Discussion

Frequent references are found in the literature emphasizing the synergistic or antagonistic effect of a particular substance upon the biological activity of other, chemically unrelated compounds. In the case of the germicides, it has been shown by Domagk (1935), Baker, Harrison, and Miller (1941), Valko and DuBois (1944), and many others that cationic and anionic detergents will neutralize each other. Such reversal of the activity of quaternary ammonium compounds have been demonstrated with soaps, sodium taurocholate, sodium lauryl sulfate, suramin sodium and many other anionic agents. However, it should be emphasized that these observations are based upon experiments which utilized, as test organisms, only bacterial species. In view of our own findings—that an enhancement of antifungal activity occurs with mixtures of both classes of compounds—it becomes clearly evident that one cannot speak in general terms of synergism or antagonism without at least specifically defining the nature of the test organism.

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Occurrence of Enterococci: Bud, Blossom, and Soil Studies[1]

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Abstract

Mundt, J. Orvin (University of Tennessee, Knoxville). Occurrence of enterococci: bud, blossom, and soil studies. Appl. Microbiol. **9**:541–544. 1961.—The occurrence of enterococci (group D streptococci) on buds and flowers of plants and in soils has been studied. They were recovered from 27.5% of the flowers of seven species of plants, and from 6.8% of the buds of the same plants. They were recovered from 34% of the flowers of nonagricultural plants, from 32.2% of the flowers of ten species of agricultural dicotyledonous plants, and from 10.4% of the flowers of five species of grasses and cereals. The enterococci were invariably present or invariably absent from all samples taken from very few species. They occurred in small numbers on enclosed tassels and silks of corn of 22 of 60 samples, and in greater numbers on 90% or more of these after their floral parts had emerged. Interposition of a mechanical barrier reduced the incidence of recovery from flowers. The occurrence in soil, generally at a low level of population, may be correlated with occurrence on the plant growing on the soil or with nearby enterococcal-bearing plants.

It is concluded that enterococci may be regarded as temporary residents on plants, capable of limited reproduction, and that they are disseminated among plants by the action of insects and wind, and spread to the ground by these agencies, gravity, and rain.

Little is known about the relationship of enterococci (group D streptococci) to plants and to soil. They occur commonly on plants, as reported by Sherman (1937) and by Mundt, Johnson, and Khatchikian (1958). At times they are dominant organisms in the

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natural fermentation of orchard grass and alfalfa silage (Langston and Bouma, 1960). Randall (1956) and Medrek and Litsky (1960) isolated them in small numbers from all soils examined.

Shapiro and Holder (1960) did not include enterococci among the isolates, many of which are presumed to be epiphytic, from salad greens. Burri (1903) and Dueggeli (1904) defined a bacterial plant epiphyte as one which accompanies all parts of the growing plant. Voznyakovskaya and Khudykov (1960) suggested that to be considered an epiphyte, a bacterium should compose at least 10% of the natural flora isolated from the plant. It is known that enterococci do not survive long in soil (Mallmann and Litsky, 1951; Guthof, 1959); however, Dueggeli did not consider presence in the soil to be an essential property of an epiphyte. They should be recovered from few, if any, samples of plants of species upon which they are not epiphytic. This approach is used in an attempt to clarify the relationship of these bacteria to plants.

Material and Methods

Small buds and blossoms were excised beneath the calyx and introduced intact into tubes of azide-dextrose (AD) broth. The inner floral structure of buds of *Hibiscus syriacus* (althea), *Nicotiana* (tobacco), and *Abelmoshus* (okra) were taken for culture after removal of the calyx; stamens and pistils only of the opened flowers of these species were taken.

Corn plants containing enclosed tassels and silks were removed to the laboratory. Emerged structures were excised and placed into sterile containers. Glassine bags were slipped over emergent ear buds of corn, and over buds of *Hibiscus*. Samples of soil were transferred from the root zones into sterile containers in a manner to include both surface and subsurface soil.

Weighed portions of tassels and silks of corn were disintegrated with 9 volumes of water in a blender. Decimal dilutions were introduced into tubes of AD broth for estimation of population of enterococci by most probable numbers, and to plates for estimation of viable aerobic counts. Separate portions were reduced overnight to oven-dry weight at 100 C.

From all tubes of AD broth showing growth, transfers were made into ethyl violet broth. AD agar was streaked from tubes with typical growth for recovery of colonies. Selected colonies were taken for study of identity.

Results

Corn flowers. Enterococci were recovered from 9 of 32 samples of enclosed tassels, and from 13 of 28 enclosed silks (Table 1). They also were recovered from 34 of 38 emerged tassels taken during antheresis, and from 27 of 28 emerged, receptive, and postreceptive silks. The difference between occurrence on enclosed and emerged on floral parts is significant at the 1% level as determined by the *chi*-square test. Levels of enterococcal populations were relatively low on enclosed structures, up to 10,000 per g when present, but were much greater (to 300,000 per g) on emerged tassels and silks. In comparison, nutrient agar counts indicated viable aerobic populations as great as 8,000,000 bacteria per g from enclosed tassel, 430,000 per g from enclosed silk, and as many as 20,000,000 per g from emerged floral parts. All counts are expressed on the basis of dry weight of plant material.

Sixteen of 23 samples of pollen gathered during antheresis, were free of enterococci. Anthers fell into

TABLE 1. *Recovery of enterococci from flowers of corn*

Flower part	No. sampled	Enterococci recovered			Nutrient plate	
		No. of samples	Per cent	MPN* maximum	No. plated	Range of counts per g × 10^{-3}
Tassel, enclosed	32	9	28.1	10,000	9	9–8,000
Tassel, in pollen	38	34	89.6	300,000	1	20,000
Pollen grains	23	7	30.4		1	5
Silk, enclosed	28	13	46.7	170	2	20–430
Silk, emerged	23	22	95.6	>100,000	2	5,000
Silk, bagged	14	10	71.4	100,000	6	2–5,000

* MPN = Most probable numbers, on dry weight per gram.

TABLE 2. *Recovery of enterococci from corn by half-monthly time periods*

Half-month period	No. of samples	No. containing enterococci	Per cent containing enterococci
July 1–15	33	22	66.7
July 16–31	26	17	65.6
Aug. 1–15	21	12	57.2
Aug. 16–31	26	19	73.2
Sept. 1–15	14	9	64.3
Totals	120	79	
Per cent			65.8

TABLE 3. *Recovery of enterococci from buds and blossoms of 7 genera of plants*

Genus of plant	No. of structures sampled	Samples containing enterococci	Per cent	No. of structures sampled	Samples containing enterococci	Per cent
Phaseolus sp	187	11	5.8	298	52	17.4
Abelmoschus sp	44	6	13.6	108	35	32.4
Nicotiana sp	13	0	0.0	53	17	32.1
Hibiscus syriacus	23	2	8.7	31	25	80.6
Aesclepias sp	5	0	0.0	10	3	30.0
Rubus niger	3	0	0.0	20	8	40.0
Lonicera semperviridans	4	0	0.0	6	5	83.3
Totals	279	19		526	145	
Per cent of total			6.8			27.5

the collecting vessels frequently, however, and these may have been the source of enterococci in the 7 positive samples.

Bagged flowers. Despite bagging, insects had free access to emergent silks, although mechanical contamination from anthers was prevented. The incidence of 71.4% recovery from bagged silks is compared with recovery from 95.6% exposed silks. None of eight *Hibiscus* flowers developing in glassine bags yielded enterococci, whereas they were recovered from 80.6% of unprotected flowers (Table 3). Many bagged buds of *Hibiscus* failed to develop.

Distribution on corn during the growing season. The percentage of recovery from corn flowers during half-month intervals is shown in Table 2. Except for greater frequency during one time period, the second half of August, the percentage of incidence is relatively constant.

Buds and flowers of other species. Enterococci were recovered from 19 (6.8%) of 279 buds, and from 145 (27.5%) of opened flowers of seven species of plants, as shown in Table 3. The difference is significant at the 1% level, as shown by the *chi*-square test. They were present on some blossoms of all species studied, but absent from the buds of four species. The 20 blossoms of *Rubus niger* were taken from a cultivated area; an additional 38 blossoms taken from a forest area failed to yield enterococci on culture. Although enterococci were present in significant percentages on the blossoms of all species examined, they were not invariably present on all samples of a species.

Miscellaneous flowers. Numbers of blossoms cultured and percentage recovery of enterococci occurring on three groups of plants are shown in Tables 4 and 5. All plants were growing in inhabited or agricultural areas. Enterococci were absent from two species, *Berbera* and button clover. Enterococci were obtained from all samples of only one species, *Prunus*. The percentage of occurrence on flowers of all other species lies at some intermediate value. Results with two species indicate the influence of pollution. Twenty-five flowers of *Ligustrum* and *Lonicera* growing near a wet septic field yielded enterococci, whereas only 12 of 30 samples of these plants taken from a remote area yielded enterococci on culture.

The percentage of occurrence of enterococci on cultivated agricultural plants is at some intermediate value for all except the tomato. Eaves and Mundt (1960) have commented that enterococci could be recovered from insects taken in corn fields, but not from the same insects taken in tomato fields.

The low incidence of recovery from cereals and

TABLE 4. *Recovery of enterococci from flowers of nonagricultural plants*

Genus of plant	No. of samples	Per cent with enterococci
Ilex cornuta (Holly)	25	32.0
Malus sp. (flowering crab)	25	12.0
Japonica sp. (flowering quince)	25	52.0
Prunus sp. (sour cherry)	25	100.0
Cercis sp. (red bud)	25	20.0
Spirea sp.	25	28.0
Berbera sp. (Juliana barberry)	50	0.0
Hemerocallis sp. (day lily)	17	5.8
Clover, dutch	10	90.0
Clover, button	10	0.0
Weed flowers, miscellaneous	43	34.8
Aesclepias sp.	10	30.0
Ligustrum sp.	10	60.0
Lonicera sp. (honeysuckle)	20	30.0
Chrysanthemum leucanthemum	15	86.6
Total number of samples	335	
Average per cent recovery		34.0

TABLE 5. *Recovery of enterococci from flowers of agricultural plants*

Genus of plant	No. of samples	Per cent with enterococci
Cucumeris sp. (cucumber)	11	45.4
Gossypium sp. (cotton)	16	81.2
Phaseolus sp. (lima bean)	40	55.0
Raphanus sp. (radish)	26	77.0
Lycopersicon sp. (tomato) leaf	25	8.0
Lycospersicon sp., flower	100	1.0
Cucurbita sp. (zuccini)	38	41.0
Nicotiana sp. (tobacco)	39	41.0
Ipoemaea sp. (sweet potato)	6	33.0
Medicago sp. (alfalfa)	6	50.0
Secale cereale (rye grass)	15	20.0
Poa praetensis (blue grass)	15	7.0
Sorghum sudanensis (sudan grass)	25	4.0
Sorghum sp.	3	67.0
Avena sativa (oats)	15	0.0
Dactylis glomerata (orchard grass)	15	13.0
Totals:		
Broad-leaved plants	279	
Average per cent recovery		32.2
Cereals and grasses	88	
Average per cent recovery		10.4

TABLE 6. *Recovery of enterococci from soils*

Crop plant	No. of soil samples	No. of entero-cocci recoveries
Tobacco	3	1
Alfalfa	8	4
Sweet potato	6	1 (1:10)
Zucchini	3	1
Tomato	30	7
Okra	8	2
Cotton	5	0
Snap beans	20	14
Corn	19	4
Totals	102	34

grasses, 10.4% (Table 5), may be compared with an incidence of 34.0% on the broad-leaved group of woody and annual flowers (Table 4) and 32.3% on agricultural plants (Table 5).

Enterococci in soils. Table 6 records the numbers of samples of soils cultured and the numbers from which enterococci were obtained. Numbers seldom exceeded 1,000 per g of soil, and frequently were as low as 10 per g, when present. No enterococci were recovered from 1-g samples of cotton field soil, although 81.2% of the cotton flowers sampled (Table 5) yielded enterococci. The incidence in corn field soils is low, 26.6%, in view of the high percentage of recovery from corn flowers. Conversely, the recovery from soils of tomato fields, 23.3%, is high, as compared with recovery from tomato plants. This may be explained by proximity to, and the leeward location of, the tomato to corn fields.

Enterococci were recovered both from plants and from soils in which the plants grew in eight fields; they were not recovered from either plants or soil of four fields. They were present in the soils of two fields, but not the plants the fields supported; were found on the plants of three fields, but not in the soils supporting them.

Identity of isolates. Isolates were identified as *Streptococcus faecalis*, a proteolytic variant of *S. faecalis*, and *Streptococcus faecium*. The ratio of the strains identified was very similar to that described earlier by Mundt et al. (1957).

Discussion

From results obtained, it appears that enterococci (group D streptococci) are temporary residents on plants, that they must be reintroduced during each growing season, and are capable of reproduction during periods when conditions are favorable. In most instances they are not invariably present on a given species of plant, nor are they invariably absent. They are found less frequently on buds than on blossoms. Through interference with mechanical access, the number of flowers in which they may be found can be reduced.

Most samples of the agricultural plants as well as the other plants were grown in extensive, cultivated areas which may have been adjacent to, but did not support simultaneously, domestic animal life. Most samples were taken well above the ground, thus minimizing the possibility of contact with wild animals. Samples of plants bearing enterococci were distributed throughout large, cultivated fields.

The potential role of insects has been indicated by Eaves and Mundt (1960), and this is further substantiated in this work. Enterococci were isolated with greater frequency from corn tassels while well wrapped within the sheath of leaves, if these were infested with insects of the family *Nitidulidae*. In the earlier work, enterococci were isolated relatively frequently from insects taken in corn fields, but not from those of the same species captured in tomato fields.

The simultaneous occurrence or absence of enterococci in soils and on the plant grown on the soil of 12 of 17 fields suggests also the role of wind, gravitational flow, and rain from the plant downward. Enterococci were rarely recovered from all samples taken in the same field, and frequently the level of population was between 10 and 100 per g of soil. Pollution from wild animals, although a possibility, is remote because of the very low populations in cultivated fields.

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