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On the effects of secretions of saprophytic bacteria on the course of mitosis in adventitious roots of *Allium cepa* L.

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Abstract

The influence of 21 strains of saprophytic bacteria isolated from onion cultures on the course of mitosis and on the level of chromosome aberrations in adventitious roots of *Allium cepa* L. was studied. Liquid cultures of all bacterial strains caused no changes in divisions of the cells. However, ten of the strains were responsible for disturbances in this process. The nature of these disturbances depended to a large extent on the kind of medium in which the bacteria were grown.

INTRODUCTION

The investigations of the last few years show that chromosome aberrations can be caused by infection with pathogenic microorganisms, such as: viruses (Hampar et al. 1961; Nichols et al. 1965, Heneen et al. 1970), rickettsiae (Halkka et al. 1970) and bacteria of mycoplasma class (Paton et. al. 1965). According to initial observations made by Cebrat et al., (1972) saprophytic microorganisms can also be responsible for chromosome aberrations. These authors have established an increased level of chromosome aberrations in adventitious roots of onions cultured in liquid media idiopathically infected with bacteria. Their further studies on the effects of sterile isolated strains showed that the supernatant of bacteria cultured on liquid Davis medium causes strong disturbances in the course of mitosis. These disturbances are of a different nature from those which occur after an idiopathic infection with bacteria. The differences in action could be due to the conditions of culturing the bacteria and therefore to changes in their metabolic activity, to different effects of the nature of bacteria and of pure strains and to the fact that Ceberat et al. in their experiments studied only bacterial strains which multiplied the most quickly on nutrient agar. Investigation of the influence

of 21 bacterial strains isolated from onions cultured on liquid media on the course of mitosis in adventitious roots of the onion was the subject of this work. Various conditions of culturing the bacteria were considered during the experiments. The problem was whether the occurring increased level of chromosome aberrations in adventitious roots of the onion is caused by bacterial secretions or by the products of degradation of their cells.

MATERIALS AND METHODS

Bacteria were isolated from the water on which the onions were cultured. The number of bacteria in water was determined by planting appropriate dilutions on nutrient agar. 21 bacterial strains were isolated. In order to determine the influence of the individual strains on the mitoses in adventitious roots of the onion they were treated with supernatant from liquid bacterial cultures on Davis medium (Lederberg 1950). The course of mitosis was normal. Among 21 examined strains there were 10 responsible for changes in the course of mitosis. These bacteria were distinguished on the base of their morphological characteristics and biochemical and physiological tests (Mackie and McCartney 1956).

Onions with the external scales peeled off were sterilized with 0,1% $HgCl_2$ for 10 minutes. After sterilization they were washed for about 2 hours in running water. Then they were placed in tap water. When the roots reached 3—5 cm in length the onions were transferred to an examined medium. The media tested were:

a) supernatant from bacteria cultured on liquid Davis medium,

b) supernatant from bacteria cultured on an onion extract (onion juice was diluted with distilled water in ratio 1 : 20),

c) washings, supernatants and washed bacteria cultured on solid media: nutrient agar and Davis medium.

Washings — solution obtained after transferring bacterial cultures showing a homogeneous growth on solid medium to sterile water.

Supernatant — liquid obtained after centrifugation of the washings.

Washed bacteria — bacterial suspension obtained after centrifugation of the washings, then washed with sterile water, centrifuged for the second time and again suspended in water.

d) bacterial homogenate obtained after breaking of the cells with ultrasounds.

Bacteria were incubated for 48 hours at 37°C in all the experiments. Each experiment was done three times. Several roots were taken from every onion before transferring to a different medium (control) and after

incubation in it for 2, 4, 24, 48 hours. Roots were fixed in acetic acid with ethyl alcohol (1 : 4). The slide preparations were stained by the nigrosin method.

RESULTS

Bacteria responsible for changes in the course of mitosis were distinguished on the base of their morphological characteristics and biochemical and physiological tests as belonging to the following genera: *Agrobacterium, Flavobacterium, Pseudomonas* and *Escherichia*. Supernatants of these bacteria, cultured on Davis medium, caused strong disturbances in the multiplication of the meristematic cells of adventitious roots of the onion. A grained structure of chromatin in interphase nuclei (due to the influence of *Agrobacterium* and *Flavobacterium* strains (Plate I, Photo 1), decline of the chromosome structures in prophase (Plate I, Photo 2), strong sticking and loss of the chromosome structures in metaphase (Plate I, Photos 3, 4), bridges and lagging chromosomes in anaphase and telophase (Plate I, Photos 6, 7, 8, 9) (under the influence of *Agrobacterium, Flavobacterium* and *Pseudomonas* strains), strong bulging of chromosomes in metaphase (Plate I, Photo 5) (under the influence of *Escherichia* strain) could be found in this case.

In the next experiment bacteria were cultured on diluted onion extract (in ratio 1 : 20), which did not affect mitosis (Plate II, Photo 10). This extract could be similar to the natural medium, which exists in water with cultured onion. Supernatants from bacteria of the genus *Agrobacterium* cultured on the onion extract after 4 hour action caused the shortening of chromosomes and their loose disposal in a cell. After 8 hour treatment with supernatant c-mitoses predominated in the cells of the roots which could probably be an effect of inactivation of the mitotic spindle (Plate II, Photo 11).

Bacteria were cultured on solid media in order to eliminate possible influence of the liquid medium components. The solid media were: nutrient agar and Davis medium united with agar. Washings, supernatants and bacteria washed in water (see above) were used in the experiments.

The influence of bacteria cultured in such a way on the course of mitosis in adventitious roots of the onion was completely different than the above mentioned.

The effects of the washings from bacterial cultures of *Agrobacterium, Flavobacterium* and *Pseudomonas* genera could be seen after 8 hours as a shortening of the chromosomes, their loose disposal in a cell and irregularity of the chromosome separation in anaphase (Plate II, Photos 12, 13, 14). After 24 hours the number of the mitoses became smaller and the chromosomes were distinctly shortened. Besides a great number

of the cells with micronuclei could be observed. This can be the evidence that chromosome aberrations occured earlier (breaking of the chromosomes and chromosome bridges) or that some disturbances of the spindle occured (elimination of the chromosomes from the telophase nuclei). An increased number of chromosome aberrations, fragments, and bridges was observed under the influence of the washings from bacteria of *Agrobacterium* genus cultured on nutrient agar. Also under the influence of the supernatant of the same bacteria cultured on nutrient agar and on solid Davis medium the same irregularities could be observed after 48 hours (Table 1, 2). The similarity in the action of washings and supernatants could be explained by the fact that in both cases there were substances exuded by bacteria into the medium. Bacterial suspension washed in sterile water caused no disturbances in mitosis but the number of mitoses became smaller. This fact could be the result of the action of the substances due to disintegration of the cells.

In order to define the role of these substances onion roots were treated with a homogenate of bacteria from the *Agrobacterium* genus. Bacteria were suspended into 0,5% glucose solution which by itself did

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Plate I

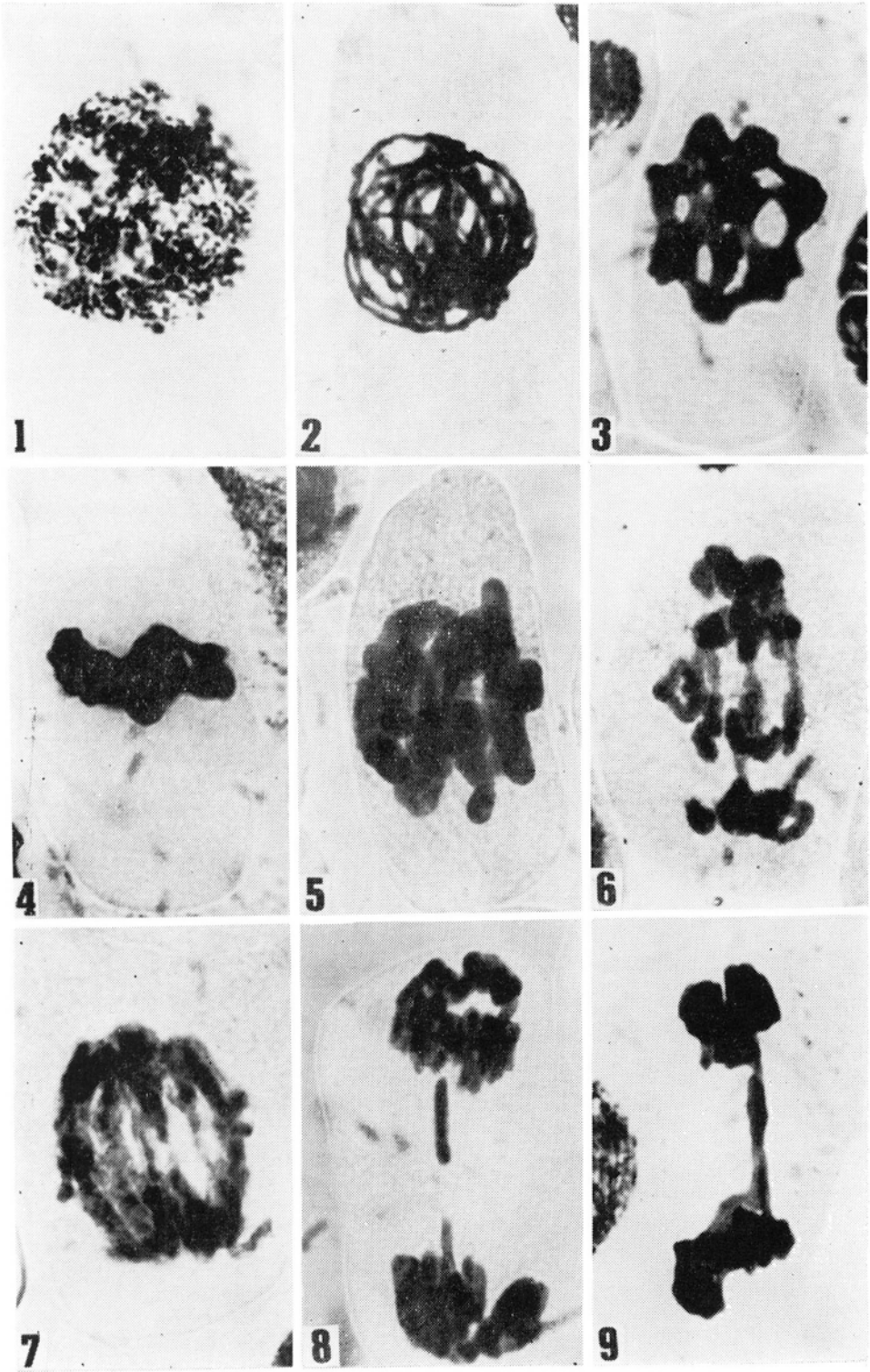
The course of mitosis in adventitious roots of the onion *Allium cepa* L. after 4 treatments with supernatant of bacteria of the different genera cultured on liquid Davis medium.

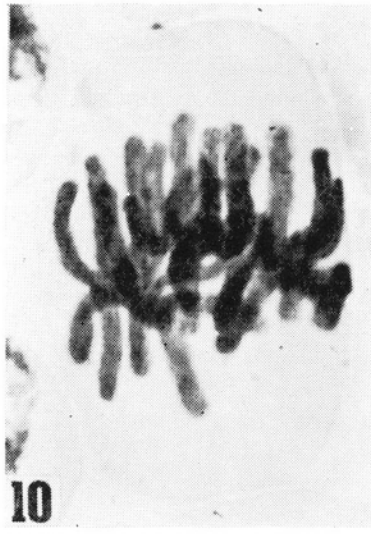
1 — interphases with grained structure of chromatin (*Agrobacterium*) 2 — prophases with homogeneous chromosome structure (*Flavobacterium*) 3, 4 — metaphases with shortened and thickened chromosomes and with disappearance of chromosome structures (*Agrobacterium, Flavobacterium*) 5 — metaphases with shortened and strongly bulging chromosomes (*Escherichia*) 6, 7 — 8 — anaphases with bridges, lagging chromosomes and with lost fragments of chromosomes (*Agrobacterium, Flavobacterium, Pseudomonas*) 9 — telophases with chromosome bridge and with disappearance of chromosome structure (*Agrobacterium*).

Plate II

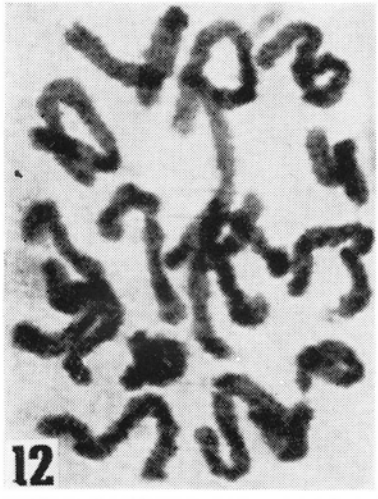
Mitosis in adventitious roots of the onion (*Allium cepa* L.) after treatment with bacteria of *Agrobacterium* genus cultured on onion extract and on nutrient agar. 10 — metaphases after 8 hour treatment with sterile onion extract 11 — c-metaphases fater 8 hour treatment with supernatant of liquid bacterial culture on an onion extract 11, 13, 14 — prophases, metaphases, anaphases with loosely disposed chromosomes after 8 hour treatment with washings of bacteria from nutrient agar 15, 16 — anaphases with lost chromosomes and with fragments of chromosomes after 48 hour treatment with washings of bacteria from nutrient agar.

Plate I




10


11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13


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15


16

Table 1

Level of chromosome aberrations in adventitious roots of the onion (*Allium cepa* L.) after treatment with washings, supernatant and washed bacterial suspension of bacteria from the *Agrobacterium* genus cultured on nutrient agar

Acting agent	Concentration	Level of chromosome aberrations (%) after a time (hours)				
		0	4	8	24	48
Washing	10/150	2,0	4,4	2,7	2,9	7,5
Number of examined anaphases		196	250	183	70	133
Washing	40/150	0,0	2,1	1,4	0,0	5,7
Number of examined anaphases		176	288	140	35	141
Supernatant	10/150	3,3	1,1	0,0	6,5	12,1
Number of examined anaphases		217	267	102	92	157
Bacteria suspension in water	10/150	2,2	1,8	3,1	2,3	×
Number of examined anaphases		134	163	97	43	0

The way of preparing of washing, supernatant and bacterial suspension has been described in "materials and methods".
Concentration: 10/150 or 40/150 — bacteria gathered from 10 or 40 plates and put into 150 ml of sterile water.
× — no date due to lack of mitoses.

Table 2

Level of chromosome aberrations in adventitious roots of the onion (*Allium cepa* L.) after treatment with supernatant of bacteria of *Agrobacterium* genus cultured on a solid Davis medium

Supernatant in concentration	Level of chromosome aberrations (%) after a time (hours)			
	0	8	24	48
5/150	0,6	0,6	×	4,0
Number of examined anaphases	710	300	0	600
20/150	0,0	0,5	×	2,6
Number of examined anaphases	200	200	0	300

The way of preparing the supernatant has been described in "materials and methods".
Concentration: 5/150 or 20/150 — bacteria gathered from 5 or 20 plates nad put into 150ml of sterile water.
× — no data due to lack of mitoses

Table 3

Level of chromosomes aberrations in adventitious roots of the onion (*Allium cepa* L.) after treatment with supernatant of bacteria of *Agrobacterium* genus cultured on nutrient agar

Supernatant in concentration	Level of chromosome aberrations (%) after atime (hours)				
	0	4	8	24	48
10/150	0,0	0,3	×	×	5,0
Number of examined anaphases	110	300	0	0	160
20/150	1,0	5,3	1,3	×	×
Number of examined anaphases	277	245	220	0	0

The way of preparing the supernatant has been described in "materials and methods".
Concentration: 10/150 or 20/150—bacteria gathered from 10 or 20 plates and put into 0,5% glucose dilution.

not cause disturbances in the divisions of the cells. In all cases the homogenate caused shortening of the chromosomes and the number of mitoses became smaller. After 48 hours no mitoses could be seen.

At the same time the effects of supernatants and bacterial suspension washed in 0,5% glucose solution were studied. Under the influence of supernatant the shortening of the chromosomes and increased number of chromosome aberrations could be observed (Table 3). The level of the chromosome aberrations after treating with the suspension of the washed bacteria was normal as well as the appearance of mitoses.

Thus in this paper it has been established that secretions of saprophytic bacteria of *Agrobacterium*, *Flavobacterium* and *Pseudomonas* genera can be responsible for the disturbances in separation of chromosomes.

DISCUSSION

The results of the expariments show that secretions of some saprophytic bacteria can be responsible for chromosone aberrations and for other disturbances in the divisions of the nuclei of adventitious roots of the onion.

The nature of these changes depend on the genus of the bacteria and on the medium in which their multiplication took place. Every genus of the bacteria has a slightly different metabolism, so that the differences in their action seem to be obvious. These phenomena can be explained by the change in the cell metabolism or by the change of the concentration of the mutagenically acting secretions. The character of these substances has not yet been studied. Generally it is known that bacteria exude: toxins, antibiotics and enzymes. It has been established that some of these compounds are mutagenic. Among toxines exuded by bacteria which act on the plants we know marasmines which cause

among others the withering of the plants (Gäumann, Jaag 1950). It is possible that at least in some cases the action of the marasmines on the plants can be due to their influence on the genetic apparatus of the meristematic cells of roots. It is known that coumarine (Querciolli 1955), sulfur amino acids (Woll according to Dubinin 1967), phenols and their derivatives (Levan 1948), amines, amides and urea (Woll according to Dubinin 1967) are responsible for the chromosome aberrations. Gramicidine, produced by *Bacillus brevis*, is an example of an inhibiting action of antibiotics on the plants (Krasilnikov 1947). We know that some antibiotics (as penicillin and streptomycin) cause mutations (Dubinin).

The enzymes liberating active compounds include nucleases and proteases because it is clear that nucleic acids and their precursors, and albumines and products of their disintegration can be responsible for chromosome aberrations (Dubinin 1967; Ahnström, Natarajan 1966), An important part is played also by cellulolytic and tinolytic enzymes which cause the liberation of the phenol compounds (Husoin, Dimond 1960). We know that phenols and quinones and products of their condensation are not only growth inhibitors (Pridham according to Rubin, Arichowska 1971) but also induce mutations.

SUMMARY

In this work the influence of 21 bacterial strains, isolated from onions cultured on liquid media, on the course of mitosis in adventitious roots of *Allium cepa* L. was studied. Ten of these strains belonging to *Agrobacterium, Flavobacterium, Pseudomonas* and *Escherichia* genera caused changes in chromosome structure and in the course of mitosis. The nature of these changes depended on the medium on which bacteria multiplied. Supernatants of bacteria cultured on liquid Davis medium were responsible for the occurrence of the grain structure of chromatin in interphase nuclei, shortening and sticking of the chromosomes in metaphases and the appearance of the lagging chromosomes and bridges in anaphases.

Supernatants of the bacteria of *Agrobacterium* genus cultured on onion extract caused c-mitoses. Water suspension of the bacteria cultured on solid media, on nutrient agar and on Davis medium, caused an increase of the number of chromosome aberrations. Supernatants from these suspensions acted in the same way. Suspensions from the washed bacteria did not cause changes of the level of chromosome aberrations. After treating roots with homogenate no divisions of the cells occured.

The results of these experiments show that secretions of some saprophytic bacteria can affect the genetic apparatus of roots' cells.

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Wpływ wydzielin bakterii saprofitycznych na przebieg mitozy w korzeniach przybyszowych *Allium cepa* L.

Streszczenie

W pracy przebadano wpływ 21 szczepów bakterii, wyizolowanych z wodnych kultur cebul, na przebieg mitozy w korzeniach przybyszowych *Allium cepa* L. Dziesięć z tych szczepów, należących do rodzajów *Agrobacterium*, *Flavobacterium*, *Pseudomonas* i *Escherichia* powodowało zmiany w strukturze chromosomów i prze-

biegu mitozy. Charakter tych zmian zależał od podłoża, na którym były one namnażane. Supernatanty z hodowli bakterii w płynnej pożywce Davisa były przyczyną pojawiania się ziarnistej struktury chromatyny w jądrach interfazowych, silnego skrócenia i sklejenia się chromosomów w metafazie oraz występowania wlokących się chromosomów i mostków chromosomowych w anafazie. Supernatant z bakterii z rodzaju *Agrobacterium*, hodowanych na wyciągu z cebuli, wywoływał c-mitozy. Wodne zawiesiny bakterii na pożywkach stałych; agarze odżywczym i pożywce Davisa, powodowały wzrost poziomu aberracji chromosomowych. Supernatanty z tych zawiesin wykazywały takie samo działanie. Zawiesina przepłukanych bakterii w wodzie nie wpływała na poziom aberracji chromosomowych. Po działaniu homogenizatem z bakterii nie obserwowano podziałów komórkowych.

Wyniki tych doświadczeń wskazują, że wydzieliny niektórych bakterii saprofitycznych mogą wpływać na aparat genetyczny komórek korzeni.