

PARTIAL ANTIBIOTIC SPECTRUM OF TOMATIN,¹ AN ANTIBIOTIC AGENT FROM THE TOMATO PLANT²

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The clinical attractiveness of antibiotic agents, as typified by penicillin and streptomycin, lies in their ability to effect striking and specific bacteriostatic action *in vivo* without the simultaneous production of severe toxic symptoms. However, since penicillin and streptomycin are limited in their usefulness because of their ineffectiveness against certain important groups of pathogenic organisms, the search for new antibiotic agents continues in the hope that additional substances having sufficiently low toxicity will be found whose high antibiotic activity against the penicillin- and streptomycin-resistant organisms will permit their therapeutic use in the conquest of the diseases caused by these pathogens.

Although the fungi, including *Actinomyces* and related forms, and bacteria have been the most fruitful sources of antibiotic agents, antibiotic activity has also been attributed to the juices of certain green plants. Many plant families have been examined for antibiotic activity (Osborn, 1943; Huddleson *et al.*, 1944; Lucas and Lewis, 1944; Seegal and Holden, 1945), and several plant constituents that possess antibiotic activity have been isolated in crystalline form. Among these antibiotic agents are a substance from garlic (*Allium sativum*) that has been tentatively identified as the sulfoxide of diallyl disulfide (Cavallito, Buck, and Suter, 1945); a substance from common burdock (*Arctium minus*) that has not been identified but which appears to be a lactone having the empirical formula $C_{15}H_{20}O_5$ (Cavallito, Bailey, and Kirchner, 1945); and a substance designated "crepin" from *Crepis taraxacifolia* that has the empirical formula $C_{14}H_{18}O_4$ (Heatley, 1944).

It is the purpose of this paper to describe some of the antibiotic properties of what is believed to be a new antibiotic agent from a plant source. This substance occurs in the tomato plant and has been designated "tomatin." Tomatin has not yet been crystallized, but preparations of sufficient potency have been obtained to warrant a preliminary investigation of its antibiotic spectrum. Because of the probable impurity of the tomatin preparation used in the present investigation, the data to be presented have only qualitative or, at best, semi-

¹ In a recent publication (Irving, Fontaine, and Doolittle, 1945) this substance was referred to as "lycopersicin." Inasmuch as it has since been learned that the term "lycopersicin" was once used (Duggar, 1913) as a synonym for "lycopene," the red pigment of the tomato, the designation of the antibiotic agent has been changed to "tomatin" to avoid possible confusion.

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quantitative significance. Investigation of the activity of tomatin with respect to the organisms employed in the present work will be repeated when purer tomatin preparations are isolated, and experimentation will be extended to include other pathogens.

EXPERIMENTAL

Preparation of tomatin. Details of the procedure for the isolation of potent tomatin preparations from the tomato plant and a discussion of the chemical and physical properties of tomatin will be presented elsewhere. The tomatin preparation used in the present investigation was prepared in the following manner: The mechanically expressed juice of thoroughly washed, mature, Red Currant tomato plants (*Lycopersicon pimpinellifolium*) was autoclaved, and the clear extract obtained by centrifuging was concentrated to dryness *in vacuo* at 60 C. The extract obtained by thorough extraction of the residue with absolute methanol was concentrated to dryness *in vacuo*, and an aqueous solution of the residue was sterilized and stored in the cold for use in these experiments. This solution (pH 4.0) contained approximately 70 tomatin units per ml when assayed by the procedure previously described (Irving, Fontaine, and Doolittle, 1945).

Procedure. Sterile, 90-mm petri dishes, containing 20 ml of solidified nutrient agar,³ were warmed to 45 C and flooded with 3 ml of a suspension of bacterial cells or fungus spores in the same medium. The inoculum was prepared by adding to 10 ml of melted medium (cooled to 45 C) 1 ml of a suspension obtained by washing the surface of a vigorously growing agar slant culture of the organism with 5 ml of sterile water. Five porcelain cylinders (8 mm by 10 mm high) were dropped on the solidified, inoculated surface of the plate; suitable dilutions of the sterile tomatin stock solution were pipetted into three of the cylinders; and the plates were incubated at 28 or 40 C until growth of the organism was sufficiently advanced to permit accurate measurement of the inhibition zones produced. In nearly all instances dilute solutions of penicillin ("penicillin-sodium," Chas. Pfizer and Co., Inc.) were placed in the two remaining cylinders on each of the plates for comparison. The penicillin solutions were standardized by assay against *Staphylococcus aureus* (Schmidt and Moyer, 1944).

Results. The effectiveness of tomatin and of penicillin in inhibiting cultures of four bacteria and ten fungi is shown in table 1. To facilitate comparison, only the results for solutions containing 5 units of tomatin and 4 units of penicillin per ml are given in the table. Experiments have also been conducted at various times with tomatin concentrations of 1 and 10 units per ml and penicillin concentrations of 2.5 and 20 units per ml. In all instances the diameters of the inhibition zones produced by lower or higher concentrations of tomatin corresponded closely with the values that would be expected on the basis of the figures given in the table. Since the tomatin solutions used had pH values of approximately 4.0, it was desirable to test the effect of weak acid solutions alone

³ Medium: bacto yeast extract dehydrated, 5 g; bacto peptone, 5 g; glucose anhydrous Squibb, 2.5 g; bacto agar, 15 g; distilled water to 1 liter; pH 6.4.

on each of the organisms. None of the organisms listed in table 1 was inhibited when a solution containing 0.5 g KCl, 0.5 g Mg SO₄, and 1.0 g KH₂PO₄ and having a pH of 3.5 was used in place of tomatin in a cylinder of the test plate.

Typical cylinder plates, which illustrate the effect of tomatin on *Trichophyton mentagrophytes* and the three *Fusarium* species, are shown in figure 1. The

TABLE 1
Antibiotic effects of tomatin and penicillin on certain bacteria and fungi

ORGANISM*	INCUBATION PERIOD	DIAMETER OF INHIBITION ZONE, MILLIMETERS	
		Tomatin	Penicillin
	<i>hr</i>	<i>5 u/ml</i>	<i>4 u/ml</i>
<i>Staphylococcus aureus</i> (NRRL B-313)†	18	17	28
<i>Bacillus subtilis</i> (NRRL 558)†	18	22	29
<i>Escherichia coli</i> (NRRL B-210)†	18	0	0
<i>Phytomonas solanacearum</i>	20	16	0
<i>Penicillium notatum</i> (NRRL 1249B21)	48	0	0
<i>Aspergillus clavatus</i> (ATCC 1007)	19	23	
<i>Aspergillus clavatus</i> (ATCC 9192, Waksman 129)	19	21	
<i>Fusarium oxysporum</i> f. <i>lycopersici</i> (R-5-6)	23	23	0
<i>Fusarium oxysporum</i> f. <i>pisi</i> (SPD 340)	48	24	0
<i>Fusarium oxysporum</i> f. <i>conglutinans</i> (SPD 341)	42	32	0
<i>Candida albicans</i> (ATCC 2091)	23	20	0
<i>Trichophyton mentagrophytes</i> (ATCC 9533)	89	36	0
<i>Epidermophyton floccosum</i> (ATCC 9646)	120	34‡	0
<i>Microsporum audouini</i> (ATCC 9082)	43	§	

* NRRL, Northern Regional Research Laboratory; ATCC, American Type Culture Collection; R-5-6, highly virulent strain from collection of Dr. F. L. Wellman (Wellman, 1942); SPD, Doolittle collection, originally obtained from the collection of Dr. J. C. Walker. The culture of *P. solanacearum* was a fresh isolate taken from a severely diseased tomato plant.

† Incubated at 40 C; all others incubated at 28 C.

‡ Inhibition zone produced by 1 unit tomatin per ml.

§ Growth too slow for satisfactory application of cylinder-plate technique. However, this organism is strongly inhibited when tomatin is added to the culture medium (see figure 2).

effectiveness of tomatin, when added to the culture medium, in inhibiting several organisms is illustrated in figure 2.

As was to be expected, penicillin was effective only against the two gram-positive organisms, *Staphylococcus aureus* and *Bacillus subtilis*. Like penicillin, tomatin was found to be effective against these two organisms and ineffective against gram-negative *Escherichia coli*. However, unlike penicillin, tomatin was found to be effective against the gram-negative, bacterial plant-wilt pathogen *Phytomonas solanacearum*. Tomatin appeared to be without significant action upon the fungus *Penicillium notatum*, but it exhibited marked fungistatic

activity toward two strains of *Aspergillus clavatus*. On the *Aspergillus clavatus* plates, after 19 hours of incubation, the inhibition zones were perfectly clear and devoid of all growth, indicating the possible fungicidal action of tomatin upon this organism. After 47 hours of incubation there appeared wide halos of appressed growth surrounding the clear zones.

The most striking antibiotic effects of tomatin were observed, however, in the case of the three plant-wilt pathogens, *Fusarium oxysporum* f. *lycopersici* (tomato

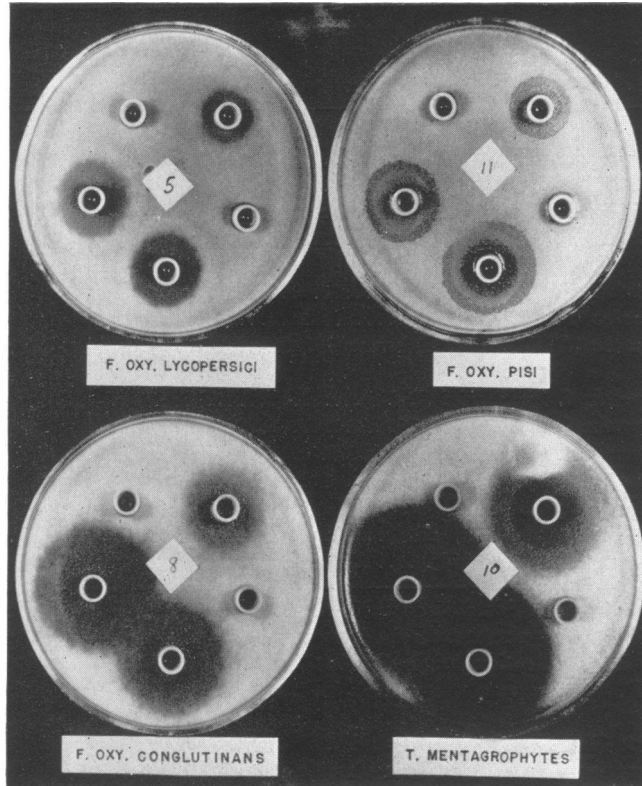


FIG. 1. EFFECT OF TOMATIN AND OF PENICILLIN ON SEVERAL FUNGI

Reading clockwise from bottom, the cylinders on plates 5, 8, and 10 contain tomatin 5 u per ml; tomatin, 10 u per ml; penicillin, 2.5 u per ml; tomatin, 1 u per ml; penicillin, 4 u per ml. The cylinders on plate 11 contain tomatin, 10 u per ml; tomatin, 5 u per ml; penicillin, 2.5 u per ml; tomatin, 1 u per ml; penicillin, 4 u per ml.

wilt), *F. oxysporum* f. *pisi* (pea wilt), and *F. oxysporum* f. *conglutinans* (cabbage yellows), and in the human dermatophytes, *Candida albicans*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum audouini*. It will be recalled that all of these fungi are representatives of the so-called *Fungi Imperfecti*, whose "perfect" or sexual forms are not known. Most of the fungi parasitic in man are in this group (Zinsser and Bayne-Jones, 1934). Infections by these fungi may occur in various parts of the body and include, among others, such established clinical entities as favus, ringworm, eczema, thrush, and sprue. Many of these and similar infections are not always serious, but some are wide-

spread, often incapacitating, persistent, recurrent, and resistant to treatment. The marked activity of tomatin with respect to the representatives of the *Fungi Imperfecti* listed in table 1 suggest that tomatin may be effective against many other pathogenic fungi within this group. A more thorough investigation of the fungistatic and fungicidal activity of tomatin in relation to the imperfect fungi is in progress.

It is noteworthy that tomatin not only strongly inhibits a highly virulent strain of *Fusarium oxysporum* f. *lycopersici*, the organism that causes wilt in the tomato plant, but it also inhibits, to an equal or greater degree, the *Fusarium* species that cause similar wilt diseases in peas and cabbage. The role played by tomatin in the natural wilt resistance exhibited by some varieties of tomatoes



FIG. 2. EFFECT OF ADDITION OF TOMATIN TO THE MEDIUM ON THE GROWTH OF SEVERAL ORGANISMS

Plate on left contains no added tomatin; growth vigorous. Plate on right contains 1 unit of tomatin per ml of medium; growth insignificant. F, *Epidermophyton floccosum*; A, *Microsporium audouini*; M, *Trichophyton mentagrophytes*; S, *Phytophthora solanacearum*. Both plates incubated for 43 hours at 28 C.

and the significance of the antibiotic activity of tomatin toward other plant-pathogenic species of *Fusarium* and the bacterial wilt organism will be discussed elsewhere.

DISCUSSION

The marked fungistatic and possibly fungicidal powers of tomatin *in vitro* encourage speculation concerning its possible therapeutic applicability in human and animal fungus infections. However, such a possibility can be entertained only if subsequent investigations, now in progress, prove tomatin to be sufficiently nontoxic to permit local or perhaps internal application in man and animals. A highly active nontoxic fungistatic agent would be of value in the parenteral or oral treatment of certain fungus infections in cases in which fungistatic agents like actinomycin and gliotoxin (Reilly, Schatz, and Waksman, 1945) are of limited usefulness because of their toxicity.

It is difficult to make a true comparison of the relative fungistatic powers of

tomatin and certain other fungistatic agents, since units of measurement differ between investigators. With the best tomatin preparations so far obtained, definite fungistatic activity toward *T. mentagrophytes* can be demonstrated by the cylinder-plate method with a solution containing approximately 5 micrograms of tomatin per ml. In other words, according to one widely used method of evaluation, such a tomatin preparation would contain approximately 200,000 dilution units per gram. The published figures for the two very active fungistatic agents actinomycin and gliotoxin (Reilly, Schatz, and Waksman, 1945), both of which are presumably pure compounds, indicate that each contains approximately 5 to 6 million dilution units per gram when *T. mentagrophytes* is used as the assay organism. Tomatin, therefore, even in the impure preparations now available, approaches within a factor of approximately 25 the fungistatic activity of these crystalline antibiotic agents.

SUMMARY

Tomatin, an antibiotic agent that occurs in the tomato plant, has been shown to inhibit effectively cultures of *Staphylococcus aureus*, *Bacillus subtilis*, *Phytomonas solanacearum*, *Aspergillus clavatus*, *Fusarium oxysporum* f. *lycopersici*, *Fusarium oxysporum* f. *pisi*, *Fusarium oxysporum* f. *conglutinans*, *Candida albicans*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporium audouini*. It is without effect upon cultures of *Escherichia coli* and *Penicillium notatum*. These results suggest the possibility that tomatin may be useful in the treatment of certain human and animal fungus infections, provided current investigations prove tomatin to be effective *in vivo* and of sufficiently low toxicity to permit local or preferably oral or parenteral administration.

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