STUDIES ON CELLULOLYTIC ACTIVITY OF FUSARIA WITH REFERENCE TO BACTERIAL AND OTHER CELLULOSE SUBSTRATES

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INTRODUCTION

In recent years numerous investigations have been carried out on the cellulolytic activity of *Fusarium* species with reference to the part played by these fungi in the decomposition of cotton fabrics under various exposure conditions (White *et al.*, 1948; Marsh and Bollenbacher, 1949; Marsh *et al.*, 1949). Other reports list a few species which were found capable of decomposing cellulose (Thaysen and Bunker, 1937; Siu, 1951). White *et al.* (1948) found twenty-seven isolates of Fusaria, out of the thirty-four investigated, to be active in pure-culture cotton fabric tests for cellulolytic activity. These tests were carried out on strips of cotton fabrics and were aimed mainly at indexing the economic importance of the fungi in causing deterioration of the fabrics under field conditions from the point of industrial and military considerations. Evidence has been presented by Tracey (1953) to show that activity of cellulolytic enzyme preparation obtained from fungi is dependent upon the nature of the cellulose substrata. It would seem important, therefore, to test fungal species on different cellulosic substrates in evaluating the cellulolytic activity of the species. So far, no attempt seems to have been made to estimate the activity of fungi on bacterial cellulose membranes biosynthesized by *Acetobacter xylinium*. These membranes are known to be the purest form of cellulose (Gortner, 1949) and have been used in the isolation of cellulose-splitting bacteria from soil (Aschner, 1937). The present investigation was undertaken as a comparative study on the cellulolytic activity of twenty-three species of *Fusarium* on bacterial as well as other cellulose substrates.

MATERIALS AND METHODS

All the species of Fusaria employed in this investigation were obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland, and monoconidial cultures of these species were tested for cellulolytic activity. The inoculum was prepared as follows: Strips of filter paper were placed in half-strength Richards solution (sugar omitted) in a test-tube and autoclaved. In each case, a spore suspension of any one fungus was placed on the protruding but moist portion of the strip and allowed to grow at 25–29°C. for 10 days. After incubation, a portion of the growing colony on the filter paper was used for inoculation in determining cellulolytic activity.

Cellulolytic activity of the species on filter paper: A central core was cut out from discs of Whatman No. 1 filter paper and one such disc (1100 mg.) was folded up in the form of a fluted cone and introduced into 250 ml. conical flasks containing 50 ml. medium in such a way that approximately one-third of the filter paper remained immersed in the solution. After autoclaving, the flasks were inoculated by placing the inoculum on the unsubmerged portion of the paper. The weight of the residual filter paper, left over after fungal decomposition, was determined after 15 and 30 days' incubation at 25–29°C.

Cellulolytic activity of the enzyme preparation: Suspension of regenerated filter paper (Scales, 1915) was taken in Sorensen's phosphate buffer at *p*H 5·0, 5·5, 6·0, 6·5, 7·0, and 7·5, and in each case, 5 ml. of the culture filtrate, obtained after 3 weeks' growth of different Fusaria on filter paper, was added to 5 ml. aliquots of the suspension in test-tubes maintained at 37°C. Turbidometric determinations of the density of the cellulose suspension were made after 0, 4, 16, 22, 28 and 40 hours in Spekker Absorptiometer using H.2 neutral filter. The increase in percentage light transmission plotted against time interval indicated enzymic activity of the filtrate.

Cellulolytic activity of Fusaria on bacterial cellulose: Acetobacter xylinium was grown on yeast-extract-sucrose medium (Aschner, 1937) and after 5 days' growth at 27°C., the bacterial membranes were washed thoroughly in tap water, immersed in 20% sodium hydroxide for 2 days to dissolve out the bacterial protein, washed again in running tap water, rinsed in dilute HCl to remove the last traces of the alkali and finally washed again in tap water and distilled water. These membranes were soaked in half-strength Richards mineral solution in flasks, autoclaved and after 2-3 days one such membrane was transferred to a 10-cm. Petri dish and the surface moisture on the membrane removed with a filter paper. The dishes were autoclaved and the membranes inoculated in the centre in a manner similar to that of agar plates. The radial spread of the Fusaria on the cellulose membrane (Plate XX, Fig. 9) resulted in the liquefaction of the cellulose and indicated cellulolytic activity of the species. To determine the area of liquefaction, the surface of the membrane covered with the fungal colony was gently rubbed with a glass spatula when the liquefied area separated from the rest of the membrane into a jelly-like mass (Plate XX, Figs. 11 and 12). It was further observed that the area of liquefaction was slightly less than that covered by the fungal growth. Approximately, 2 mm. of the peripheral region of the fungal colony was not liquefied. This was probably due to the fact that enzymic activity in this area had not progressed sufficiently. In the present work, all results are expressed as the diameter of the fungal colony on the cellulose membrane.

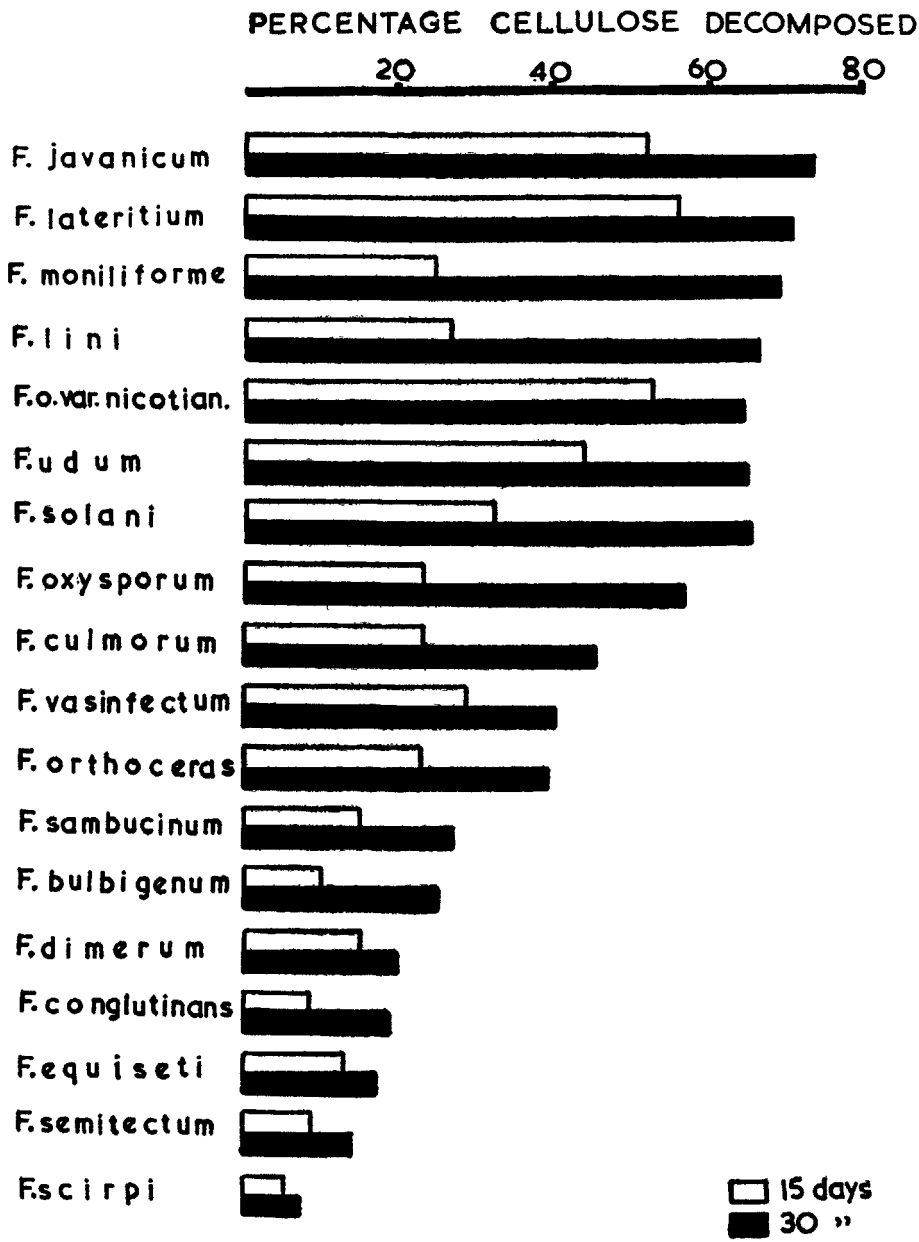
Results

Text-fig. 1 shows the results of the cellulolytic activity of the Fusaria on filter paper. No correction has been made here for the weight of the fungal mat and it is deemed that these results would provide sufficient index for the purpose of comparing the cellulolytic potentiality of the different species.

Degradation of cellulose by the Fusaria differed strikingly with the species (Plate XX, Figs. 1–8). Whilst most of the Fusaria digested filter paper to varying extent, five species, *F. buharicum*, *F. caucasicum*, *F. chlamydosporum*, *F. sporotrichioides* and *F. poae*, did not decompose filter paper. *F. javanicum*, *F. lateritium*, *F. lini*, *F. moniliforme*, *F. solani*, *F. oxysporum* var. *nicotianae*, *F. udum* and *F. oxysporum*, in the order mentioned, were strongly cellulolytic, whereas *F. bulbigenum* var. *lycopersici*, *F. conglutinans*, *F. dimerum*, *F. equiseti*, *F. sambucinum*, *F. scirpi* and *F. semitectum* were poor decomposers of filter paper (Text-fig. 1). *F. vasinfectum*, the cotton wilt pathogen, *F. orthoceras* and *F. culmorum* utilized the cellulose to a moderate extent. Test for glucose in the 15-day-old cultures undergoing degradation of filter paper were negative, but traces of glucose were detected in the metabolic filtrate obtained 30 days after cellulose degradation had progressed.

In correlating cellulolytic activity of the *Fusarium* with its species, the strength of enzyme (cellulase) activity of the filtrate of Fusaria growing on filter paper was determined as an index of the cellulolytic activity of the species on filter paper cellulose; results are presented in Text-fig. 2 and Table I.

Excepting *F. chlamydosporum*, cellulase activity was observed in the filtrates of the other species tested. The data obtained here showed that enzymic strength of the metabolic filtrate was directly related to cellulolytic activity of the species on



TEXT-FIG. 1. Cellulolytic activity of Fusaria on filter paper.

filter paper. Filtrates of *F. moniliforme*, *F. javanicum* and *F. lateritium*, which caused maximum breakdown of filter paper (Text-fig. 1), had highest cellulase activity (Text-fig. 2); conversely, low enzyme strength was observed in the filtrates of *F. scirpi* and *F. dimerum*, which were found to be poor decomposers of cellulose.

The strength of the enzyme activity of the filtrate varied with the *p*H of the medium in which it was tested (Text-fig. 2). For the filtrate of most species tested,

TABLE I

Showing enzyme activity of Fusarium *culture filtrates on re-precipitated cellulose by turbidometric measurements*

Fusarium species	*p*H of medium	Light transmission after					
		0 hr.	4 hr.	16 hr.	22 hr.	28 hr.	40 hr.
F. lateritium	5·0	0·270	0·280	0·325	0·345	0·370	0·405
	5·5	0·268	0·285	0·335	0·360	0·390	0·425
	6·0	0·268	0·280	0·320	0·345	0·378	0·410
	6·5	0·268	0·290	0·315	0·335	0·362	0·388
	7·0	0·265	0·268	0·290	0·308	0·335	0·365
	7·5	0·270	0·270	0·295	0·318	0·341	0·360
F. bulbigenum var. *lycopersici*	5·0	0·270	0·285	0·305	0·335	0·345	0·355
	5·5	0·270	0·290	0·315	0·350	0·365	0·375
	6·0	0·275	0·290	0·300	0·335	0·345	0·360
	6·5	0·260	0·290	0·305	0·320	0·330	0·345
	7·0	0·255	0·275	0·285	0·300	0·312	0·325
	7·5	0·265	0·275	0·285	0·310	0·320	0·335
F. lini	5·0	0·280	0·290	0·305	0·350	0·365	0·395
	5·5	0·285	0·295	0·315	0·368	0·388	0·415
	6·0	0·280	0·290	0·300	0·355	0·375	0·395
	6·5	0·275	0·301	0·310	0·335	0·350	0·365
	7·0	0·270	0·310	0·290	0·320	0·345	0·375
	7·5	0·285	0·285	0·310	0·315	0·330	0·350
F. solani	5·0	0·250	0·315	0·280	0·300	0·310	0·335
	5·5	0·250	0·270	0·290	0·312	0·328	0·335
	6·0	0·260	0·280	0·305	0·330	0·348	0·362
	6·5	0·250	0·275	0·302	0·335	0·365	0·378
	7·0	0·260	0·280	0·305	0·325	0·362	0·370
	7·5	0·255	0·275	0·300	0·325	0·345	0·358
F. vasinfectum	5·0	0·140	0·175	0·205	0·245	0·255	0·275
	5·5	0·145	0·178	0·215	0·258	0·270	0·295
	6·0	0·140	0·165	0·200	0·240	0·255	0·275
	6·5	0·140	0·165	0·205	0·225	0·240	0·255
	7·0	0·145	0·160	0·180	0·220	0·240	0·255
	7·5	0·140	0·140	0·180	0·210	0·215	0·230
F. oxysporum	5·0	0·255	0·280	0·290	0·325	0·350	0·370
	5·5	0·250	0·280	0·295	0·335	0·365	0·385
	6·0	0·250	0·272	0·292	0·325	0·350	0·370
	6·5	0·250	0·270	0·290	0·320	0·335	0·355
	7·0	0·250	0·272	0·290	0·320	0·340	0·355
	7·5	0·250	0·255	0·290	0·320	0·335	0·350
F. equiseti	5·0	0·255	0·290	0·315	0·330	0·355	0·370
	5·5	0·260	0·295	0·318	0·335	0·365	0·385
	6·0	0·255	0·285	0·290	0·320	0·355	0·368
	6·5	0·260	0·280	0·280	0·320	0·345	0·365
	7·0	0·265	0·280	0·290	0·320	0·355	0·368
	7·5	0·270	0·275	0·290	0·320	0·335	0·365
F. scirpi	5·0	0·260	0·260	0·275	0·300	0·310	0·320
	5·5	0·260	0·265	0·278	0·305	0·310	0·320
	6·0	0·260	0·262	0·282	0·310	0·320	0·330
	6·5	0·260	0·270	0·280	0·320	0·328	0·340
	7·0	0·265	0·268	0·268	0·295	0·310	0·335
	7·5	0·270	0·270	0·290	0·310	0·325	0·340

TABLE I—*contd.*

Fusarium species	*p*H of medium	Light transmission after					
		0 hr.	4 hr.	16 hr.	22 hr.	28 hr.	40 hr.
F. dimerum	5·0	0·260	0·265	0·285	0·310	0·320	0·335
	5·5	0·260	0·265	0·285	0·320	0·325	0·335
	6·0	0·260	0·275	0·290	0·330	0·335	0·345
	6·5	0·255	0·280	0·295	0·320	0·340	0·355
	7·0	0·265	0·275	0·300	0·325	0·340	0·355
	7·5	0·265	0·265	0·295	0·325	0·340	0·355
F. javanicum	5·0	0·150	0·170	0·220	0·235	0·245	0·265
	5·5	0·150	0·170	0·220	0·245	0·255	0·275
	6·0	0·150	0·175	0·230	0·265	0·375	0·290
	6·5	0·155	0·165	0·230	0·280	0·303	0·330
	7·0	0·160	0·185	0·232	0·265	0·290	0·320
	7·5	0·155	0·178	0·235	0·270	0·285	0·305
F. moniliforme	5·0	0·270	0·295	0·330	0·360	0·375	0·410
	5·5	0·270	0·295	0·335	0·375	0·390	0·435
	6·0	0·265	0·290	0·325	0·365	0·385	0·415
	6·5	0·270	0·290	0·328	0·365	0·380	0·398
	7·0	0·270	0·285	0·315	0·345	0·365	0 390
	7·5	0·270	0·275	0·315	0·340	0·355	0·375

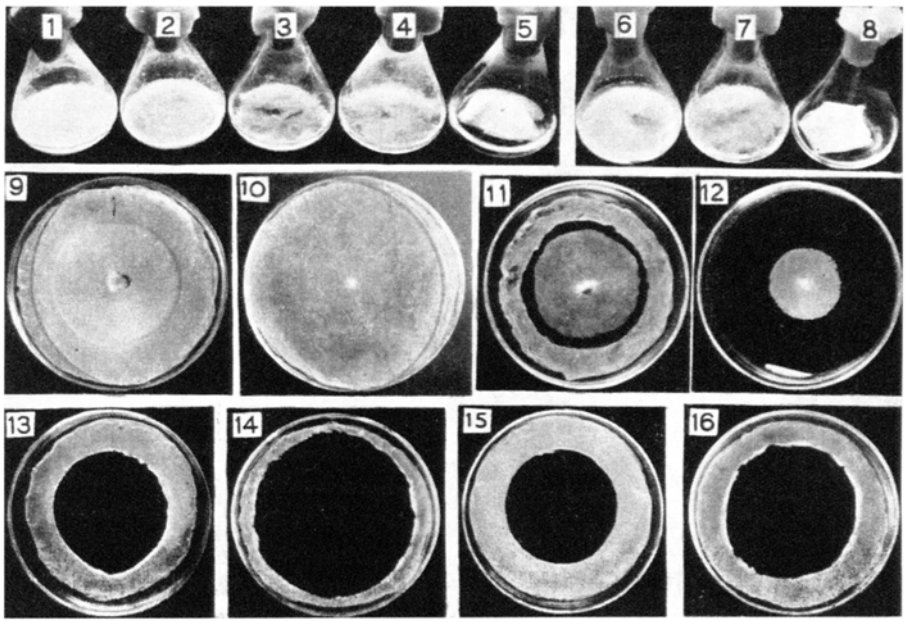
*p*H 5 5 seemed to be the most favourable for enzymic activity and the enzyme strength was lower when measured at higher *p*H levels. In contradistinction, filtrate activity of *F. dimerum*, *F. javanicum*, *F. solani* and *F. scirpi* was maximum at *p*H 6 5 and decreased at the lower *p*H levels.

On the bacterial cellulose membranes all the Fusaria manifested good cellulolytic activity (Text-fig. 3) with the exception of *F. caucasicum* (Plate XX, Fig. 10), which was also unable to digest filter paper. All the other species which had no cellulolytic activity on filter paper, i.e. *F. buharicum*, *F. chlamydosporum*, *F. poae* and *F. sporotrichioides*, were able to decompose the bacterial cellulose and the extent of decomposition was striking in the case of *F. chlamydosporum* and *F. sporotrichioides*, both of which made 6·2 cm. diametrical spread on the membrane (Text-fig. 3).

Out of seven species, which were poor decomposers of filter paper, only *F. semitectum* failed to make any appreciable growth on the bacterial cellulose membrane, whilst the other six species, *F. bulbigenum* var. *lycopersici*, *F. conglutinans*, *F. dimerum*, *F. equiseti*, *F. sambucinum* and *F. scirpi*, decomposed the membranes markedly. *F. udum* and *F. lateritium*, in which strongest degradation of filter paper was observed, did not make commensurate growth on the cellulose membranes, whereas *F. culmorum*, which had only moderate cellulolytic activity on filter paper, made the maximum growth on the membrane (Plate XX, Fig. 14; Text-fig. 3). *F. javanicum*, *F. lini*, *F. moniliforme*, *F. solani*, *F. orthoceras*, *F. oxysporum*, *F. oxysporum* var. *nicotianae*, *F. udum* and *F. vasinfectum* grew well on the bacterial cellulose (Text-fig. 3).

DISCUSSION

It is now well recognized that degradation of cellulose by fungi depends largely on the chemical composition of the substrate (Siu and Reese, 1953). In the experiments reported, with the exception of one species, *F. caucasicum*, all others were able to decompose bacterial cellulose. On the other hand, five species, *F. buharicum*, *F. caucasicum*, *F. chlamydosporum*, *F. poae* and *F. sporotrichioides*, were unable to digest filter paper, whilst seven others, namely *F. bulbigenum* var. *lycopersici*,



FIGS. 1–8. Show the disintegration of filter paper by Fusaria 3 weeks after inoculation; *F. lateritium* (1), *F. sambucinum* (2), *F. lini* (3), *F. conglutinans* (4), *F. caucasicum* (5), *F. vasinfectum* (6), *F. culmorum* (7) and *F. chlamydosporum* (8).

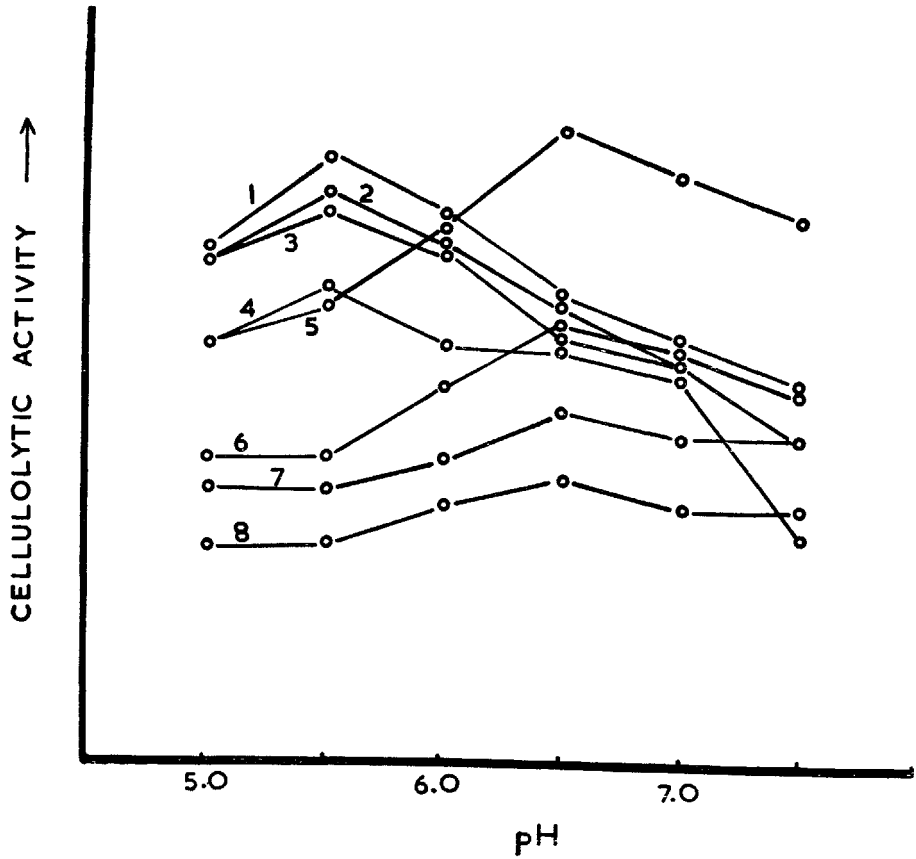
FIG. 9. Characteristic growth of Fusaria on bacterial cellulose membrane.

FIG. 10. Inability of *F. caucasicum* to grow on bacterial cellulose.

FIG. 11. Decomposed and undecomposed parts of the cellulose membrane separated by rubbing the surface of the *Fusarium* colony with a glass spatula.

FIG. 12. The fungal colony left behind after the removal of the undecomposed part of the cellulose membrane.

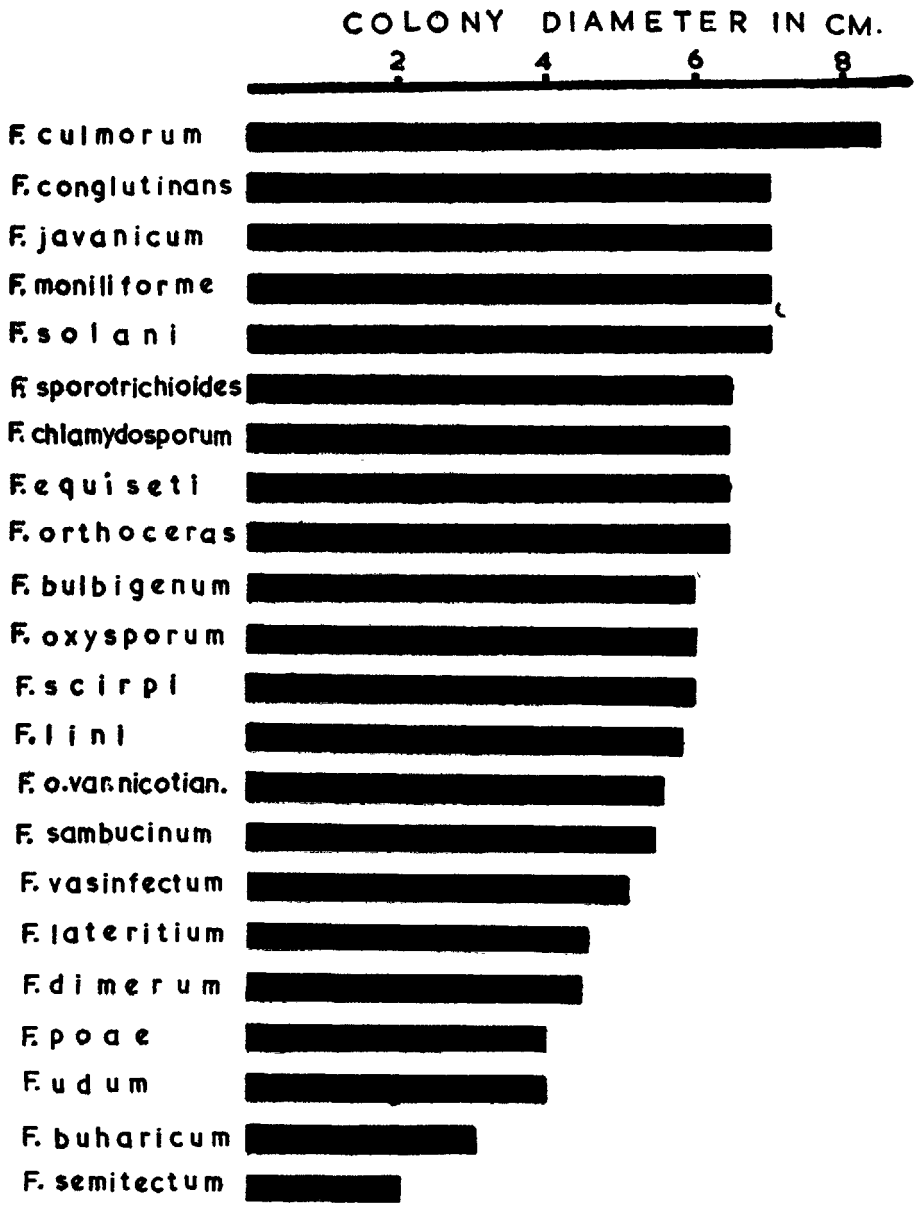
FIGS. 13–16. Show the digestion of bacterial cellulose by Fusaria: cellulose liquefied as the result of fungal growth has been removed. *F. orthoceras* (13), *F. culmorum* (14), *F. chlamydosporum* (15), and *F. solani* (16).



TEXT-FIG. 2. Effect of *p*H on cellulolytic activity of *Fusarium* culture filtrates on re-precipitated filter paper; *F. moniliforme* (1), *F. lateritium* (2), *F. vasinfectum* (3), *F. lini* (4), *F. javanicum* (5), *F. solani* (6), *F. dimerum* (7) and *F. scirpi* (8).

F. conglutinans, *F. dimerum*, *F. equiseti*, *F. sambucinum*, *F. scirpi* and *F. semitectum*, were capable of only weak cellulolytic activity on this substratum. According to Siu and Reese (1953), the non-cellulosic components in the substrate determine its susceptibility to microbial decomposition. This factor may operate in two directions: one, in which the availability of the non-cellulosic constituents increases its susceptibility, and, in the other case, the resistance of the non-cellulosic ingredients to microbial attack protecting the cellulose substrate from decomposition (Basu, 1948). The inability of *F. chlamydosporum*, *F. sporotrichioides*, *F. poae* and *F. buharicum* to decompose filter paper, while causing degradation of bacterial cellulose, may have been due to either the resistant substances in the filter paper being probably toxic to these species or due to their inability to produce the specific enzyme, C_1 (Siu and Reese, 1953), which is concerned in the splitting of native cellulose. Further observations on the nature of the bacterial cellulose synthesized by *A. xylinium* which is being undertaken elsewhere (Minor *et al.*, 1954), utilizing C^{14} labelled carbon source, will no doubt throw much light on the question of the greater susceptibility of bacterial cellulose to Fusaria than filter paper.

The response of cellulase activity to *p*H is known to vary strikingly with different sources of enzyme. Optima for cellulolytic activity of *Aspergillus niger* and *A. oryzae* were *p*H 4·7 and 4·5, respectively, whereas *Myrothecium verrucaria* has an



TEXT-FIG. 3. Growth of Fusaria on cellulose membranes biosynthesized by *Acetobacter xylinium*, six days after inoculation.

optima around *p*H 5·5 (Siu, 1951). The enzymic strength of *Fusarium* culture filtrate was found to be directly related to the cellulolytic activity of the species on filter paper and also varied with the *p*H of the medium. Enzyme activity of filtrate of most of the species was maximum at *p*H 5·5 (Table I and Text-fig. 2), while filtrates of *F. dimerum*, *F. javanicum*, *F. solani* and *F. scirpi* had an optima of 6·5 (Text-fig. 2). These optima are in reference to re-precipitated cellulose and enzyme activity of the *Fusarium* filtrates is likely to vary with the nature of the

cellulose substrate used, as observed by Reese and Levinson (1952) in the case of other organisms.

It is obvious from this work that Fusaria manifest considerable variation in relative ability of a particular species to attack cellulose (Text-figs. 1 and 2), with the exception of *F. caucasicum*. This is in keeping with similar observations, as for example, within the genus *Aspergillus* only certain species are cellulolytic, and even within the same species of *Penicillium citrinum* only some isolates are cellulolytic (Siu and Reese, 1953). One difficulty in probing deeper into this problem has been in obtaining the fungal enzyme in a pure state; the production of these 'adaptive' enzymes only on cellulose substrates is also a limiting factor in determining the exact status of a fungal isolate in respect of cellulolytic activity and only further work in this direction could clarify the overall picture.

Summary

Twenty-three species of *Fusarium* were tested for cellulolytic activity on filter paper and bacterial cellulose. Considerable variation was observed in the relative ability of a particular species to attack these two cellulose substrata. Four species, *F. buharicum*, *F. chlamydosporum*, *F. poae* and *F. sporotrichioides*, did not digest filter paper, but decomposed bacterial cellulose. Only *F. caucasicum* was inactive on both filter paper and bacterial cellulose; all the other species decomposed these cellulose substrata. Culture filtrates of Fusaria growing on filter paper were active on re-precipitated cellulose and the strength of the enzymic activity of the filtrate was directly related to the ability of the species to decompose filter paper. The activity of the enzyme preparation of most of the species was maximum at *p*H 5·5, whilst four species had an optima of *p*H 6·5.

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