

Profiling of Microbiota in Sport-Drink and Orange Juice After Drinking Directly From Plastic Bottles



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Introduction

Plastic Bottles

- Regarding remaining beverages in plastic bottles
→ Limited scientific information on the concentration and composition of bacteria in the remaining drinks is available.
- It is recommended that any remaining beverage in plastic bottles should be discarded after drinking.
- However, some consumers may be inclined to store or leave the remaining beverages in plastic bottles after drinking and then drink them later, especially in the summertime or in hot weather to prevent dehydration.
- To understand the characteristics of the bacteria in the remaining beverages in plastic bottles after drinking directly from the bottles, analyses of the bacteria at the mouth of bottles and in the remaining beverages in the bottles both immediately after drinking from the bottles and after storage at 37°C for 24 h were performed.

It is suspected that oral bacteria can be transferred to drinks in plastic bottles and can multiply in the bottle after drinking

To elucidate the effect of bacteria on drinks after drinking,

- The present study examined that
- The transfer of oral bacteria
 - Their multiplication and survival in drinks

In this study, we used Sport-drink and Orange juice →



Materials & Methods

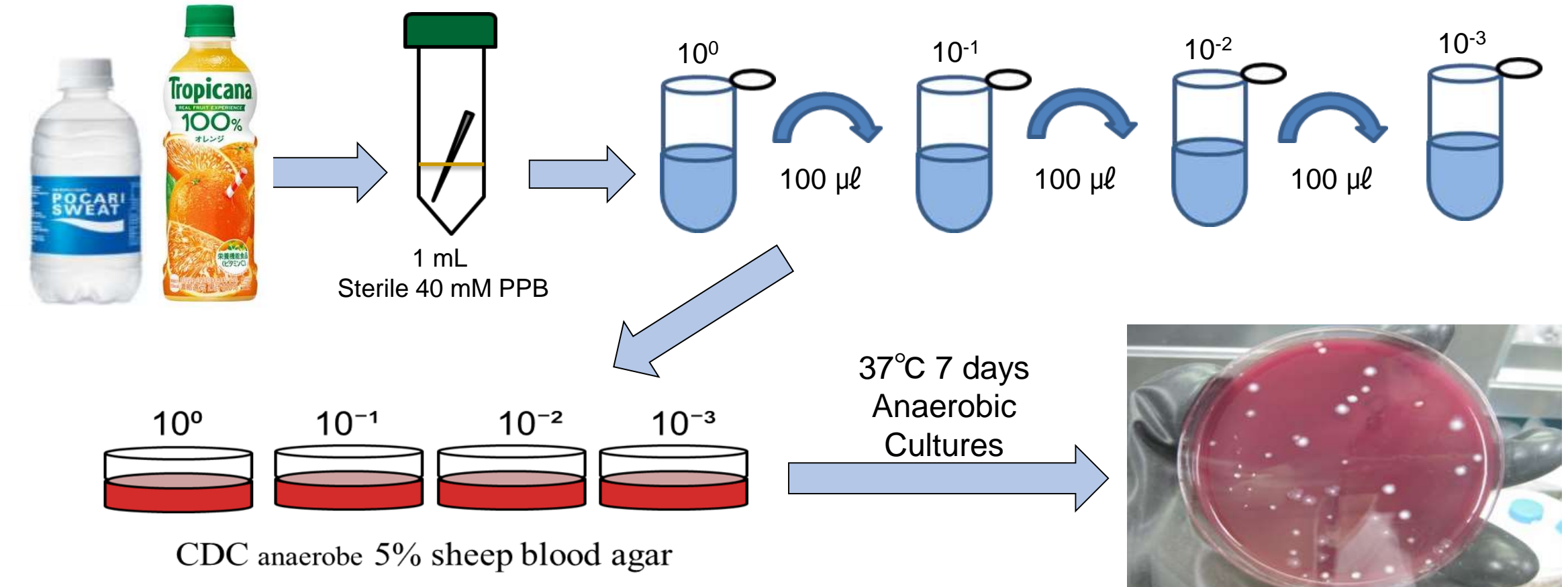
I Subjects

After informed consent, 9 healthy human subjects (14-23 years old) were asked to drink approximately 100mL of a sport-drink or orange juice from a plastic bottle.

II Samples

The mouth of the bottles were swabbed with sterile cotton. After suspended and vortexed in a buffer, serial-diluted samples were inoculated onto CDC blood agar plates, and incubated anaerobically and aerobically at 37°C.

Samples of the remaining drinks immediately after drinking and after storage 37°C for 24h were also cultured. Baby-Drinks after drinking (immediately after and 3-h later) were also inoculated. Salivary samples from each subject were also cultured.



PCR-PFLP

Genomic DNA was then extracted from single colonies using InstaGene Matrix kit (Bio-Rad Laboratories). The 16S rRNA gene were individually digested with *Hpa* II, and the digested products were separated on 2% agarose gels. Isolates were identified according to the PCR-RFLP analysis and sequence.

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1 ttcttggttt gatctggct caggaagac gctggggg tgcataac agcaagatg
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1441 gaggtaact ttagagagc agcgtcaca ggtggatg atgattggg ttagctcga
1501 caagtcagc cgttggaa
    
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16S rRNA Gene (ca. 1,500bp)

Program

Initial heat activation	95°C	5min	} 30cycles
Denaturation	94°C	1min	
Annealing	55°C	1min	
Extension	72°C	1.5min	
Final extension	72°C	10min	

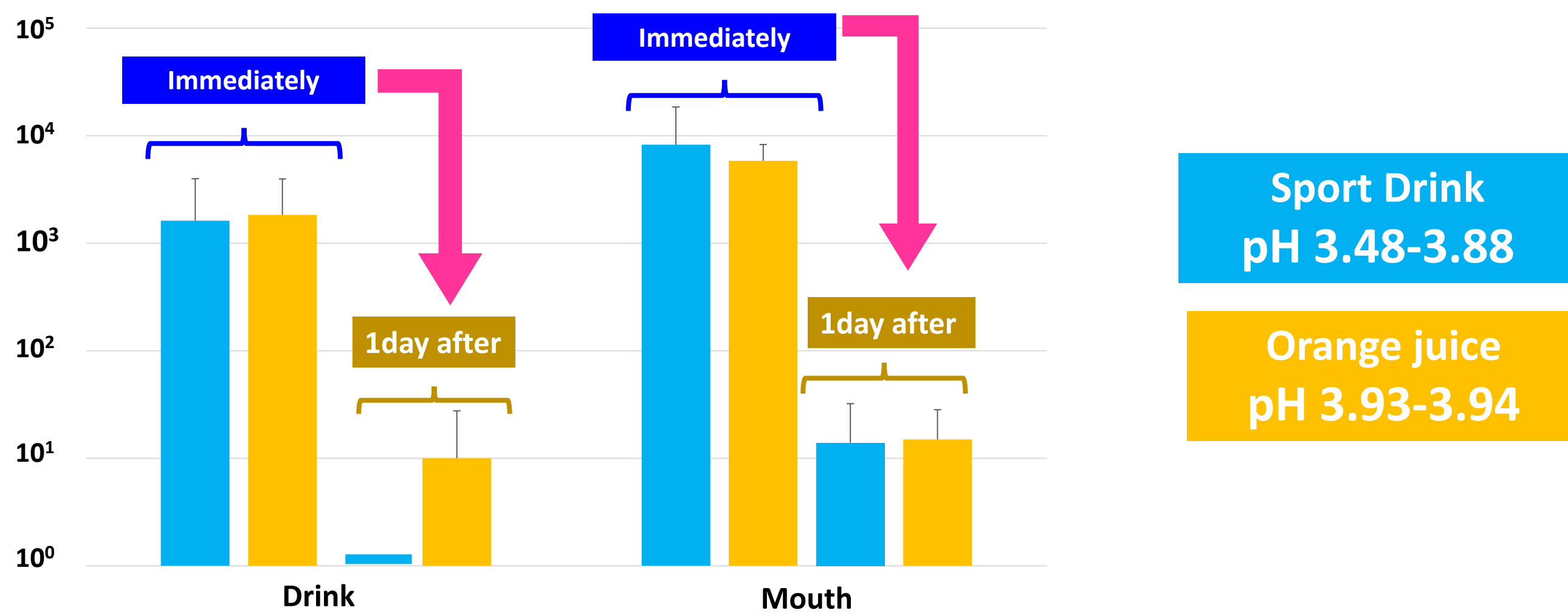
Universal primers

27F: 5'- AGA GTT TGA TCM TGG CTC AG -3'
1492R: 5'- TAC GGY TAC CTT GTT ACG ACT T -3'

Stained with Ethidium Bromide

Actinomyces oris
Streptococcus mitis/oralis

Results 1: Bacterial amounts (CFU) & pH



The mean amounts of bacteria were $(1.6 \pm 2.3) \times 10^3$ CFU/mL and $(2.9 \pm 3.3) \times 10^3$ CFU/mL from the remaining sport-drink and orange juice immediately after drinking, respectively. In contrast, $(2.5 \pm 5.5) \times 10^4$ CFU/mL and $(5.8 \pm 2.4) \times 10^3$ CFU/mL were recovered at the mouth of the bottles of a sport drink and orange juice immediately after drinking, respectively. Little bacteria were recovered from the samples 24h after drinking.

Results 2: Bacterial Compositions

	Sport drink		Orange juice		Saliva (n=11)
	Drink (n=9)	Mouth (n=6)	Drink (n=8)	Mouth (n=8)	
Total	349	228	309	294	633
Anaerobes	44 12.6%	33 14.5%	59 12.9%	40 11.6%	118 18.6%
<i>Veillonella</i>	9 2.6%	14 6.1%	33 10.7%	26 8.8%	54 8.5%
<i>Prevotella</i>	9 2.6%	2 0.6%	2 0.7%	2 0.7%	29 4.6%
<i>Fusobacterium</i>	5 1.4%	1 0.3%	1 0.3%	7 1.1%	7 1.1%
<i>Megasphaera</i>	2 0.6%	1 0.2%	1 0.2%	1 0.2%	1 0.2%
<i>Porphyromonas</i>	1 0.3%	4 1.8%	2 0.7%	7 1.1%	7 1.1%
<i>Oribacterium</i>	1 0.3%				
<i>Selenomonas</i>	4 1.1%		1 0.3%		
<i>Leptotrichia</i>	1 0.3%				1 0.2%
<i>Peptostreptococcus</i>			2 0.6%		
<i>Solobacterium</i>		1 0.4%		2 0.7%	3 0.5%
<i>Eubacterium</i>			2 0.6%	1 0.3%	5 0.8%
<i>Propionibacterium</i>					7 1.1%
<i>Atopobium</i>					4 0.6%
<i>Finegoldia</i>					2 0.3%

Aerotolerant anaerobes

	Drink (n=9)	Mouth (n=6)	Drink (n=8)	Mouth (n=8)	Saliva (n=11)
<i>Cutibacterium</i>	12 3.4%	14 6.1%	19 6.1%	5 1.7%	1 0.2%
Facultative anaerobes	290 83.1%	182 79.8%	233 75.4%	231 77.9%	475 75.0%
<i>Streptococcus</i>	195 55.9%	87 38.2%	107 34.6%	106 36.1%	306 48.3%
<i>Actinomyces</i>	36 10.3%	53 23.2%	83 26.9%	70 23.8%	30 4.7%
<i>Neisseria</i>	22 6.3%	28 12.3%	12 3.9%	6 2.0%	24 3.8%
<i>Rothia</i>	18 5.2%	9 2.9%	16 5.4%	19 3.0%	19 3.0%
<i>Schaalia</i>	7 2.0%	6 2.6%	6 1.9%	10 3.4%	63 10.0%
<i>Gemella</i>	7 2.0%	4 1.8%	7 2.3%	6 2.0%	20 3.2%
<i>Staphylococcus</i>	3 0.9%	4 1.8%	6 1.9%	12 4.1%	6 0.9%
<i>Corynebacterium</i>			1 0.3%	3 1.0%	2 0.3%
<i>Capnocytophaga</i>			2 0.6%		5 0.8%
<i>Campylobacter</i>	2 0.6%				

- ✓ *Streptococcus* (55.9%), *Actinomyces* (10.3%), *Neisseria* (6.3%), *Rothia* (5.2%), *Veillonella* and *Prevotella* (5.4%) were predominant in the remaining Sport drink (n=9).
- ✓ *Streptococcus* (38.2%), *Actinomyces* (23.3%), *Neisseria* (12.3%) and *Veillonella* (6.1%) were predominant at the mouth of Sport drink (n=6).
- ✓ *Streptococcus* (34.6%), *Actinomyces* (26.9%), *Veillonella* (8.8%) and *Rothia* (5.4%) were predominant in the remaining Orange juice (n=8).
- ✓ *Streptococcus* (36.1%), *Actinomyces* (23.8%) and *Veillonella* (6.1%) were predominant at the mouth of Orange juice (n=8).
- ✓ From saliva, *Streptococcus* (48.3%), *Schaalia* (10.0%), *Actinomyces* (4.7%) and *Neisseria* (3.8%) were detected as predominant species (n=11).
- ✓ These results indicate that oral bacteria flow into the remaining drinks through the mouth of plastic bottles.

Discussion

- More than 10³ cells/mL of oral bacteria, including *Streptococcus*, *Actinomyces*, *Neisseria* and *Veillonella*, were found in the remaining beverages in the bottles immediately after drinking and at the mouth of plastic bottles.
- The bacterial levels decreased 100-fold after storage at 37°C for 24 h both in the remaining beverages and at the mouth of plastic bottles, likely due to the lower pH (less than pH 4) of sport drink and orange juice.
- The findings of the present study suggest that the remaining beverages (less than pH 4, such as sport drink and orange juice) may be preserved safely in a room temperature for a specified amount of time.