SOME FUNGI OF THE CACHE LA POUDRE RIVER, COLORADO

by

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SAMPLE AREAS

The Cache la Poudre River originates in Poudre Lake (elevation of 10.725 feet) in the Rocky Mountains of Colorado, on the east side of the Continental Divide. The lake is part of Rocky Mountain National Park and has considerable recreational value because it is well-stocked with fish and is followed for its length by Trail Ridge Road (U.S. Highway 34), the only trans-mountain highway across the Park. From Poudre Lake, the Cache la Poudre River flows in a generally northern direction, draining a long valley that is relatively inaccessible. A tributary brings water from Barnes Meadow Reservoir to which water has been brought by a small canal from Chambers Lake at or near the head of the Laramie River. Further downstream a tunnel brings additional water to the Cache la Poudre River directly from the Laramie River. Camp grounds and occasional summer homes attest to man's use of these watersheds. At about a point called Spencer Heights (marked by a small grocery and supply shop) along Colorado State Route 14, the river turns northeastward and later almost due eastward through a canyon in which are found, as the topography permits, summer homes, camp grounds, group camps, and other recreational areas, as well as limited attempts at mountain farming; these increase as the River descends the canyon. The broad flood plain of the river supports increased farming as the river leaves the canyon and the mountains and starts its journey across the Great Plains of Colorado. Bellvue and Laporte are farm communities upstream from Fort Collins through which the Cache la Poudre River flows. Just below Bellvue

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the river receives water from Horsetooth Reservoir, a large lake to which water from the Big Thompson project has been brought from the Colorado Riverbasin through a series of storage dams and pumping stations and stored for irrigation usage. The lakes and streams in system also are used, sometimes heavily, for all types of recreation.

Downstream from Bellvue, the Cache la Poudre River receives a variety of effluents. Above Fort Collins these are relatively light since the communities are small, served by septic tanks or privies, and there is little industry except agriculture. At and below Fort Collins the stream receives the effluents of various industries, such as aggregate production and meat processing plants, and a municipal sewage treatment plant of low efficiency. Further downstream, in addition to runoff from fields and feed lots, the stream receives the effluents form a sugar refinery. The Cache la Poudre River joins the South Platte River just downstream from Greeley, Colorado.

Technique

In a survey of the biota of the Cache la Poudre River to determine the effects of polluting substances on the biota and the effects of the biota on the polluting sustances, Lowell Keup on Oct. 25, 1964 took a set of samples at five stations between Bellvue and Greeley and returned them to Cincinnati for processing. On July 22 and 23,

Table I

Fungus colonies in Cache la Poudre River samples

Station	Season	Location	Habitat			
			Water	Sediment	Bank Soil	Upland Soil
1	F	Poudre Lake				
	S		52,000	5,200	4,800	16,400
		Cache la				
2	F	Poudre River at outlet				
	S	of Poudre Lake	15,000	2,600	4,300	89,000
3	F	Spencer Heights				
	S		17,000	2,800	1,500	544,000
5	F	Below Fort Collins				
	S	water filtration plant	13,000	7,600	1,600	19,000
6	F	Bellvue	83,000	205,000	88,000	92,000
	S		16,000	123,000	2,900	9,400
7	F	Below Fort Collins	7,800	280,000	4,600	20,000
	S	sewage treatment plant	13,000	4,000	1,100	75,000
8	F	Near Windsor, above	1,200	2,500	45,000	25,000
	S	sugar plant	8,400	1,900	6,600	23,000
9	F	Near Bracewell, below	22,000	180,000	44,000	34,000
	S	sugar plant	9,600	3,700	2,600	22,000
10	F	Spanish Colony	15,000	180,000	11,000	34,000
	S		6,800	4,500	2,000	17,000

1965, Dr. L. W. DURRELL, Colorado State University, and the author obtained a second set of samples at these five stations, and took a first set from five stations (only four of which were processed) upstream from Bellvue (two sets at the Poudre Lake headwaters and two sets along the river at Spencer Heights and near the Ft. Collins water filtration intake.

Five types of material were sampled at each point. In the first set of samples taken by KEUP when the river was at about its lowest autumn flow, sampling at each station included water in the flowing stream, sediment in the stream bottom, bank soil at the interface of the water and the soil, floodplain soil subject to occasional overflow and upland soil subject to overflow of the stream only at highest flood stages. When the second set of samples was obtained, the river was swollen with summer rainwater at most points and only four types of samples were collected water, bottom sediment, stream-bank interface, and upland soil. Because of the higher water, the bottom sediment of the second sampling possibly corresponded with the stream-soil interface samples of the first, and the stream-soil interface samples of the second period may have corresponded with the floodplain samples of the first.

All samples collected were brought to the laboratory at Cincinnati for examination. Here, 15 ml of each sample was added to 135 ml distilled water and shaken at 150 oscillations per minute on the rotary shaker for approximately 30 minutes. Following this, samples containing soils were diluted 1:1000 by adding 5 ml of shaken sample to 45 ml distilled water for 1:100 dilution and repeating the process for the final dilution. Upland soils were diluted one step farther to 1:10,000. For all types of samples plate counts were corrected to number of colonies per gram dry weight of soil by oven drying, at 100° C, pairs of 15-ml samples of the undiluted sample, and correcting for dilutions and the average number of colonies obtained for five replicate plates poured from each sample. Pour plates for colony counts were prepared in neopeptone – dextrose – rose bengal – aureomycin agar. Some of the undiluted sample was plated with hemp seed in distilled water to determine whether true aquatic fungi were present. To recover yeasts, 1 ml of the 1:10 dilution of each sample was added to 50 ml yeast – nitrogen – base (YNB-Difco) to which, in one instance, 1 % dextrose was added, and in another 20 % dextrose. After 60 hours on the shaker the amount and type of growth were recorded and, if yeast-like organisms were present, a loopful of cells was streaked on yeast extract – malt extract – dextrose agar plates for rapid growth. Developing colonies were restreaked on the same medium and finally purified on Diamalt agar plates.

RESULTS

Colony counts on poured plates of samples collected in both fall (October) and summer (July) are given in Table I. In general, in

TABLE II.

Distribution of fungal species in Cache la Poudre River Basin

Species	Station and habitat								
	1	2	3	5	6	7	8	9	10
Penicillium lilacinum	WSB U	WSB U	WSB U	WSB U	WSB*U	WSB	WSB U	WSB U	W B U
Unidentified molds	WSB U	WSB U	SB U	WSB U	WSB*U	WSB*U	WSB*U	WSB*U	WSB U
Phialophora jeanselmei	WSB U	WSB U	S	S	WSB	WSB*U	WSB*U	WSB*U	WSB U
Cephalosporium ssp.	WSB	W B U	W	B	WSB	SB*U	WSB*U	WSB U	WSB U
Fusarium oxysporum	W	B U	B U	B U	SB*U	S U	B U	WSB*U	W U
Trichoderma viride	W B	U	U	WSB U	WSB*U	WSB*	SB*U	SB*U	WSB U
Penicillium spp. indet.	SB U	SB U	B U	WSB U	WSB*U	WSB*U	WSB U	WSB*U	WSB U
Unidentified yeasts	S	S U	W B	SB	WSB*U	WSB U	WSB U	WSB*U	WS U
Phoma ssp.	B	–	W B U	W B U	S U	W *	S	SB U	WS *U
Gliocladium roseum	W	–	B	U	B*U	*U	WSB*U	B*U	SB*U
Rhodotorula sp. indet.	U	B	–	B	S	U	W *U	WS U	S
Mucor hiemalis	B U	U	–	WSB U	SB	B	S U	B U	B U
Mucor ssp. indet.	SB U	B	–	WS U	SB*U	SB*U	SB*U	SB*U	WSB*U
Penicillium funiculosum	W	W	–	B	WSB U	B	B	SB	S
Aspergillus niger	U	S	–	B	S U	U	B	–	B U
Penicillium corymbiferum	SB U	S	–	S	–	*	U	–	WS U
Mucor alternans	S	B	–	S	B	WSB U	S	–	–
Fusarium roseum	U	U	–	–	W *	W	W B*U	W	S U
Epicoccum purpurascens	WS	W	WS	W	B*	–	–	–	W B*
Pestalotia heterocornis	B	–	U	U	B	–	–	–	–
Penicillium expansum	B	–	W	W	–	–	–	S	–
Penicillium rubrum	B	–	–	SB U	–	B	–	S	S
Trichoderma alba	S	–	–	W	B U	*	–	W U	–
Penicillium purpureogenum	B	–	–	S	–	–	–	–	–
Aspergillus ustus	U	–	–	–	S *U	–	B	W B	*
Alternaria tenuis	WS	–	–	–	*U	–	U	U	*
Penicillium nigricans	W	–	–	–	B	–	–	S	–
Memnoniella echinata	U	–	–	–	–	S	–	–	–
Cryptococcus luteolus	B	–	–	–	–	–	B	–	–
Actinomucor elegans	S	–	–	–	–	–	–	S	B*
Aphanomyces sp.	S U	–	–	–	–	–	–	B U	–

Cladosporium cladosporioides	–	W U	W	W U	W	W	WS U	W U	W U
Aspergillus fumigatus	–	W	–	S	S *	WS *U	WSB*	WSB	W B*U
Trichosporon spp. indet.	–	B	–	W –	S	S	W	–	W
Aspergillus flavus	–	B	B	–	–	S	W *	WS	W
Oidiodendron sp.	–	W	W	–	U	W	–	W B	W
Torulopsis famata	–	B	WSB	U	–	–	–	–	–
Penicillium implicatum	–	B	–	B	–	W	–	–	–
Beauvaria bassiana	–	B U	–	–	–	–	–	–	–
Sepedonium sp.	–	B	–	–	–	–	–	–	–
Verticillium sp.	–	B	–	–	–	–	–	–	–
Aspergillus versicolor	–	–	W	U	B	W *	B	W B	WS *
Rhodotorula mucilaginosa	–	–	U	B U	*U	W B	B	–	SB*U
Aspergillus ochraceus	–	–	W	–	S U	W	S	SB U	SB
Geotrichum candidum	–	–	B	–	B	WSB*	B*U	WS	SB
Aureobasidium pullulans	–	–	W	–	W	U	U	SB	–
Verticillium lateritium	–	–	W	–	–	S *	*	WSB*	W B*
Mucor plumbeus	–	–	U	WS	S	–	–	–	–
Achlya klebsiana	–	–	U	–	–	–	–	SB	–
Cryptococcus diffluens	–	–	B	–	–	–	–	–	–
Penicillium aurantiacum	–	–	U	–	–	–	–	–	–
Penicillium puberulum	–	–	SB	–	–	–	–	–	–
Rhizopus ssp.	–	–	–	S U	B U	B	SB	*U	*U
Candida krusei	–	–	–	B	–	W B	SB	WS	SB
Rhizopus arrhizus	–	–	–	S	–	–	U	SB U	SB U
Penicillium variabile	–	–	–	B	–	W	–	B U	S U
Rhizopus chinensis Sect.	–	–	–	S	–	U	–	–	S
Fusarium aquaeductuum	–	–	–	W	W	–	–	B	W
Penicillium simplicissimum	–	–	–	W	B	–	–	U	–
Stachybotrys atra	–	–	–	U	U	–	–	W B U	SB*
Paecilomyces marquandii	–	–	–	U	*	B	–	–	W U
Cunninghamella elegans	–	–	–	–	S U	W U	B	B	U
Rhodotorula glutinis	–	–	–	–	S	U	S U	WS	U
Fusarium ssp. indet.	–	–	–	–	SB U	W U	S *U	U	W B
Aspergillus wentii	–	–	–	–	*	–	B	S U	*
Paecilomyces varioti	–	–	–	–	B U	WSB	–	–	B
Rhodotorula texensis	–	–	–	–	SB	S	–	–	S
Penicillium oxalicum	–	–	–	–	S	WS	–	SB	–

TABLE II. (cont.)

Species	Station and habitat								
	1	2	3	5	6	7	8	9	10
Cryptococcus laurentii	–	–	–	–	*	–	B	–	S *
Penicillium janthinellum	–	–	–	–	S	–	–	–	WS U
Penicillium martensii	–	–	–	–	U	–	W B	W	–
Candida spp. indet.	–	–	–	–	U	–	–	S	–
Gliomastix convoluta	–	–	–	–	S	–	–	–	–
Myrothecium verrucaria	–	–	–	–	*	*U	–	–	–
Candida utilis	–	–	–	–	B	–	–	–	–
Penicillium javanicum	–	–	–	–	B	–	–	–	–
Penicillium sclerotiorum	–	–	–	–	S	–	–	–	–
Torula sp. indet.	–	–	–	–	*	–	–	–	–
Torulopsis aeria	–	–	–	–	B	–	–	–	–
Torulopsis inconspicua	–	–	–	–	B	–	–	–	–
Ulocladium atrum	–	–	–	–	*	–	–	–±	–
Aspergillus terreus	–	–	–	–	–	U	B	B	WSB U
Penicillium chrysogenum	–	–	–	–	–	*	U	W B	WSB U
Paecilomyces sp. indet.	–	–	–	–	–	*	*	S U	W
Monosporium apiospermum	–	–	–	–	–	S	B	–	U
Candida tropicalis	–	–	–	–	–	WS	–	–	U
Phoma herbarum	–	–	–	–	–	W	–	–	B*
Aspergillus spp. indet.	–	–	–	–	–	W	–	B	–
Trichosporon cutaneum	–	–	–	–	–	B	–	W	–
Zygorhynchus moelleri	–	–	–	–	–	U	S	–	–
Aspergillus chevalieri	–	–	–	–	–	S	–	–	–
Candida curvata	–	–	–	–	–	W	–	–	–
Cladosporium sphaerosporum	–	–	–	–	–	*	–	–	–
Doratomyces microsporus	–	–	–	–	–	B	B	–	–
Gymnoascus vinaceus	–	–	–	–	–	U	–	–	–
Papulospora sp.	–	–	–	–	–	U	–	–	–
Penicillium kapuscinskii	–	–	–	–	–	S	–	–	–
Penicillium velutinum	–	–	–	–	–	B	–	–	–
Phialophora fastigiata	–	–	–	–	–	*	–	–	–
Torulopsis versatilis	–	–	–	–	–	W B*	–	–	–
Scopulariopsis brevicaulis	–	–	–	–	–	S	–	–	–
Ulocladium tuberculatum	–	–	–	–	–	U	–	–	–

Fusarium solani	–	–	–	–	–	–	B U	SB*U	SB U
Stemphylium consortiale	–	–	–	–	–	–	U	B	WS *
Absidia glauca	–	–	–	–	–	–	S	W	–
Aspergillus flavipes	–	–	–	–	–	–	U	S	–
Candida parapsilosis	–	–	–	–	–	–	U	S	–
Aspergillus terreus v. *floccosus*	–	–	–	–	–	–	B	–	–
Doratomyces stemonitis	–	–	–	–	–	–	B	–	–
Penicillium canescens	–	–	–	–	–	–	U	–	–
Prototheca sp.	–	–	–	–	–	–	S	–	–
Curvularia geniculata	–	–	–	–	–	–	–	B	S
Torulopsis sp. indet.	–	–	–	–	–	–	–	S U	B
Aspergillus tamari	–	–	–	–	–	–	–	U	–
Cladosporium herbarum	–	–	–	–	–	–	–	U	–
Gliocladium catenulatum	–	–	–	–	–		–	S U	–
Penicillium ochrochloron	–	–	–	–	–	–	–	B U	–
Rhodotorula pennaeus	–	–	–	–	–	–	–	U	–
Scopulariopsis spp.	–	–	–	–	–	–	–	U	–
Trichosporon pullulans	–	–	–	–	–	–	–	U	–
Ascomycetes	–	–	–	–	–	–	–	–	WS
Aspergillus nidulans	–	–	–	–	–	–	–	–	*
Aspergillus sydowii	–	–	–	–	–	–	–	–	W
Candida intermedia	–	–	–	–	–	–	–	–	S
Curvularia lunata	–	–	–	–	–	–	–	–	*
Helminthosporium sp.	–	–	–	–	–	–	–	–	*
Phialocephala heterospora	–	–	–	–	–	–	–	–	S
Stilbella bulbicola	–	–	–	–	–	–	–	–	W
Xylaria sp.	–	–	–	–	–	–	–	–	W
Allomyces arbusculus	–	–	–	–	–	–	–	–	U

FOOT NOTE:

W = Water
S = Bottom sediment
B = Bank soil

* = Flood plain soil
U = Upland soil

For habitat location data see Table I.

TABLE III.

Number of species at each sampling station by habitat

HABITAT	STATION								
	1 Head of Poudre Lake	2 Poudre River below Lake Outlet	3 Spencer Heights	5 Below Water Filtration Plant Poudre Canyon	6 Bellvue	7 Fort Collins below sewage treatment plant	8 Windsor	9 Bracewell	10 Spanish Colony
Running Water in River	11	9	13	15	12	28	14	25	31
Sediment in bottom of stream	14	7	6	17	27	24	22	35	34
Soil from bank at interface of water	15	16	12	17	28	22	30	35	27
Upland Soil, from above usual high water level	13	12	11	18	24	23	27	25	31
(Flood plain soil)					20	20	14	11	20
Total species at each station	31	27	28	40	57	66	54	65	68

the five downstream locations, larger numbers of colonies appeared in the October than in the July samples. Two factors may account for this apparent discrepancy. First, July samples were obtained when the river was carrying heavy runoff from upstream. This serves as a scouring action, which removes sediments and sludges from the stream bottom and those portions of the inundated floodplain that were sampled. Second in the lower parts of the stream, the pollutants resulting from seasonal processing of such plant products as beet roots had not yet started in July, but was in full progress in October.

TABLE IV.

Occurrence of yeasts in Cache la Poudre River

Species	Oct.	July	Total number of stations
Candida curvata		*	1
intermedia	*		1
krusei	*	*	5
lambica	*	*	7
parapsilosis		*	2
tropicalis	*		2
utilis	*		1
ssp.		*	2
Cryptococcus diffluens		*	1
laurentii	*		3
luteolus	*	*	2
Prototheca sp.	*		1
Rhodotorula glutinis	*		5
mucilaginosa	*	*	6
pennaeus	*		1
texensis	*		3
ssp.	*	*	8
Torulopsis aeria		*	1
famata		*	3
inconspicua	*		1
versatilis	*		1
ssp.	*	*	2
Trichosporon cutaneum	*	*	2
pullulans	*		1
ssp.	*	*	4
Unidentified	*	*	9

The kinds of fungi isolated by the several techniques used are listed in Table II. This includes 128 species of filamentous soil fungi, water molds, and yeasts. The number of species isolated from each habitat in each location are summarized in Table III. The occurrence of yeast species is summarized in Table IV. Since some isolates picked from plates were not completely identified to species, partly because of lack of sporulation, loss of the culture before com-

pletion of the analysis, or insufficient growth details for diagnosis, a number of categories appear in Table II as "sp" or as "unidentified molds", etc. Including those incompletely identified or unidentified groups, eight species or species groups were present at all nine stations. While some species were common to each of the five habitats sampled at each station, many species were restricted in our present experience to one, two, or three of these habitats. Possibly, with more frequent and thorough sampling some of the gaps may be filled.

Note that in the four stations at higher elevation fewer species were recovered. This may have resulted from the fact that only one set of samples from these stations was processed, or that the apparent pollution levels were lower at each station except at station 1. Where more samples were studied, larger numbers of species were recovered; this may be attributed to some degree, however, to the presence of larger amounts of added organic matter or polluting substances.

The list in Table II may be presented in several ways. The yeasts that were isolated and identified are listed in Table IV; while yeasts occurred at every station, no single species has yet been found at all nine stations in this system. Without more sampling, it would be difficult to try to explain this. Some of the yeasts listed in Table IV are known to be associated with human health problems, but usually these are restricted to tropical regions. In general, these yeasts parallel those found in other streams that have been contaminated with sanitary sewage.

Such species of fungi as *Phialophora jeanselmei* (LANGERON) EMMONS, *Aspergillus fumigatus* FRESENIUS, *Geotrichum candidum* LINK ex PERSOON, and *Monosporium apiospermum* SACCARDO (*Allescheria boydii* SHEAR) are associated to greater or lesser extent with human disease. In the tropics, *Phialophora jeanselmei* causes a mycetoma in man, but it is not known to do this in temperate regions. *Allescheria boydii* is an etiological agent of Madura foot. Usually this disease is not contracted unless infested soil reaches the bone tissue of the lower extremities through a deep wound. *Aspergillus fumigatus* and *Geotrichum candidum* may cause lung disorders similar to pneumonia if large enough quantities of spores capable of causing aspergillosis or geotrichosis are inhaled. Few, if any, cases of disease are known to have been produced by these fungi in the region of the Cache la Poudre River basin. *Rhodotorula* species, species of other yeast genera, and *Aureobasidium pullulans* (DE BARY) ARNAUD, as well as other fungi, may on occasion enter wounds and cause a secondary infection or irritation, but these fungi are widespread and the same situation may be true for the populations of the cleanest water or soil areas.

A more important group of organisms in this list may be considered. These cause the disease of plants that grow in the soil and may contact the stream waters especially through irrigation. If spores

capable of causing disease in plants are present in irrigation water they may be added to those already present in the soil and thus increase the chance of producing an unhealthy crop. In some if not all cases, species of fungi capable of causing disease in plants are represented by strains that can produce severe disease problems; other strains cannot produce disease. Some of the species listed in Table II that can cause diseases include: *Fusarium oxysporum* SCHLECHTENDAHL, *F. solani* (MARTIN) APPEL & WOLLENWEBER, and *F. roseum* LINK, strains of all of which are capable of causing vascular wilt diseases or leaf spots on a variety of crop plants; a species of *Cephalosporium*, possibly represented here, which can cause severe disease of grasses and grains; species of *Rhizopus*, *Penicillium*, *Aspergillus*, *Aureobasidium* and other fungi listed that can cause severe problems in the storage and shipment of various kinds of produce; *Alternaria tenuis* NEES ss. lat., species of *Stemphylium* and *Curvularia*, and species of *Phoma*, which cause mild to serious infection in leaves and roots of some crop plants, or which may attack only the senescent leaves. Other fungi on the list cause damage of one sort or another to crop plants.

DISCUSSION

On the basis of preliminary, rather casual sampling of waters, river bed materials, and river bank soils, we found that in Colorado, as elsewhere, relatively large numbers of viable fungal cells can be recovered with the use of nutrient agars and solutions. Among the fungi recovered are species that can cause disease in man if conditions are favorable, and species that can cause disease in the plants of man's crops especially if pathogenic strains are present. Many of these organisms are present in our environment, as shown by their occurrence in locations not contaminated, or presumably not contaminated, by man's activities. When organic matter in the form of various wastes is added to these sites, these organisms use these wastes as food materials and their populations increase, resulting in larger inocula for downstream areas. When waters containing such spores are added to fields with susceptible crops, a potential hazard is created.

At present, to what extent the organic enrichment of the stream or adjacent soils causes an increase in potentially pathogenic fungus populations is not known. Here, reference has been made to organisms pathogenic for man, his livestock, and his crops or ornamental plants. As organic pollution increases, however, we may expect that some change will occur in the balances set up by nature that hold some of these organisms in check.

Summary

Samples of water, bottom sediment, bank soil, and upland soil from nine stations on the Cache la Poudre River, Colorado, from

Poudre Lake (elevation 10,752 feet) in Rocky Mountain National Park to a point near its confluence with the South Platte River near Greeley, Colorado, on the Great Plains were examined for fungal populations. A total of 128 species of fungi were recovered on four types of bait, agar, and liquid media. While human, animal, and plant pathogenic fungi were present, species found were of widespread occurrence and no serious involvement was noted. If streams such as this continue to receive organic enrichment, increasing loads of potentially pathogenic fungi possibly may be found.