and shades of green. -Erect conidiophores.	-Conidia are one celled, smooth or rough walled. -Conidiophores consists of whorl of phialids.	*Aspergillus candidus*
7	-Black in color. -Powdery texture. -Elevated growth.	-Large dark brown conidial heads. -Conidiophores are smooth-walled and hyaline. -Conidia are globose to subglobose , dark brown to black and rough walled.	*Aspergillus niger*
8	-Produced white, yellow, green and brown color colonies. -Cottony in texture. -Slightly elevated.	-Conidial heads are short columnar. -Conidiophores are usually short , brownish and smooth walled. -Conidia are globosend rough walled.	*Aspergillus nidulans*
9	-Cottony growth. -Center wrinkled. -Mycelium white to orange white. -Conidiogenesis moderate, grayish green in color.	-Colorless hyphae. -Thallus highly branched. -Constricted conidiophores.	*Peniciilium pinophillum*
10	-Light green in color and yellowish conidia scattered throughout the plate.	-Conidia are globose. -Phialids are slender. -Branched conidiophores.	*Trichoderma viride.*
11	Grey, green brown ,Regular, Wooly, Flat	Septate brown hyphae. -Conidiophores are Septate, brown, simple or branched. -Conidia are Septate, brown, muriform, oval, chains or single.	*Alternaria*

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Fungal diversity is very important because of their economic importance as well as their Pathogenicity. Soon the basis of reported fungus and their ability of degradation and enzymatic activity of fungal strain they may use in commercial sector. A large number of fungal diversity associated with sugarcane industrial effluent and this database created a novel record of the fungal diversity associated with sugarcane industrial effluent. Preparation of database provided a base in solving the problems associated with pollution of sugarcane industry and may become a basis for the management of sugarcane industrial effluent. Table 3 shows the Microorganisms their site and their activity performed.

TABLE 3. Microorganisms their site and their activity

Microorganism	**Site**	**Activity**
Xanthomons	Cane	Organic acid
Aerobacter	Cane	Organic acid
Aspergillus fumigatus	Air	Organic acid
Brevibacterium imperiale	Cane	Red pigment ,Organic acid
Lactobacills fermentum	PJ, MJ	Lactic acid
Lactobacillus Cellubioscus	PJ, MJ	Lactic acid
Leuconastoc mesenteroids	PJ, MJ	Lactic acid, Dextran
Pichia spp	MJ	Fermentation
Hansenula spp	MJ	Fermentation
Saccharomyces spp	MJ	Fermentation
Bacillus spp	MJ	Levan production
Leuconostoc dextranium	MJ	Dextran
Pleocyta sacchari	MJ	Fermentation
Candida spp	MJ	Fermentation
Saccharo coccus thermophilus	Diffuser	Lactic acid
Bacillus spp	Syrup Massecuite	Leven Prodn.
Bacillus stearothermophilus	Syrup -80^{o}C	Organic acid
Thermophilic actinomycetes	80^{o}C	Lactic acid
Staphylococcus spp	Syrup Massecuite	Fermentation
Aspergillus spp	Syrup Massecuite	Organic acid , Aflatoxin
Bacillus megatherium	Diffuser 80^{o}C	Organic acid
Micrococcus spp	Syrup	Oxidation

MJ (Mixed Juice); PJ (Primary Juice)

IV. CONCLUSION

The present investigation was carried out to isolate the most frequently occurring and optimally performing microorganisms from Sugar Industry. Fungi and bacteria were traced through each stage of the manufacturing process and found in greatest numbers in the mixed juice, molasses, press mud and bagasse. The Bacterial isolates observed were *Leuconastoc mesenteroids, Leuconostoc dextranium E.coli, Bacillus.subtilis, Straphylococcus aureus, Salmonella spp, Pseudomonas Putida were present in various samples.* The fungi isolated on a variety of samples collected from sugar factory showed a wide range viz; *Alternaria gaisen, Aspergillus flavus, Aspergillus candidus, Aspergillus niger, Aspergillus nidulans, Peniciilium pinophillum , Trichoderma reesei, Trichoderma viride along with Pichia spp,. Hansenula spp, Pleocyta sacchari, Saccharomyces spp,* and *Candida tropcalis* belonged chiefly to the Aspergilli.

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Seema Paroha, Shubham Awadhiya, and D. Chandra, "Sugar Cane Industry – Source for Microbial diversity," *International Research Journal of Advanced Engineering and Science*, Volume 5, Issue 4, pp. 81-85, 2020.