ANTIBACTERIAL SUBSTANCE IN SEEDS OF FRAXINUS EXCELSIOR L.

By
L. FERENCZY
Institute for Plant Physiology of the University of Szeged
(Received March 26, 1956)

Examinations relating to the resting period of the seeds of *Fraxinus excelsior* L. led to the conclusion that the dormancy is produced by the inhibitory substance contained in the inner mucinous layer of the seeds (1).

Now the question arose: has the inhibitor of the seeds of *Fraxinus excelsior* L. — which markedly reduces the germination of other seeds as well — its static effect also on microorganisms?

To answer this question the effect on 18 different Gram positive and negative bacterium species, partly pathogenic, partly saprogenic, has been studied and observed.

Material and methods

The seeds of the *Fraxinus excelsior* L. were harvested in the forest of *Tös*, County of Bács-Kiskun. The investigations began a month after harvest.

To determine the bacteriostatic sensitivity the agar-diffusive method was used (2). The experiments were carried out in Petri-dishes at 8 mm thickness, pH 7, broth medium containing 2% agar.

A thin suspension from the broth (24 hours) culture of the bacterium species has been transferred on the hardened culture medium and having dried the surface (about 10 minutes) the seeds were transversally cut in two halves and immersed edgewise into the culture medium, thus rendering possible the diffusion of the inhibitor from the mucinous layer. Then the dishes were placed in the thermostat at 30° C. Readings were made after 20 hours.

In the case of antibacterial effect the bacterium responsive to inhibitor is not able to increase within the diffusion zone, the intensity of the effect is shown by the size of the clear zone developed during the incubation.

The results are taken from the data of 20 parallelly made experiments.

Results

As the mucinous layer is in contact with the culture medium on only one side of the seed opened, while on the other side the endosperm and the seed coat prevent the diffusion of the substance of the mucinous layer, consequently

the inhibitory zone appears only on the open surface of the seeds, beside the mucinous layer, in a semicircle.

The table below shows the bacteriostatic effect of the seeds of *Fraxinus excelsior* L. relating to various bacterium species and strains.

1. *Aerobacter aerogenes*	—
2. *Agrobacterium tumefaciens*	4
3. *Bac. cereus*	3
4. *Bac. megatherium 208*	3
5. *Bac. Morgani*	2
6. *Bac. cereus var. mycoides*	4
7. *Bac. New Castle*	2
8. *Bac. subtilis* var. *panis*	2
9. *Erwinia carotovora*	2
10. *Escherichia coli 1*	—
11. *Proteus X 19*	—
12. *Pseudomonas aeruginosa 3*	—
13. *Shigella Flexneri VI*	2
14. *Shigella Shiga*	2
15. *Staphylococcus albus*	2
16. *Staphylococcus Buttle*	3
17. *Xanthomonas malvacearum*	1
18. *Xanthomonas solanacearum*	2

The numbers denote the radius of the clear zones in mm.

The data of the table explicitly prove the presence of the antibacterial inhibitor in the seeds. The effect — though of different intensity and selective — is clearly shown up in a wide bacteriological spectrum. The inhibitor arrests the increase of all the Gram positive and of several Gram negative species.

No inhibition os in the increase of *Pseudomonas aeruginosa 3*, whereas the pyocyanin formation, characteristic of the species, beside the mucinous layer, within the 3 mm zone, fails.

Experiments made in different periods of the stratification of the seeds showed that the decrease of the antibacterial efficacy is proportionate to the effect inhibiting the germination. Observations obtained from both the localisation of the substance and the preliminary studies of the isolation indicate that the natural substance producing the dormancy and the substance of the antibacterial effect is in all probability identical. To clear up the chemical nature of the substance the isolation has been commenced.

Summary

The mucinous layer producing the dormancy of the seeds of *Fraxinus excelsior* L. possesses also antibacterial effect; the 18 bacterium species used in the experiment reduce the increase of all the Gram positive and of several Gram negative species. *Pseudomonas aeruginosa 3* produces no pyocyanin in the presence of the inhibitor. The decrease of the effect is proportionate to the time of stratification.

Reference

1. *Ferenczy, L.* Acta Biol. Szeged, **1**, 17—24, (1955).
2. *Köhler, H.* Nachr. Deutsch. Pflschutzdienst **8**, 1—9, (1954).