

# Human Bifidobacteria from Walnut Creek

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The coliform and *E. coli* counts on Des Moines' urban streams often are moderately high, and counts can soar following rain events. Studies show that Walnut Creek and Beaver Creek both significantly effect the bacterial counts of the rivers into which they flow: the Raccoon and Des Moines Rivers, respectively.

Because high *E. coli* numbers indicate probable fecal contamination, it is desirable to characterize fecal sources when the condition threatens a drinking water facility.

Differentiating between human and animal sewage is instructive because human waste is more likely to cause disease. Animal sources include cattle, hogs, cats, dogs, and wild animals such as birds, rats, deer, and urban raccoons.

This study's purpose was to determine whether the fecal influence on Walnut Creek included human waste. The study was conducted two days following a moderate rainfall. Stream flow had returned to normal. This study indicates water table horizontal drainage is a more significant contributor to *E. coli* than is surface runoff.

*Bifidobacterium* species are common inhabitants of human and animal intestines and waste. They ferment many different carbohydrates. The carbohydrate sorbitol is used primarily in human food and therefore bifidobacteria in human intestines adapt enzymes to ferment it. Animals are seldom given foods with sorbitol, and therefore their intestinal *Bifidobacterium* species do not develop sorbitol-fermenting abilities.

Recovery of bifidobacteria that ferment sorbitol from waterways strongly implies the presence of human waste. The fewer sorbitol-fermenting bifidobacteria that are isolated, the less likely that a significant amount of animal waste is present.

## **METHOD:**

Seven Walnut Creek sites were sampled (map sites: 1, 2, 3, 4, 6, 7, and 10) in addition to two Raccoon River sites upstream and downstream from the confluence with Walnut Creek (sites RRA and RRB respectively).

Each sample was processed by membrane filtration. The filter was placed onto HBSA (Human Bifidobacterium Sorbitol Agar) and incubated anaerobically at 35 °C for 48 hours.



The culture plate was then examined for yellow colonies, which were subcultured to sheep blood agar. Anaerobic incubation was once again performed for 24 hours. Each subculture was studied for purity and gram-stained to determine microscopic morphology. Those colonies that were apparently of one species and that stained as thin gram positive non-spore-forming rods were once again subcultured to sheep blood agar in preparation for speciation.

Anaerobic biochemical strips (API 20A) containing twenty different substrates including sorbitol, were inoculated with suspensions of the chosen subcultured bacteria.

After another 24-hour incubation period, the biochemical strips were examined, and the species and sorbitol-fermenting capabilities were determined.

### Results:

Site WC1 had three sorbitol-fermenting *Bifidobacterium*, WC4 had three, WC6 had two, WC7 had seven, and WC10 had two. Sites RRA and RRB grew no *Bifidobacterium*. The viability of *Bifidobacterium* in the environment is likely limited, therefore it may have been missing from the Raccoon River samples because it did not survive the journey from sites upstream.

Site	Rural versus Urban	Sorbitol Fermenting Bifidobacteria isolated
WC1	Main channel Combined Rural & Urban	3
WC4	Urban	3
WC6	Main channel Combined Rural & Urban	2
WC7	Combined Urban & Living History Farms	7
WC10	Combined Urban & Rural most tributaries	2
RRA	Combined Above Walnut Creek	0
RRB	Combined Below Walnut Creek	0

### Discussion:

Cultures from all sites recovered sorbitol-fermenting bifidobacteria, with site WC7 having the most. Such results strongly suggest that *part* of the bacterial load that we experience from Walnut Creek comes from human sewage. There is apparently a general sewage infiltration into the ground, which is subsequently washed into the creek following rainfall. This study does not rule out animal fecal contribution.

A follow-up study was performed on 9/22/99 during a drought period. No sorbitol fermenting bifidobacteria were isolated, suggesting no contributions from leaking sanitary sewers or septic tanks.

