Actinomycetes in the Elbow River Basin, Alberta, Canada

BERYL ZAITLIN,1* SUSAN B. WATSON,2 JAMIESON DIXON3 AND DEBORAH STEEL4

1Department of Biological Science, University of Calgary, Calgary, Alberta T2N 1N4
2Aquatic Ecosystem Management Research Branch, National Water Research Institute, P.O. Box 5050, Burlington, Ontario L7R 4A6
3City of Calgary Waterworks and Wastewater, Laboratory Services, Watershed Protection, P.O. Box 2100, Stn. M., Calgary, Alberta T2P 2M5
4RR #1, Hubbards, Nova Scotia BOJ 1T0

Actinomycetes can produce significant amounts of the earthy-muddy odour compounds geosmin and 2-methylisoborneol (MIB). These filamentous bacteria are found in both terrestrial and aquatic environments, and are particularly abundant in soil. They can enter freshwater systems via terrestrial runoff and subsequently cause taste and odour outbreaks in drinking water. Since it is well known that actinomycete growth and odour production is modified strongly by environmental factors such as moisture and nutrient levels, we hypothesized that watershed and stream characteristics should influence the potential odour impact of soil runoff on surface water. In this study, 1) the relationship between actinomycete abundance and characteristics such as stream discharge, turbidity and Escherichia coli levels was investigated, and 2) actinomycetes from contrasting terrestrial sources were examined for differences in their geosmin and MIB production. Actinomycetes and stream characteristics were sampled from the Elbow River, an important drinking water source for the City of Calgary (Alberta, Canada), and three tributary streams. Actinomycetes from forested regions and agricultural land were tested for taste and odour compound production. Actinomycete levels in streams were found to correlate closely with E. coli levels and to a lesser extent with turbidity, suggesting that actinomycetes are particularly abundant in runoff from terrestrial sources with fecal contamination. Most of the 18 actinomycete isolates tested were able to produce geosmin and/or MIB regardless of their terrestrial sources, suggesting that taste and odour outbreaks due to actinomycetes may be more influenced by differences in abundance than differences in source.

Key words: actinomycetes, land use, runoff, geosmin, 2-methylisoborneol

Introduction

Actinomycetes are gram-positive filamentous bacteria that are abundant in soils (Goodfellow and Williams 1983), but are often found in freshwater (Cross 1981; Wnorowski 1992). Some actinomycete species may be resident in freshwater environments (Roach and Silvey 1958; Willoughby...
1974; Zaitlin et al. In press), but large numbers of these taxa also enter freshwater from land with soil runoff (Persson 1980; Niemi et al. 1982).

Actinomycetes from terrestrial environments and those isolated from aquatic environments are often capable of producing highly potent volatile odourous compounds, particularly geosmin (trans-1,10-dimethyl-trans-9-decalol) and MIB (2-methylisoborneol) (Gerber 1979; Izaguirre 1992; Juttner 1995; Stahl and Parkin 1996). These compounds are responsible for a significant number of taste and odour outbreaks in drinking water supplies (Izaguirre 1992; Juttner 1995; Sugiura and Nakano 2000). Cyanobacteria, which are also major geosmin/MIB producers, have usually been considered responsible for most of these outbreaks (Izaguirre 1992; Juttner 1995), but in some cases, actinomycetes have been implicated (Jensen et al. 1994; Sugiura and Nakano 2000).

Actinomycetes tend to be most abundant in soils that are warm, dry and slightly acidic (Goodfellow and Williams 1983). Their abundance and diversity also appears to be influenced by the levels and type of organic matter in agricultural soils (Weyman-Kaczmarkowa and Pedziwilk 1996) or by the composition and depth of forest soil (Davies and Williams 1970). In vitro culture work has shown that production of geosmin/MIB can vary considerably among actinomycete species and with growth conditions (Sivonen 1982; Aoyama 1990).

In surface waters, actinomycetes often are found associated with sediment (Johnston and Cross 1976; Cross 1981). However, the type of soil or land use that contributes significant numbers of actinomycetes to streams has not been examined. This research focused on the relationship of actinomycete abundance in freshwater streams to major stream and watershed characteristics. The hypothesis was that the numbers of actinomycetes found in these streams would be correlated with turbidity, streamflow, nutrients, and differences in watershed development. Actinomycetes from different land-use areas were also tested for their taste and odour compound production patterns. In order to test these ideas, 3 streams were selected, draining into the Elbow River (Alta., Canada) from catchments with: i) undisturbed forest, or ii) agricultural/pasture land with some urban development. The river downstream of the 3 stream inflows was sampled, and actinomycetes were isolated from agricultural and forest soil, and their in vitro odour production was characterized. These data were used to assess whether there is any relationship between the numbers and/or the odour production potential of actinomycetes entering streams and associated catchment basin characteristics, which clearly has significant implications for surface water odour levels.

**Materials and Methods**

**Sampling Sites**

The Elbow River is a major source of drinking water supplies for the City of Calgary, serving the water needs of approximately half the current
population of over 900,000. The Elbow River originates at Elbow Lake in the Front Range of the Canadian Rocky Mountains of southwestern Alberta (50°37'20"N; 115°00'15"W), draining a watershed of 1220 km² (Fig. 1). The river extends from a largely forested headwater region in Kananaskis Country through alpine, sub-alpine, boreal foothills, and aspen parkland ecoregions, to a predominantly agricultural mid-region of improved pasture with dispersed cattle grazing and accompanying forage crop production from the hamlet of Bragg Creek to the City of Calgary corporate limits, and thereafter through the city under the influence of the urban environment. In the southwest quadrant of the city, the river is impounded to form the Glenmore Reservoir from which the Glenmore Water Treatment Plant receives its drinking water supply. Several kilometres downstream, the Elbow River joins the Bow River.

Dominant vegetation in the upper Elbow River drainage basin includes trembling aspen (Populus tremuloides Michx.), balsam poplar (P. balsamifera L.), lodgepole pine (Pinus contorta Loud.) and white spruce (Picea glauca Moench). Soils in the Elbow watershed are primarily black chernozemics, orthic gray luvisols, eutric brunisols, and coarse loam overlying glaciofluvial gravels (Mitchell and Prepas 1990).

Land use in the upper Elbow watershed in Kananaskis Country is centred primarily on recreation, including camping, hiking, mountain

Fig. 1. The Elbow River watershed of southwestern Alberta, Canada, showing the location of the mainstem river site (Weaselhead) and three tributary sites (Prairie Creek, Pirmez Creek, and Springbank Creek).
biking, equestrian, and some limited off-road vehicle activity. Logging, oil and gas production, and cattle grazing leases are also present. The hamlet of Bragg Creek is the only municipality in the Elbow River watershed upstream of Calgary; however, country residential estate and acreage lot development is increasing throughout the area. Within the City of Calgary, two storm sewer outfalls draining urban residential catchments flow to the Elbow River above the Weaselhead site; several others drain directly to the Glenmore Reservoir.

One mainstem site and three tributary sites of the Elbow River were selected for this study. The mainstem site is located in the Weaselhead Natural Environment Area of North Glenmore Park, immediately upstream of Glenmore Reservoir (50°59′31.1″N; 114°08′51.7″W). Prairie Creek, the uppermost of the tributary sites, drains a small and largely forested watershed of 43 km². Prairie Creek was sampled near the mouth at 50°52′00.9″N; 114°47′20.3″W. Pirmez Creek, the smallest of the sampled tributary watersheds at 2.5 km², arises from a small spring in a ranch yard, and flows through primarily pasture land, the grounds of a private residential retreat, and a forested zone near the confluence with the Elbow River. Pirmez Creek was sampled at about mid-reach at 51°02′26.0″N; 114°25′11.6″W. Springbank Creek drains a moderately sized watershed of 32 km² of mixed agricultural, country residential estate developments, and the small estate/acreage community of Springbank. Springbank Creek was sampled near the mouth at 51°02′06.9″N; 114°19′12.5″W. Sampling for all sites was done during a period of no detectable odours.

In the second phase of the work, actinomycetes selected for geosmin/MIB analysis were taken from forest soil in the Kananaskis region, and from agricultural soils in south-central Alberta, at the Lacombe Research Station, Lacombe, Alberta.

**Water Sampling and Analysis**

Samples were taken at weekly intervals during June and July in 1999. At each site, subsurface water samples were taken from the centre of the stream at mid-depth by direct grab, returned within 2 h to the laboratory and stored at 4°C prior to analysis. Samples for chemical analysis were collected by filling clean, pre-rinsed 1-L polyethylene bottles. Samples for bacterial analysis were taken by filling sterile 250-mL polyethylene bacteriological bottles. Stream temperatures were measured on site using a digital thermometer.

Stream discharge (Q) was calculated for the tributaries by measuring stream velocity, v, (Global Flow Probe), and depth, d, at set intervals across the stream width, w, as the product of $v \cdot d \cdot w$ (in m³/s). Elbow River discharge at the Weaselhead was estimated as mean daily Glenmore Reservoir inflows based on a reservoir stage-discharge relationship developed for the dam. The calculation considers reservoir level, plant raw intake pump rates, and monitored downstream flows (City of Calgary Waterworks, unpublished).
Turbidity, total phosphorus (TP), sulphate (SO$_4$), nitrate (NO$_3$), and nitrite (NO$_2$) were measured in the laboratory according to Standard Methods (APHA 1998). Turbidity was measured using a Hach 2100N® turbidimeter. TP was analyzed after persulphate digestion using the automated stannous chloride method with a Technicon AAII® continuous flow analyzer. SO$_4$, NO$_3$ and NO$_2$ were measured by ion chromatography using a Dionex DX-120 Ion Chromatograph®.

Total coliform and *E. coli* were measured using a Colilert® Quantitray 2000® system (Edberg et al. 1991; IDEXX Laboratories Inc.), enumerated using the MPN (Most Probable Number) method (APHA 1998).

For actinomycete determination, three replicate bottles were shaken vigorously, and then 0.1 mL per bottle was plated on chitin agar with cycloheximide (0.005 g L$^{-1}$), a medium semiselective for Streptomycetes (Hsu and Lockwood 1975). Plates were incubated under ambient laboratory conditions (cf. 23°C) and counted after 10 days to allow for development of slow-growing forms.

**Terrestrial Sampling**

Samples were taken from the F, H, and A horizons at five locations in the Kananaskis region in July 2001 and from four fields used for barley and other grain production at the Lacombe Research Station, Lacombe, Alberta, on 8 May 1998. Isolations were made by diluting 1 g of soil in sterile distilled water to 0.1 mg L$^{-1}$ concentration, and plating the diluted soil on chitin agar amended with cycloheximide. Actinomycete colonies were serially plated on Czapek’s agar (CZ) and nutrient agar until free of contamination. The ten most common actinomycetes from the Kananaskis region (D. Jayasingh, University of Calgary, Calgary, Alta., pers. comm.) and common actinomycetes from Lacombe were selected for further study. Plates were incubated under ambient laboratory conditions (~23°C).

Eighteen of these terrestrial actinomycete isolates were tested for geosmin/MIB production using a headspace microextraction GC-MS protocol (HSPME/GC-MS; Watson et al. 2000) that has a detection limit of 2 ng L$^{-1}$, which is below the human threshold odour concentrations of 10 to 30 ng L$^{-1}$ for geosmin and MIB (cf. Young et al. 1996). The routine replicability of the measurements was ± ca. 10 to 12%. At 14 days growth, plates were sampled by removing approximately 1 cm$^2$ of agar with the associated biomass, which was then added to 25 mL distilled water in a septum-capped glass vial containing a stir bar and 6 g of pre-baked NaCl. Biphenyl-D$_{10}$ was added at 200 ng L$^{-1}$ as an internal standard. The vials were sealed with a septum screw cap, stirred and extracted for 30 min using a polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 µm) SPME fibre (Supelco; Sigma-Aldrich Canada), inserted through the septum and extended into the headspace. Samples were measured using a Hewlett Packard 6890 GC coupled to a Hewlett Packard 5972® mass selective detector. GC-MS analyses were carried out after 1 min desorption in splitless mode. Samples were run in full scan mode and geosmin and MIB
were identified and quantified using retention times and mass spectra derived from analytical standards (Sigma-Aldrich Canada).

**Data Analysis**

Total actinomycete, total coliform and *E. coli* counts, turbidity, flow rate, temperature, total phosphorus, sulphate, and nitrate were analyzed for linear relationships by scatterplot analysis, then a simple regression was done to determine if actinomycete counts could be predicted by the other parameters. Analysis was done using the SPSS software package (SPSS 11.0, SPSS Inc., Chicago, Ill.).

**Results**

Over the duration of the experiment, the Elbow River had the highest and lowest single-day actinomycete counts, with a mean count of 256 colony-forming units (CFU mL⁻¹) on 5 July and a count of zero on 7 June. Overall maxima, minima and average abundances are shown in Table 1. Lognormal transformed actinomycete colony counts closely tracked ln *E. coli* counts (Fig. 2) and total coliform counts (figure not shown). Regression analysis indicated highly significant correlations between lognormal transformed levels of actinomycete and *E. coli* (n = 15, P < 0.001), and between lognormal transformed actinomycete and total coliform counts (n = 15; P = 0.005). Significant correlations were found between lognormal transformed actinomycete counts and turbidity (n = 14, P = 0.1). No significant correlations were found between actinomycetes and all other measured parameters (stream discharge, temperature, TP, SO₄, NO₃, and NO₂). Taken individually, there was a negative correlation between lognormal transformed actinomycete counts and turbidity in Pirmez creek (n = 3, P = 0.007), but not in the Elbow River (n = 3, P = 0.145). Prairie Creek and Springbank Creek could not be analyzed due

<table>
<thead>
<tr>
<th>Site</th>
<th>Average actinomycete counts in CFU/mL</th>
<th>Highest mean actinomycete count in CFU/mL (date)</th>
<th>Lowest mean actinomycete count in CFU/mL (date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prairie Creek</td>
<td>48.4</td>
<td>137 (5 July 1999)</td>
<td>10 (7 June 1999)</td>
</tr>
<tr>
<td>Pirmez Creek</td>
<td>4.5</td>
<td>37 (5 June 1999)</td>
<td>0.3 (7 June 1999)</td>
</tr>
<tr>
<td>Springbank Creek</td>
<td>84.1</td>
<td>230 (5 July 1999)</td>
<td>13 (21 June 1999)</td>
</tr>
<tr>
<td>Elbow River</td>
<td>84.1</td>
<td>257 (5 July 1999)</td>
<td>0 (7 June 1999)</td>
</tr>
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to the small sample size. Increased turbidity in all cases during the study was due to precipitation and subsequent runoff. Actinomycete counts, *E. coli* counts, total coliform counts and TP peaked or were elevated in Springbank Creek, Pirmez Creek and the Elbow River on 5 or 6 July, but only actinomycete counts peaked on this date in Prairie Creek (Table 2).

There were no significant differences between overall actinomycete totals at any of the stream sites; however, the 5/6 July sampling date had significantly more actinomycetes (as lognormal transformed data) than the other three sampling dates (7–8 June, 21–22 June, 19–20 July). Other stream characteristics also did not differ significantly between the sites, except the Elbow River site had significantly higher SO₄ levels (n = 15, P = 0.016).

The majority of the 18 forest and agricultural soil actinomycete isolates tested produced geosmin and/or MIB (Fig. 3) but there were no differences in odour patterns between actinomycetes from any of the different soil types.

**Discussion**

In this study, the pristine creeks Prairie Creek and Pirmez Creek both had low levels of *E. coli* and lower levels of actinomycetes than Springbank Creek and the Elbow River. However, actinomycetes decreased with increasing turbidity in Pirmez Creek, suggesting they were diluted out with increased water input in this pristine creek. There was a large peak in actinomycete levels that correlated with turbidity but not *E. coli* levels in
Table 2. Actinomycetes, total coliform, Escherichia coli, and stream parameters for three tributaries of the Elbow River (Pirmez Creek, Prairie Creek and Springbank Creek), and the Elbow River at the Weaselhead Natural Environment Area

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Actinomycete total count (CFU/0.1 mL)</th>
<th>E. coli (CFU/100 mL)</th>
<th>Total coliform (CFU/100 mL)</th>
<th>Stream discharge (m³/s)</th>
<th>Temperature (°C)</th>
<th>Turbidity (mg/L)</th>
<th>TP (mg/L)</th>
<th>SO₄ (mg/L)</th>
<th>NO₃ (mg/L)</th>
<th>NO₂ (mg/L)</th>
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<td>6/7</td>
<td>3</td>
<td>20</td>
<td>613</td>
<td>0.43</td>
<td>4.7</td>
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<td>10.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>6/21</td>
<td>3</td>
<td>17</td>
<td>649</td>
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<td></td>
<td>0.002</td>
<td>8.41</td>
<td>0.229</td>
<td>0</td>
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<td>422</td>
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<td>7/19</td>
<td>11</td>
<td>31</td>
<td>613</td>
<td>1.84</td>
<td>7.8</td>
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<td>0.006</td>
<td>8.36</td>
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<td>PC</td>
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<td>1</td>
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<td>25</td>
<td>0.28</td>
<td>6.5</td>
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<td>0.93</td>
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<td>4</td>
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<td>7.9</td>
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<td>0.002</td>
<td>0.94</td>
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<td>0</td>
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<tr>
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<td>7/6</td>
<td>11</td>
<td>34</td>
<td>345</td>
<td>9.63</td>
<td>5.2</td>
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<tr>
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<td>261</td>
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<td>5.6</td>
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<td>0.023</td>
<td>0.84</td>
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<td>980</td>
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<tr>
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<td>517</td>
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<td>172</td>
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PRC; Prairie Creek.
PC; Pirmez Creek.
SC; Springbank Creek.
ER; Elbow River.
July in Prairie Creek. Prairie Creek contrasted to Springbank Creek, which drains a watershed of equivalent size. In Springbank Creek, *E. coli* and actinomycete levels had higher maxima at times of increased turbidity, and similar values to the other creeks at times of low turbidity. The Elbow River at Weaselhead, which receives inputs from the three creeks in this study, other creeks and two large storm sewers, also showed increased actinomycetes and *E. coli* levels at times of increased turbidity, and lower levels at times of low turbidity. This suggests that the actinomycetes were coming from similar areas as the *E. coli*, i.e., terrestrial sources associated with animal manure. This is similar to the findings of Al-Diwany and Cross (1978) who also reported a close correlation between levels of *Streptomyces*, *Rhodococcus coprophilus* and fecal streptococci in river water.

The majority of the actinomycetes isolated from both forest and agricultural soil were capable of producing geosmin and/or MIB, similar to actinomycetes isolated from sediment in the Lake Ontario basin (Zaitlin et al. In press) and actinomycetes isolated from Lake Kasumigaura, Japan (Sugiura and Nakano 2000). This suggests that if conditions were favorable for geosmin/MIB production and a heavy sediment load entered streams from forest or agricultural soil, significant taste and odor outbreaks could occur. The type of sediment entering the water could have a major effect on the magnitude of the odor outbreak. Odour is a function of the number of odor-producing cells and the amount of odor produced per cell. This in turn is a function of both the species producing the odor and the conditions under which the odor is produced. This research indicated that both forest- and agricultural-origin actinomycetes are capable of producing odour. Furthermore, as fecal coliform bacteria are found in low concentrations in soils that do not receive manure inputs (Faust 1982), the close correlation in this study between *E. coli* and actinomycete counts suggests that the primary source of actinomycetes in these streams is not forest soil but soil associated with animal manure.

The prevalence of actinomycetes in land used for animal pasture has never been studied. If odour-producing actinomycetes are highly prevalent in pasture soils, runoff from pasture soils may be more significant in...
taste and odour outbreaks than runoff from other areas. Clearly, this is a significant area of future investigation.

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IDEXX Laboratories Inc. One IDEXX Drive. Westbrook, Me. 04092.


Jayasingh D. University of Calgary, Calgary, Alta., pers. comm.


