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Research Article

MICROBIAL DIVERSITY IN SOFT DRINKS

A. Shiva Shanker, Ashwitha Kodaparthi and Pavan Kumar Pindi*
Department of Microbiology, Palamuru University, Mahabubnagar- 509001, A.P., India
*Dr. Pavan Kumar Pindi, Associate Professor & HOD, Department of Microbiology, Palamuru University, Mahabubnagar-509001, A.P. Email: pavankumarpindi@gmail.com

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ABSTRACT
A total of 4 carbonated soft drink (CSD) samples, from various locations in nine places of India were examined to determine their biological quality. Most samples were not in compliance with microbiological standards set by organizations like the World Health Organization (WHO). The prevailing bacterial isolates were *Gluconobacter sp., Lactobacillus sp.,* and *Leuconostoc sp.* The prevailing fungal isolates were *Penicillium expansum* and the next isolate was *Penicillium roqueforti*. Many microorganisms are found in soft drinks as environmental or raw materials contaminants, but relatively can grow within the acidic and low oxygen environment. It is crucial for soft drink manufacturers to inspect raw materials before they are mixed with other ingredients. Concern with consumer health, safety and the environment will continue to have a positive impact on trend in the soft drink industry.
Keywords: Carbonated Soft drinks, Microorganisms, Contamination.

INTRODUCTION

Soft drinks are enormously popular beverages consisting primarily of carbonated water, sugar and flavorings. Nearly 200 nations enjoy the sweet, sparkling soda with an annual consumption of more than 34 billion gallons. The roots of soft drinks extend to ancient times. Two thousand years ago Greeks and Romans recognized the medicinal value of mineral water and bathed in it for relaxation, a practical that continues to the present.

In the alcoholic beverage sector, breweries increasingly develop new low-alcohol and value-added products, such as fusion drinks mixing alcohol drinks with non-alcoholic beverages, for new and increasingly defined consumer groups[1]. Both soft drinks and alcoholic beverages have become more and more complex in composition. At the same time, consumer demands for more natural, nutritious and tasty products are directing breweries to minimize the use of additives and heat treatments, to increase juice contents in formulations, as well as to reduce the acidity of the products. Possible adverse health effects of benzoic acid have already led many soft drink manufacturers to abandon this additive. Hence, many traditional antimicrobial hurdles present in traditional soft drinks and alcoholic beverages are brought down[2], while product transport time, shelf-life and international trade as well as the use of new ingredients are increasing[3].

As flavored carbonated beverages gained popularity manufacturers struggled to find an appropriate name for the drinks. The most appealing name however was “soft drink” adapted in the hopes that would ultimately supplant the hard liquor” market. Although the idea never struck, the term soft drink did. Bacteria that have been associated with spoilage in the soft drink industry include Acetobacter, Alicyclobacillus, Bacillus, Clostridium, Gluconobacter, Lactobacillus, Leuconostoc, Saccharobacter, Zymobacter and Zymomonas. Gluconobacter is a common spoilage agent of fruit juices; it is a strict aerobe, requiring free oxygen. The low pH value of soft drinks and fruit juices, pH 2.5 to 3.8 inhibits most bacteria, but leaves yeasts unaffected. Spoilage is therefore due to facultative anaerobes, organisms that can grow with or without oxygen. In carbonated drinks, moulds and bacterial growth is very unlikely as they are very sensitive to CO_2.

Simple carbonated soft drinks still dominate the global beverage market, their market share is decreasing. Functional beverages and bottled water currently constitute the fastest growing beverage sectors[4]. In 2008, functional drinks reached global sales of 26.9 billion dollars, with average growth rates of 15–20% per annum. The energy drinks sector has experienced the greatest volume growth, which is expected to be strongest in 2007–2012[5].

Many microorganisms are found in soft drinks as environmental or raw materials contaminants, but relatively can grow within the acidic and low oxygen environment. Yeasts are the most significant group of microorganisms associated with spoilage of soft drinks and fruit juices. Spoilage will be seen as the growth and production of metabolic byproducts, for example CO_2, acid and tanning compounds.

Raw Materials

Water is the major ingredient in all soft drinks and should fulfil the criteria for drinking water.Soft drinks manufacturers usually use softened water to prevent off-tastes from chlorine residues[6]. This procedure reduces the concentration of metal ions to approx. 50 ppm Mg and Ca (Stratford and James 2003). Special carbohydrate sweeteners permitted in the EU are trehalose, isomaltulose (PalatinoseTM) and D-tagatose[6]. Isomaltulose is a natural tooth-friendly disaccharide with slow energy release and glycemic index and a mild sweetness[1]. Fruit and vegetable extracts also contain hexose and pentose sugars and polyols[7]. Thaumatin is a naturally sweet plant extract that can be applied as a flavour enhancer[6]. Carbonation is responsible for the characteristic taste of sparkling beverages. Carbonation of soft drinks is expressed as volumes or grams per litre. Carotenoids can be used as a source of natural colours, but they are also added in soft drinks for their antioxidant activity.

Energy drinks contain caffeine (360–630 mg/l), taurine (average 3 180 mg/l), caffeine-rich plant extracts (e.g. tea, ginseng, guarana, yerba mate) as typical energizing components, and B-vitamins[5]. The main constituents of sport drinks are carbohydrates in the form of glucose, fructose and maltodextrin (5.5–8.2%), salts and water[8]. Sodium and potassium concentrations are 20–30 and 5 mM, respectively.

There is also a growing trend to incorporate other functional ingredients in sports drinks.

Sample collection and Processing

A total of 4 carbonated soft drinks of different brands viz., Sip-on, Maa apple, F & N Ginger ale and Minute maid from different manufacturing beverage industries in Karnataka, Mahabubnagar (AP), Mysore and Chittoor in India were collected in the month of March, 2010.. pH values of the samples are noted. 120 µl of each sample was plated on LB agar plates and incubated at 36ºC overnight and observed for bacterial growth. Similarly samples were plated on Saboraud's agar plates at room temperature for fungal growth and on Potato Dextrose agar medium for growth of Yeasts.

Bacterial Genomic DNA Extraction

The genomic DNA from the bacterial cells was obtained using a modification of the method described by sambrock etal (1989). The bacterial cells from pure culture were harvested by centrifugation (12,000rpm) for 2min, and the cell pellets mixed with 600µl of lysis buffer (10mm tris – HCl, 1mM edta [pH 75], 0.5% SDS, and 100/g/ml proteinase c) and incubated at 37˚c for 1h. after the addition of 100 µl 5 M NaCl, and 80µl CTABL NaCl, the Samples were incubated at 65˚c for 10min. the samples were incubated at 65˚C for 10 min. the sample were cooled to room temperature, followed by extraction of the aqueous phase with an equal volume of chloroform : isoamyl alcohol [24:11, v/v] and then with an equal volume of phenol: : chloroform : isoamylalcohol (25:24:1, v/v_ which was centrifuged at 12,000rpm & 4˚c for 10 min. isopropanol (0.6x) was mixed with the aqueous phase, and centrifuged at 12,000rpm and 4˚c for 10 min. the DNA Pellets were dried under vacuum, and then dissolved in TS Buffer (10mM Tris-HCl, and 1Mm EDTA [pH.75][9]

16S rRNA gene Sequencing

The purified 1542bp PCR product was sequenced using universal primers. The resultant almost complete sequence of the 16S rRNA gene sequence of the isolate was subjected to BLAST sequence similarity search and Ez Taxon[10] to identify the nearest tax a. the entire related 16S rRNA gene Sequence were downloaded from the database (http://www.nbi.nlm.nih-gov).

Fungal DNA Isolation and Sequencing

The fungi were isolated using two different methods: membrane filtration and plating method. For the membrane filtration, 100 ml of water sample was filtered through membrane filters with a diameter of 47 mm and a pore size of 0.45 µm. The filters were placed in the center of agar plates after filtration. For the plating method, 500 µl of samples were plated on agar plates with a glass spreader. Two different plates were used: Malt extract agar and Sabouraud glucose agar plates, both supplemented with 40 mg/l gentamycin and 100 mg/l Chloramphenicol to inhibit bacterial growth. The agar plates were incubated t 22ºC for 3 and 7 days. After 7 days of growth, the numbers of the colony forming units (CFU) per 100 ml of samples were assessed and the different taxa of the cultivated fungi were subcultured on new agar plates at 22ºC for up to 10 days. The cultivated fungi were identified using routine microscopy techniques.

Whenever macro- and micro morphology failed to show unambiguous results, PCR of the gene coding for the ribosomal internal transcribed spacers (ITS) with the enclosed 5.8S ribosomal DNA and subsequent sequencing was performed. DNA from fungi was isolated. Thereafter, the ITS region was amplified by PCR using the primer set. Sequencing of the amplified ITS region was accomplished according to the Sanger-Coulson method (or chain termination method using single-stranded DNA) with subsequent analysis of the sequenced products using the Genetic analyzer ABI PRISM 3130. The ITS sequences were then compared with entries in genomic databanks using the Internet free-ware from European Bioinformatics Institute(EMBL) found under http://www.ebi.ac.uk/fasta33/nucleotide.html to identify the specific fungi

RESULTS AND DISCUSSION

A total of 4 carbonated soft drinks from different brands, from various locations in different manufacturing beverage industries located in India were collected in the month of March, 2010. pH values of the samples are noted. The *cfu / ml* was noted for each sample and the highest cf was found in F & N Ginger ale (94). The pH values and the cfu/ml of each sample are tabulated in table: 1

The bacterial isolates found were Gluconobacter, Lactobacillus, Leuconostoc, Bacillus, Saccharobacter, Zymobacter, Zymomonas, Acetobacter, Alicyclobacter and Clostridium (Fig. 2.). The prevailing bacterial isolates were ***Gluconobacter sp.***, ***Lactobacillus sp*** (26.66% each). *The next prevalent bacterium was* ***Leuconostoc sp***. The frequency of the similar isolates of the have been tabulated in table: 2

The fungal contaminants were *Penicillium expansum, Penicillium roqueforti, Penicillium digitatum* , *Aspergillus vesicolor, Fusarium oxysporum, Penicillium glabrum* (Fig. 3).The prevailing fungal isolates were ***Penicillium espansum*****(21.42%)** and the next prevalent isolate was ***Penicillium roqueforti*** (Refer table :3)

Table: 1 pH and Cfu values of the Samples

Name of the Soft drink	Date of Manufacture	Manufacturing Address	pH of the Sample	Cfu/ml
Sip-on	12/03/10	Megha fuit processing Pvt. LTD. Narimogera, Pattur-574202, Mangalore, Karantaka.	3.2	48
Maa apple	10/02/10	Cavin Industries Pvt. LTD, survey No 151(part) 155(part) Hasnabad-509350, Kodangal, Mahabubnagar, A.P.	4.5	67
F&N Ginger ale	02/01/10	ATC Beverages Pvt. LTD, Plot No 11B&11C KIADB industrial area, Nanjangud, Mysore-571302.	2.58	94
Minute maid	25/02/10	Hindustan coca-cola Beverages Pvt LTD,Sy No 127 to 131, Kopugunneri village, Srikalahasti mandal, Chitoor-517640, A.P	3.4	64

Table: 2. Spectrum of bacteria in the Samples:

S.No	Bacterial Genus	Numberof similar Isolates	Frequency of similar isolates
1	***Gluconobacter***	4	26.66
2	***Lactobacillus***	4	26.66
3	***Leuconostoc***	3	20.00
4	***Bacillus***	2	16.66
5	***Saccharobacter***	2	16.66
	Total	**15**	**100**

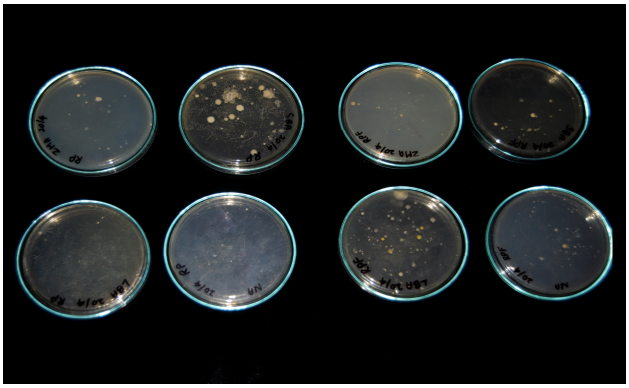

Fig. 2. The bacterial isolates from different soft drinks

Table: 3. Fungal diversity in the samples:

S.No	Name of the fungi	Number of similar Isolates	Frequency of similar isolates
1	***Penicillium expansum***	3	21.42
2	***Penicillium roqueforti***	3	21.42
3	***Penicillium digitatum***	2	14.28
4	***Aspergillus vesicolor***	2	14.28
5	***Fusarium oxysporum***	2	14.28
6	***Penicillium glabrum***	2	14.28
	Total	**14**	**100**

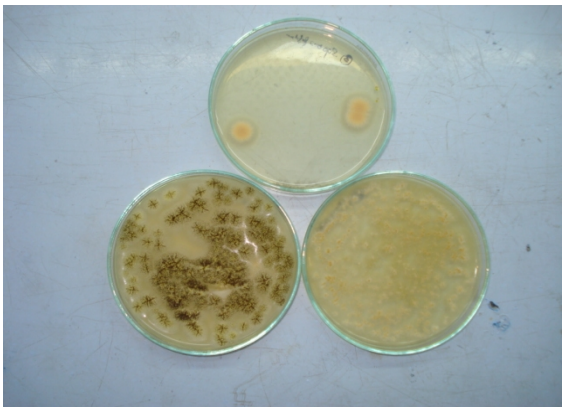

Fig. 3. Fungal colonies isolated from different soft drinks

Many microorganisms are found in soft drinks as environmental or raw materials contaminants, but relatively can grow within the acidic and low oxygen environment. Yeasts are the most significant group of microorganisms associated with spoilage of soft drinks and fruit juices. Spoilage will be seen as the growth and production of metabolic byproducts, for example CO_2, acid and tanning compounds.

CONCLUSION

Soft drink manufacturers adhere to strict water quality standards for allowable dissolved solids, alkalinity, chlorides, sulfates, iron and aluminum. Not only is it in the interest of public health, but clean water also facilitates the production process and maintains consistency in flavor, color and body. Microbiological and other testing occur regularly. The national soft drink association and other agencies set stands for regulating the quality of sugar and ingredients. If soft drinks are produced with low quality sugar, particles in the beverage will spoil it, creating floc. To prevent such spoilage, sugar must be carefully handled in dry, sanitized environments.

It is crucial for soft drink manufacturers to inspect raw materials before they are mixed with other ingredients, because preservatives may not kill all bacteria. All tanks, pumps and containers are thoroughly sterilized and continuously monitored. Cans, made up of aluminum alloy or tin coated low carbon steel, are lacquered internally to seal the metal and prevent corrosion from contact with the beverage. Soft drink manufacturers also recommend specific storage conditions to retailers to insure that the beverages do not spoil.

Significance if this study

In the future, advanced technology will lead to greater efficiency of soft drink production at all stages. New methods of water clarification, sterilization and pasteurization will improve production and minimize the need for preservatives in soft drinks. Concern with consumer health, safety and the environment will continue to have a positive impact on trend in the soft drink industry.

Competing Interests

Author has declared that no competing interests exist.

REFERENCES

1. Hausmann S. Toothfriendy beverages can now be manufactured thanks to the functional carbohydrate Palatinose creates new gategory in the soft drinks segment. Wellness Foods Europe 2009; 26–29.
2. Hutzler M, Wellhoener U, Tenge C, Geiger E. Beer mixed beverages: dangerous spoilage yeasts, susceptible beverages. Brauwelt International 2008; 26: 206–211.
3. Tribst A.A, Sant'Ana Ade S, de Massaguer P.R. Review: Microbiological quality and safety of fruit juices--past, present and future perspectives. Critical Reviews in Microbiology 2009; 35: 310-339. ISSN 1549-7828; 1040-841X. doi: 10.3109/10408410903241428.
4. Lawlor K, Schuman J, Simpson P, Taormina J. In: Sperber W.H, Doyle M.P. (eds.) Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Safety 2009; 245–283, Springer, New York.
5. Heckman M, Sherry K, Gonzales de Mejia E. Energy drinks: An assessment of their market size, consumer demographics, ingredient profile, functionality and regulations in the United States. Comprehensive Reviews in Food Science and Food Safety 2010; 9: 303–317.
6. Ashurst P.R, Hargitt R. Soft Drink and Fruit Juice Problems Solved. Woodhead Publishing. ISBN http://www.knovel.com/web/portal/browse/display? _EXT_KNOVEL_DISPLAY_bookid=2678 (12.9.2011); 2009: 326-8.
7. Statford M, James S.A. Non-alcoholic beverages and yeasts. In: Boekhout, T. and Robert, V. (eds.) Yeasts in Food, Hamburg, Germany: B. Behr's Verlag GmbH & Co; 2003: 12: 309-345.
8. Mettler S, Rusch C, Colombani P. Osmolality and pH of sport and other drinks available in Switzerland. Schweizerische Zeitschrift für "Sprtmedizin und Sporttraumatologie" 2006; 54: 92–95.
9. Sambrook J, Fritschi E.F, Maniatis T. Molecular cloning: a laboratory manual, cold Spring Harbor Laboratory Press, New York 1989.
10. Chun J, Lee J.H, Jung Y, Kim S, Kim B K, Lim Y W. EzTaxon: a web based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int J Syst Evol Microbiol 2007; 57: 2259-2261.