Occurrence of Enterococci on Plants in a Wild Environment[1]

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Abstract

Mundt, J. Orvin (University of Tennessee, Knoxville). Occurrence of enterococci on plants in a wild environment. Appl. Microbiol. **11**:141–144. 1963.—Enterococci were obtained from 14% of nearly 2,200 flowers, 3.4% of nonfloral structures of angiosperms, and from 8.3% of samples of soil, water, and lesser plants of the Great Smoky Mountains National Park, an area little influenced by the presence of man. The enterococci were recovered from one or more flowers or flower clusters of 1,515 samples in 47 taxa, but not from flowers of 67 taxa with 654 samples. The per cent of recovery was influenced adversely by dense forest cover and by increase in elevation, as compared with recovery from flowers in sunny locations in the lower elevations. The per cent recovery increased directly with rising seasonal temperature, with the maximal per cent of recovery occurring in September. In no instance did all samples of a species of flower or plant yield enterococci on culture, and with only three genera, *Cacalia*, *Delphinium*, and *Mitchella*, were the bacteria obtained from more than 50% of the samples. Approximately 11% of the cultures isolated were identified as *Streptococcus faecalis*, 64% as the soft curd producing, caseolytic variant of *S. faecalis*, 4% as *S. faecalis* var. *zymogenes*, and 20% as *S. faecium*. The per cent distribution of these species on plants was reasonably similar to the distribution within wild animals in the same environment. It was concluded that the enterococci occurring on plants arise commonly from the wild animals, and that they do not represent plant-specific species or variants of the enterococci.

A study of the occurrence of enterococci on plants growing in an area not influenced greatly by man should reflect fairly accurately the nature of the bacterium-plant relationship. An epiphytic relation with selected agricultural plants has been suggested and demonstrated on the basis of distribution (Mundt, 1961) and reproduction of the bacterium when accompanying the developing plant structures emerging from inoculated seeds (Mundt, Coggin, and Johnson, 1962). Other studies, both published (Mundt, Johnson, and Khatchikian, 1958) and unpublished, indicate that the epiphytic relationship is the result of essentially an artificial environment arising from agricultural practice.

The Great Smoky Mountains National Park provides an area for study in which the influence of man is slight. Its features were described in the preceding paper (Mundt, 1963).

Materials and Methods

Excursions for the gathering of plant samples were made regularly from early spring through late fall. Sampling was done both at random throughout the park and at several sites selected to provide contrast between high and low elevations and type of forest cover. The low open area, at an elevation of approximately 2,000 feet above sea level, was farmland until approximately 35 years ago, but now is partially overgrown. The second area at slightly higher elevation was a densely forested glen with close overhanging forest cover, frequently damp, with thick undergrowth of shrubs and annual plants. The sampling sites at the high elevation were over 4,500 feet above sea level and usually densely overgrown with low trees, shrubs, and annual plants.

Single flowers, if sufficiently large, or clusters of smaller flowers (e.g., *Mitella* sp.) constituted single samples. Entire heads of most *Compositae* and of some *Leguminosae*, lengths of fern fronds (6 to 10 cm), 1 to 10 g of fungi, usually several moss plants with appended rootlike parts, entire shoots, single buds, whole leaves, single seed pods, or one large or several small seeds constituted single samples. Usually no more than ten samples of a species were taken from any site at one time.

Most samples were introduced directly into vials of Azide Dextrose (AD) Broth with forceps sterilized with hypochlorite solution. Very large flowers, some leaves, soil, and water were gathered in sterile 6-oz preserve jars or vials and returned to the laboratory for culture.

Upon overnight incubation at 37 C, transfers were made to tubes of AD Broth. From those tubes with growth upon incubation, streaks were made on Barnes' (1956) medium for recovery of enterococci. From two to five colonies per sample were selected. Thereafter, the procedure for maintaining and identifying cultures was that described elsewhere (Mundt, 1963).

During the study, 223 cultures were isolated from human stool samples provided by a local hospital. These cultures were used in comparative studies.

Results and Discussion

Flowers. Enterococci were obtained from 308 (14.2%) of 2,169 individual flower samples representing 116 taxonomic groups. The 19 groups most abundantly sampled

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and yielding enterococci on culture are listed in Table 1. In Tables 2 and 3 are summarized the number of taxa according to range of samples per taxon, the total number of samples represented, and the number and per cent of samples from which enterococci were obtained on culture.

Enterococci were recovered from one or more of 1,515 individual samples of 49 taxa but not from 654 individual samples of 67 taxa of flowering plants. Only three genera, *Cacalia*, *Delphinium*, and *Mitchella*, yielded an excess of 50% recovery of enterococci. Recovery from between 30% and 50% was obtained from flowers of only the following 11 genera: *Campanula*, *Commelina*, *Dianthus*, *Eupatorium urticaefolium*, *Kalmia*, *Labiatae*, *Lobelia*, *Monardia*, *Phlox*, *Senecio*, and *Trisetum*. In general, enterococci were recovered from those flowers which were larger or which had a long blossom life; there were, however, exceptions. No enterococci were obtained from 16 samples of *Calycanthus*, or from 23 samples of *Rhododendron*.

The effects of forest cover and elevation are summarized in Table 4. Recovery of enterococci was greatest (22.3%) from flowers grown in the open areas exposed to sunlight; this compares fairly well with the previous reports of 34.0% from flowers growing in inhabited areas, and 32.2% from flowers of broad-leaved agricultural plants (Mundt, 1961) taken at an elevation of approximately 1,000 feet above sea level. Dense forest cover resulted in a reduction in per cent of recovery to 14.5%, and an increase of 2,500 feet in elevation resulted in a further reduction to 9.5%.

Miscellaneous plant structures. Enterococci were recovered from samples of 11 (14.4%) of 76 genera or from 14 (3.4%) of 440 samples of leaves, buds, shoots, and fruits and seeds (Table 5).

Other sources. Enterococci were obtained from 2 of 9 water samples, from 1 of 15 surface soil samples, 6 of 90 water plants, 2 of 42 mosses and liverworts, 9 of 58 fungi, and 2 of 75 fern fronds, for a total of 24 (8.3%) recoveries from 289 samples (Table 6). Mosses, particularly, provide

TABLE 1. *Taxa of flowering plants bearing enterococci in which 20 or more flower samples were cultured*

Taxon	No. cultured	No. yielding enterococci	Per cent yielding enterococci
Angelica sp.	70	9	12.9
Compositae	380	95	25.0
Erigeron sp.	40	2	5.0
Eupatorium sp.	117	20	12.5
Hydrangia sp.	36	6	16.7
Kalmia sp.	20	7	35.0
Labiatae	38	7	38.9
Monardia sp.	41	15	36.7
Orchidaceae (three spp.)	36	2	5.6
Phlox sp.	34	16	44.6
Polygonatum and *Smilacina* spp.	51	5	9.8
Prunella spp.	34	9	26.4
Rubus sp.	42	3	7.2
Senecio sp.	23	9	39.2
Solidago sp.	52	8	15.4
Thalictrum sp.	23	1	4.4
Trifolium spp.	52	8	15.4
Trillium spp.	42	1	2.4
Viola spp.	92	7	7.6
Totals	1,223	230	
Per cent		18.8	

TABLE 2. *Sampling frequency per taxa, numbers of samples, and recovery of enterococci from flowers*

Sample range (no.)	No. of taxa	Total no. of samples	No. yielding enterococci	Per cent
1–5	4	19	7	36.8
6–10	19	177	41	23.1
11–15	5	63	28	44.5
16–19	2	33	2	6.6
Total	30	292	78	26.7

TABLE 3. *Sampling frequency per taxon and numbers of samples of flowers from which enterococci were not obtained*

No. of samples per taxon	No. of taxa	Total no. of samples
1–5	14	39
6–10	37	301
11–15	3	39
16–20	8	149
21–30	4	89
Over 30	1	37
Total	67	654

TABLE 4. *Occurrence of enterococci on flowers as influenced by exposure and elevation*

	Lower elevation		Higher elevation (4,500 ft)
	Sunny (2,000 ft)	Shade (2,000 ft)	
No. of species	13	17	17
Species bearing cocci	11	8	7
No. of samples	448	373	229
Enterococci present	100	53	29
Per cent	22.3	14.5	9.5

TABLE 5. *Recovery of enterococci from nonfloral structures of spermatophytes of the Great Smoky Mountains National Park*

Structure	No. of species		No. of individuals		
	Sampled	Enterococci present	Sampled	Enterococci present	Per cent with enterococci present
Leaves	31	2	84	2	2.4
Buds	8	2	53	2	5.7
Shoots	6	0	22	0	0
Fruits and seeds	31	7	281	10	3.6
Total	76	11	440	14	
Per cent		14.4		3.4	

a cross reference to the bacterial content of the soil, for these were cultured with the rootlike parts, and the bacteria of the water plants provide a reflection of the presence or absence of the enterococci in water as well. Except for bracket fungi (*Polyporus* and related genera), fungi usually were more or less mechanically ragged, presumably the result of feeding by animals.

TABLE 6. *Recovery of enterococci from water, water plants, lesser plants, and soil of the Great Smoky Mountains National Park*

Type of sample	No. of samplings	No. of individuals		
		Sampled	Yielding enterococci	Per cent
Water	3	9	2	22.2
Soil	6	15	1	6.7
Water plants	18	90	6	6.7
Mosses and liverworts	9	42	2	4.8
Fungi	14	58	9	15.5
Ferns	13	75	2	2.67
Total	63	289	24	
Per cent		22.1	8.3	

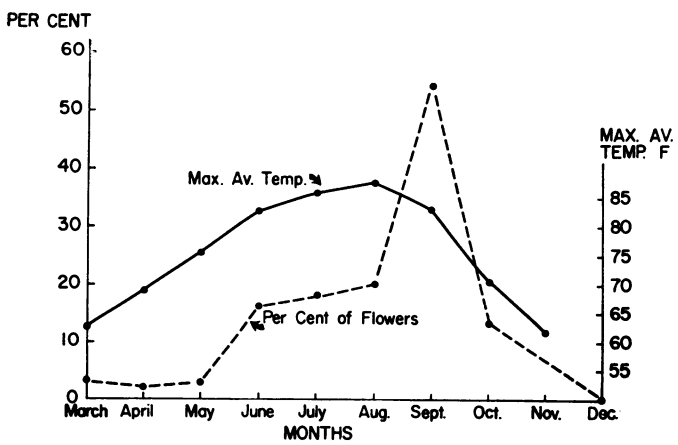

FIG. 1. *Per cent occurrence of enterococci on flowers per month correlated with the maximal average monthly temperature at Gatlinburg, Tenn.*

Seasonal distribution. Four stages in the per cent of occurrence of enterococci on flowers may be noted during the growing season (Fig. 1). From a very low per cent recovery into the month of May, there is some rise to mid-August, with abrupt rise into September, and as abrupt a decrease thereafter. The per cent recovery correlates fairly well with the maximal average temperature for these months.

Identity of enterococci. The identity of 585 cultures isolated from the plants of the Great Smoky Mountains National Park is shown in Table 7. Also, the frequency of occurrence of the major taxa was compared with the frequency in humans and in animals from the same locale (Table 7).

The soft curd producing, stratiform casein-digesting variant of *S. faecalis* occurred with the greatest frequency (81.2%) among those which reduced 2,3,5-triphenyl tetrazolium chloride strongly and which tolerated potassium tellurite. The frequency may be compared with 73% caseolytic strains isolated from frozen orange juice by Kaplan and Appleman (1952), and with 72% from plants in a general survey by Mundt and Johnson (1959). The frequency of this variant is comparable to frequency among animals (*Mammalia*, 64.1%, and *Reptilia*, 69.9%). This variant was not encountered among isolations from humans in this study, nor has it been observed in humans by Guthof or by Mieth (*personal communications*). Guthof (1957) stated that *S. faecalis* var. *liquefaciens* was present in the human in determinable numbers only if pathogenic or pathological conditions prevailed.

Of the strongly reducing cultures, 25 (5.4%) were hemolytic on human blood agar. *S. zymogenes* accounted for 5.3% of the isolations from *Mammalia*, 22.4% of the isolations from humans in this study, and 5.4% of the isolations from humans by Mieth (1961). Mieth's cultures were obtained from healthy humans, whereas in this work the cultures were obtained from stool samples of hospitalized humans.

This study presents the most conclusive evidence to date that, in general, enterococci may be chance contaminants on plants. The frequency of occurrence of

TABLE 7. *Identification of enterococci isolated from human, animal,* and plant sources*

Source	Strongly reducing								Nonreducing *S. faecium*	Other
	Total	Per cent	*S. faecalis*		Caseolytic variant		*S. zymogenes*			
			Total	Per cent	Total	Per cent	Total	Per cent		
Human	165	21.7	119	72.2	9†	5.4	37	22.4	58	0
Reptilia	53	7.0	12	22.6	37	69.9	4	7.5	8	4
Aves	6	0.8	2	33.3	3	50.0	1	16.7	2	1
Mammalia	75	9.8	23	30.6	48	64.1	4	5.3	89	13
Plants	462	60.7	62	10.6	375	81.2	25	5.4	116	7
Total	761		218		465		71		273	25

* *See* Mundt (1963).

† Acid proteolytic; see text.

S. faecalis, the caseolytic variant, *S. faecalis* var. *zymogenes*, and *S. faecium* among animals and on plants presents quite a similar picture.

In no instance, where adequate numbers of the same species of flower or plant structure were sampled, were enterococci invariably present. In some respects, this differs from the results of studies conducted with plants in agricultural environments. Here, thick stands of essentially single species of plants facilitate transfer of microorganisms from plant to plant by the elements. Some plants appear to be natural hosts to the enterococci (Mundt, 1961). A dearth of the wild animal population in the agricultural environment forces insects to be floriphagous, rather than to be both floriphagous and coprophagous, as is customary in a native environment.

The greater per cent of recovery of enterococci from flowers at lower elevations, from sunny locations, and with the advance of the season are compatible with a suggested relationship to animal life and its seasonal increase, and with the greater numbers and activity of insects. Occurrence of the enterococci in or on insects is as sporadic as it is on plants and to some extent at least is dependent upon the presence of enterococci in the environment (Eaves and Mundt, 1960).

Earlier studies (Sherman, 1937; Mundt and Johnson, 1959) suggested plant-specific variants of enterococci, because of the failure to isolate *S. faecalis* var. *zymogenes*. If a plant-specific variant were to occur, much greater uniformity among the isolates could have been expected. The recovery of this organism from plants growing in a wild environment, but failure to do so from plants growing in an agricultural environment, suggests an artificial selection when wild animal life is suppressed by agriculture.

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