

Effect of Sodium Fluoride on Growth and Some Enzymatic Activities of Four Bacteria Pathogenic to Melons

Yousry E. Saleh and Mary S. Khalil

Botany Department, Faculty of Science, Cairo University,
Cairo, Egypt.

ABSTRACT. The effect of different concentrations of sodium fluoride on growth and the enzymatic activities, *in vivo*, of *Erwinia carotovora*, var. *carotovora*; *Erwinia carotovora* var. *citrullis*; *Erwinia toxica* and *Pseudomonas lachrymans* was studied. The results show that *E. toxica* seemed to be highly tolerant to sodium fluoride (LD_{50} at $10^{-2}M$), followed by *P. lachrymans* and *E. carotovora* var. *carotovora* whereas *E. carotovora* var. *citrullis* was most sensitive.

Neither glutamic acid decarboxylase nor lysine decarboxylase was detected in the four pathogens, but arginine decarboxylase was present in *E. carotovora* var. *citrullis*. Its activity decreased with increased sodium fluoride concentration and ceased at $10^{-1} M$.

Sodium fluoride inhibited alkaline and acid phosphatase of *E. toxica* and *P. lachrymans*. Such effect was only apparent at $10^{-3} M$ or above for *E. carotovora* var. *carotovora* or at $10^{-2} M$ or above for *E. carotovora* var. *citrullis*. Lower concentrations seemed without effect for the former but were stimulatory for the latter organism. Sodium fluoride did not induce the production of the proteolytic or asparaginase enzymes.

Pollution is a very broad term. It includes the spreading of a large number of substances in the atmosphere. Fluorides are considered one of the several contaminants currently emitted. In the Rhone valley (Switzerland), fluoride emissions did not only cause drought damage to the forests but also contributed to the growth shock of the trees. Fluoride emissions raised the fluoride content of the soil especially in the water soluble components. This enhanced leaching losses of organic matter, aluminium and iron (Flueherl *et al.* 1981).

Visible damage to the lichen thalli was observed when the internal fluoride concentration exceeded 68 ppm. Internal damage started when fluoride concentration exceeded 90 ppm. Such damage was attributed to high concentrations rather

than any other pollutant (Davies 1982). Young and Broadbent (1982) reported that protease was inhibited by phenyl methyl sulphonyl fluoride (PMSF). Similarly, when PMSF (a serine protease inhibitor) was added to the tolerant strains of *Bacillus subtilis*, they became susceptible to nafcillin - induced lysis (Jolliffe *et al.* 1982).

In animals, Lavrushenko (1982) showed that sodium fluoride inhibited succinate dehydrogenase of the rat liver mitochondria whereas Farley *et al.* (1983) proved that treatment with sodium fluoride increased proliferation and alkaline phosphatase activity in chick bone cells *in vitro*. Similarly, sodium fluoride is required for optimal activity of glycosyl transferase of *Dirofilaria immitis* (Comley *et al.* 1982).

In plants, sodium fluoride significantly stimulated growth of tea pollen tubes even at 1-2 mM concentration (Konishi and Miyamoto 1983). In bacteria, Hamilton (1977) proved that fluoride inhibited glycolysis by *Streptococcus mutans*. He mentioned that bacteria, particularly oral streptococci acquire resistance to high concentrations of fluoride. Prahoveanu and Esanu (1981) showed that sodium fluoride administration reduced hemagglutination titers recorded in lung suspensions of rats experimentally infected with influenza virus.

This preliminary investigation was performed in order to throw some light on the response of some plant pathogenic bacteria to sodium fluoride administration.

Material and Methods

The following phytopathogenic organisms were tested in this investigation:

1. *Erwinia carotovora* (Jones) Holland var. *carotovora* Dye, causing wilt and soft rot of melons fruits (Saleh *et al.* 1984).
2. *Erwinia carotovora* (Jones) Holland var. *citrullis* Sizova, causing rotting of melons fruits, leaf spot and wilt of shoot system (Saleh *et al.* 1985).
3. *Erwinia toxica* Korobko, causing wilt as well as dry rot of melons and water melon fruits (Saleh and Korobko 1982).
4. *Pseudomonas lachrymans* (Smith and Bryan) Carsner, causing irregular necrotic spots on melon and water melon leaves (Saleh and Korobko 1981).

Nutrient bouillon, amended with a range of 10^{-1} - 10^{-4} M sodium fluoride, was used in this investigation. It has the following composition in g/1 solution: beef extract, 3 g; peptone, 5 g and sodium chloride, 3 g. The pH was adjusted to 7.0.

25 ml of the nutrient solution were inoculated with 1 ml of a bacterial suspension containing 5×10^8 cells and incubated, in 150 ml flasks for 12 hr at 28°C. The rate of growth was determined through the bacterial count, using the dilution plate method.

The biomass was further harvested, by centrifugation, washed several times with sterile distilled water then suspended in saline solution.

The procedures described by Bergmeyer (1974), for determination of acid and alkaline phosphatase as well as asparaginase activities were applied in this investigation.

The proteolytic activity was detected using the Srinivasan *et al.* (1964) method whereas arginine, lysine and glutamic acid decarboxylases were tested, applying Möller's medium (1955). In all these assays 0.1 ml of the bacterial suspension containing 5×10^8 cells was added to the substrate solution or medium. At least 5 replicate flasks were used in each experiment.

Results and Discussion

Figure 1 shows that the total count of *E. carotovora* var. *carotovora* and *E. toxica* was hardly affected by the presence of 10^{-4} M sodium fluoride in the medium but the total count of the remaining bacteria was attenuated. Further increase in sodium fluoride concentration lowered the total bacterial count to the extent of complete cessation of growth (*E. carotovora* var. *carotovora* and *P. lachrymans*).

Figure 2 shows that *E. toxica* was the fastest and *P. lachrymans* was the slowest grower. The rate of multiplication of *E. toxica*, in presence of sodium fluoride was least affected till 10^{-3} M above which the rate dropped by 50% of the original. On the other hand, the rate of multiplication of *E. carotovora* var. *citrullis* was severely attenuated by 10^{-4} M fluoride ion compared to either *E. carotovora* var. *carotovora* (almost unaffected) or *P. lachrymans*. Larger doses slightly affected the inhibited rate of multiplication of *P. lachrymans* but attenuated that of *E. carotovora* var. *carotovora*; 10^{-1} M sodium fluoride proved highly toxic. Not only did it arrest cell division and/or multiplication but also caused complete or partial death of the cells of the inoculum.

This indicates that *E. toxica* was most tolerant followed by *P. lachrymans* and *E. carotovora* var. *carotovora* whereas *E. carotovora* var. *citrullis* was most sensitive to fluoride ions.

In this connection it may be mentioned that according to Hamilton and Bowden (1982) addition of fluoride to the medium of *Streptomyces mitior* and *S. mutans*, though reduced the growth and metabolism of both organisms, nevertheless permitted the growth of *S. mitior* at a level that ensured its survival. Both cultures developed phenotypic resistance at the higher levels of fluoride (16-20 mM).

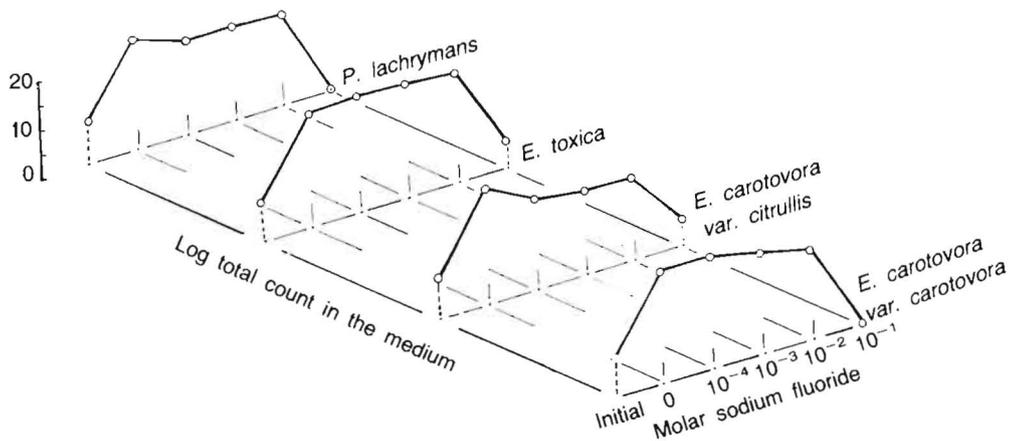


Fig. 1. Effect of various concentrations of sodium fluoride on the total count of the test organisms after 12 hr incubation

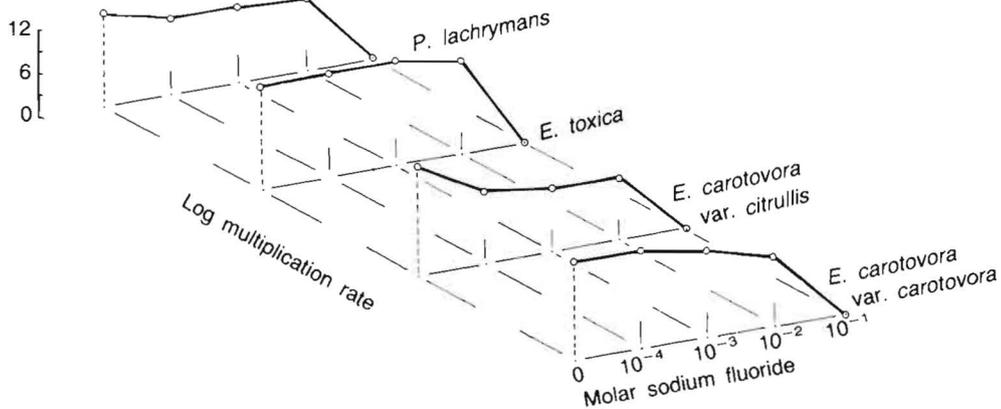


Fig. 2. Effect of various concentrations of sodium fluoride on the rate of multiplication of the test organisms after 12 hr incubation

Enzyme Activity

Trials to detect proteolytic or asparaginase activity were not successful. Similarly the four bacteria were unable to decarboxylate glutamic acid, lysine or arginine except *E. carotovora* var. *citrullis* that could decarboxylate arginine in the absence or presence of sodium fluoride. The activity decreased with increased concentration of fluoride but was totally undetected at 10^{-1} M concentration.

Figure 3 shows that *E. carotovora* var. *citrullis* possessed the highest alkaline and acid phosphatase activity followed by *E. carotovora* var. *carotovora* whereas *P. lachrymans* had the least activity. Growth of *E. toxica* or *P. lachrymans* in various concentrations of sodium fluoride highly attenuated both phosphatase activities. Similarly both phosphatases of *E. carotovora* var. *carotovora* were attenuated by 10^{-3} M or above whereas those of *E. carotovora* var. *citrullis* were stimulated by the lower sodium fluoride concentrations up to 10^{-3} M above which the activity declined. 10^{-1} M almost arrested the activity of the enzyme in the four tested organisms.

This indicates that the effect of sodium fluoride on alkaline and acid phosphatase depended on the concentration and/or tolerance of the organisms to such treatment. The highly tolerant organism (*E. toxica* and *P. lachrymans*) already had the least phosphatase activity that was suppressed even by the least applied sodium fluoride concentrations. The most sensitive organism (*E. carotovora* var. *citrullis*) already possessed the highest phosphatase activity that was further stimulated when cultured in fluoride concentrations reaching 10^{-3} M.

It is interesting to note that the most sensitive organism was the fastest grower. Such high rate of multiplication required energy to be supplied as ATP. It seems that the rate of ATP formation, by such organism was lagging behind the rate of multiplication, and thus the phosphatase activity increased in order to supply energy through hydrolysis of other phosphorylated compounds, prevailing in the cells, than ATP. Still transphosphorylation to ADP or AMP could be operating through the activity of these phosphatases and thus supplying more ATP. Several phosphomonoesterases both acid (Appleyard 1948, Axelrod 1948, Morton 1958, Boer and Steyn-Parve 1970) and alkaline (Wilson *et al.* 1964) possessed such property.

With the drop in TCA cycle, as a result of the high sensitivity of enolases (including aconitase) to fluoride (Mengel and Kirkby 1978); the stimulated glucose - 6 - phosphate dehydrogenase, catalase, peroxidase and cytochrome oxidase activity by the same ion (Lee *et al.* 1965) seemed unable to cope for enough supply of ATP thus phosphatase activity further increased.

Generally speaking, fluoride inhibition is extremely used to study the catalytic sites of various esterases (Haugen and Suttie 1974). Mornstad (1982) showed that

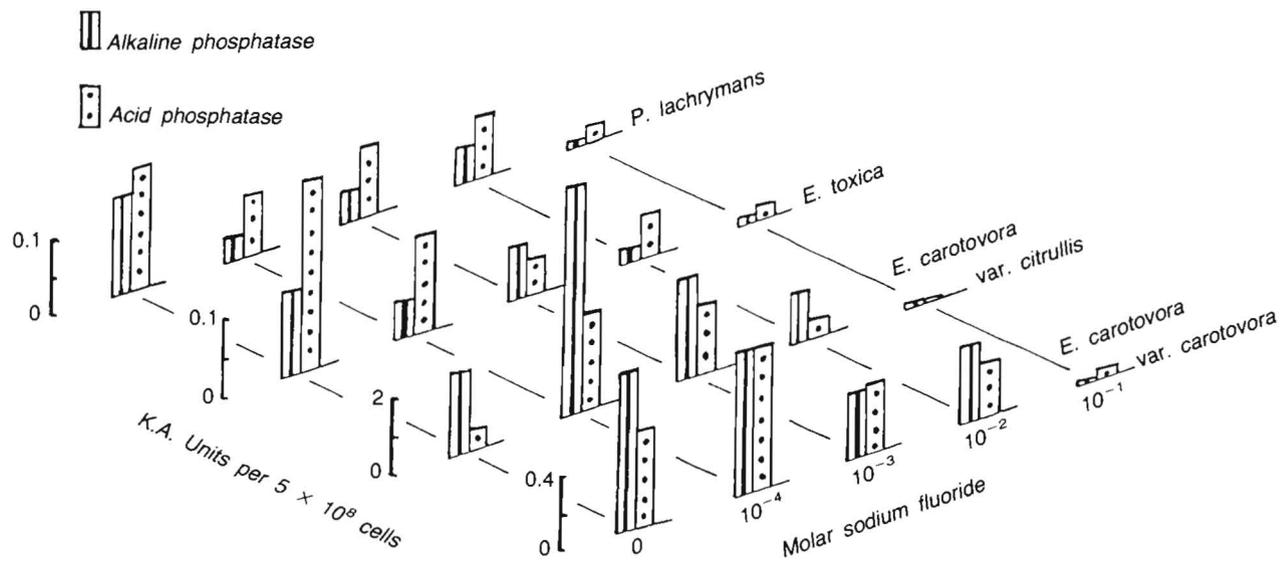


Fig. 3. Effect of various concentrations of sodium fluoride on the alkaline and acid phosphatase activity of the test organisms

crude acid phosphatase extract from the excretory enamel of rats was strongly inhibited by sodium fluoride concentrations higher than 1 mM. The purified enzyme was strongly sensitive to 10 mM concentration. Similarly, Lau and Lee (1982) showed that the activity of glucose - 1 - phosphate and glucose - 6 - phosphate phosphohydrolase of *Escherichia coli* and *Neisseria meningitidis* decreased at 10^{-4} M fluoride concentration. Maximum inhibition of glucose - 6 - phosphate hydrolysis (98%) was reached at 10^{-2} M which induced only 70% inhibition for glucose - 1 - phosphate hydrolysis. Higher concentration of fluoride lowered the inhibition of the enzyme; a phenomenon that was not observed in this study. According to Smith and Peters (1981), sodium fluoride (up to 1 mM) strongly inhibited acid pyridoxal phosphate phosphatase but had little effect against acid phosphatase of human leukocytes.

From the above observations one might apply small doses of sodium fluoride to the soil in order to suppress or totally arrest the growth of one or more of the bacterial pathogens to melons and water melons without injuring the host itself. According to Mengel and Kirkby (1978) even when the sodium fluoride levels were high, as in acid soil conditions, however, soil fluoride was not readily taken up by plant roots.

References

- Appleyard, J.** (1948) The effect of alcohols on the hydrolysis of sodium phenolphthalein diphosphate by prostatic extracts, *Biochem. J.* **42**: 596-597.
- Axelrod, B.** (1948) A new mode of enzymatic phosphate transfer, *J. Biol. Chem.* **172**: 1-13.
- Bergmeyer, H.U.** (1974) *Methods of Enzymatic Analysis*, vol. 2, Academic Press, pp. 685-690.
- Boer, P. and Steryn-Parve, E.** (1970) Further studies on the mechanism of action of an acid phosphatase from baker's yeast (*Saccharomyces cerevisiae*), *Biochim. Biophys. Acta* **206**: 281-288.
- Comley, J.C., Jaffe, J.J. and Chrin, L.R.** (1982) Glycosyl transferase activity in homogenates of adult *Dirofilaria immitis*, *Mol. Biochem. Parasitol.* **5**: 19-32.
- Davies, F.B.M.** (1982) Accumulation of fluoride by *Xanthoria parietina* growing in the vicinity of bedfordshire brickfields, *Environ. Pollut. Ser. A Ecol. Biol.* **29**(3): 189-196.
- Farley, J.R., Wergedal, J.E. and Baylink, D.J.** (1983) Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone - forming cells, *Science*, **222**(4621): 330-332.
- Flueherl, H., Felix, K., Hans, U.S., Bernhard, D., Janina, P., Theodor, K., Heinz, S., Fritz, H.S., Fellix, M. and Peter, B.** (1981) Assessment of forest damage and air pollution in the Rhone valley (Switzerland), *Eidg. Anst. Forsth. Versuchswes. Mitt.* **57**(4): 358-500.
- Hamilton, I.R.** (1977) Effect of fluoride on enzymatic regulation of bacterial carbohydrate metabolism, *Caries Res.* **11** (Suppl. 1): 262-291.
- Hamilton, I.R. and Bowden, G.H.** (1982) Response of freshly isolated strains of *Streptococcus mutans* to change in pH in the presence and absence of fluoride during growth in continuous culture, *Infect. immun.* **36**(1): 258-262.
- Haugen, D.A. and Suttie, J.W.** (1974) Fluoride inhibition of rat liver microsomal esterase, *J. Biol. Chem.* **249**: 2723-2731.
- Jolliffe, L.K., Ronald, J.D. and Uldis, N.S.** (1982) Extracellular proteases increase tolerance of *Bacillus subtilis* to nafcillin, *Antimicrob. Agents Chemother.* **22**(1): 83-89.

- Konishi, S.** and **Miyamoto, S.** (1983) Alleviation of aluminium stress and stimulation of tea (*Camellia sinensis*) pollen tube growth by fluoride, *Plant Cell Physiol.* **24**(5): 857-862.
- Lau, B.W.C.** and **Lee, Y.** (1982) Effect of fluoride on sugar phosphate phosphohydrolase from *Escherichia coli* and *Neisseria meningitidis*, *Int. J. Biochem.* **14**: 565-567.
- Lavrushenko, L.F.** (1982) Effect of sodium fluoride on succinate dehydrogenase and NADH: Cytochrome c - reductase of rat liver mitochondria, *Ukrabiokhim. Zh.* **54**(1): 86-88.
- Lee, C., Miller, G.W.** and **Welkie, G.W.** (1965) The effects of hydrogen fluoride and wounding on respiratory enzymes in soybean leaves, *Air Water Pollut. Int. J.* **10**: 169-181.
- Mengel, K.** and **Kirkby, E.A.** (1978) *Principles of Plant Nutrition*, Der Bund AG (Bern Switzerland, pp. 510-511, I.P.I. Publication.
- Moller, V.** (1955) Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system, *Acta Path. Microbiol. Scand.* **36**: 158-172.
- Morton, R.K.** (1958) The phosphotransferase activity of phosphatases, *Biochem. J.* **70**: 150-155.
- Mornstad, H.** (1982) Multiple forms of acid phosphatase (E.C. 3, 1, 3, 2) in rat secretory enamel organ, *Scand. J. Dent. Res.* **90**(4): 263-270.
- Prahoveanu, E.** and **Esanu, V.** (1981) The effect of sodium fluoride on experimental influenza virus infection in mice, *Rev. Roum, Med. Virol.* **32**(4): 293-295.
- Saleh, Y.E.** and **Korobko, A.P.** (1981) Some biological aspects of bacterial disease of melons and water-melons in Ukrainian SSR, *Egypt. J. Bot.* **24**: 219-229.
- Saleh, Y.E.** and **Korobko, A.P.** (1982) Some biological aspects of bacterial diseases of melons and water-melons in Ukrainian SSR. 3-E. *toxica* Korobko, *Egypt. J. Bot.* **25**: 14-23.
- Saleh, Y.E., Naguib, M.I.** and **El-Hindawy, H.H.** (1984) Biological studies on bacteria pathogenic to melons in Egypt caused by *E. carotovora* (Jones) Holland var. *carotovora*, *Egypt. J. Bot.* **27**: 14-28.
- Saleh, Y.E., Naguib, M.I.** and **El-Hindawy, H.H.** (1985) Biological studies on bacteria pathogenic to melons in Egypt caused by *Erwinia carotovora* (Jones) Holland var. *citrullis* Sizova, *Proc. Egypt. J. Bot. Soc.* **4**: (Ismailia conf.).
- Smith, G.P.** and **Peters, T.J.** (1981) Subcellular localization and properties of pyridoxal phosphate phosphatase of human polymorphonuclear leukocytes and their relationship to acid and alkaline phosphatase, *Biochim. Biophys. Acta* **661**: 287-294.
- Srinivasan, R.A., Iyengar, M.K.K., Babbar, I.Y., Chakraborty, S.C., Dudania, A.T.** and **Iya, K.K.** (1964) Milk clotting enzymes from microorganisms, *Appl. Microbiol.* **12**: 476-478.
- Wilson, I.B., Dayan, J.** and **Cyr., K.** (1964) Some properties of alkaline phosphatase from *Escherichia coli*. Transphosphorylation, *J. Biol. Chem.* **239**: 4182-4185.
- Young, D.B.** and **Broadbent, D.A.** (1982) Biochemical characterization of extracellular protease from *Vibrio cholerae*, *Infect. Immun.* **37**(3): 875-883.

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تأثير فلوريد الصوديوم على النشاط الانزيمي لأربعة أنواع من البكتيريا الممرضة للشمام

يسري السيد صالح و ماري صبحى خليل

قسم النبات - كلية العلوم - جامعة القاهرة - القاهرة - مصر

درس تأثير تركيزات مختلفة من فلوريد الصوديوم على النشاط الانزيمي لبكتيريا الاروينيا كاروتوفورا سلالة كاروتوفورا واروينيا كاروتوفورا سلالة سيتروليس واروينيا توكسيكا وبسيد وموناس لاكلريمانس . وقد أثبتت النتائج أن فلوريد الصوديوم يكون مشبهاً لتكوين انزيمات الفوسفاتيز الحمضية والقلوية لبكتيريا الاروينيا توكسيكا والبسيد وموناس لاكلريمانس ، وقد أتضح هذا التأثير فقط عندما يصل التركيز إلى 10^{-3} جزىء أو أكثر بالنسبة لبكتيريا الاروينيا كاروتوفورا سلالة كاروتوفورا وعند تركيز 10^{-2} جزىء أو أكثر بالنسبة لبكتيريا الاروينيا كاروتوفورا سلالة سيتروليس . كما أثبتت الدراسة أن التركيزات المنخفضة لم يكن لها تأثيراً واضحاً بالنسبة للنوعين الأولين في حين أنها كانت منشطة بالنسبة للنوعين التاليين . كما وجد أن فلوريد الصوديوم ليس له تأثير على تكوين انزيمات البروتوليز والاسبرجينيز . بالنسبة لانزيمات الليزين والجليوتاميك ديكر بوكسيليز فلم تتكون في الأربعة أنواع من البكتيريا ، في حين أن أنزيم الأرجنين ديكر بوكسيليز تتكون فقط في بكتيريا الأروينا كاروتوفورا سلالة سيتروليس ، ويقل نشاط تكوين هذا الأنزيم بزيادة تركيز فلوريد الصوديوم ويقف تماماً عند تركيز 10^{-1} جزىء . كذلك وجدت الدراسة أن بكتيريا الاروينيا توكسيكا لها درجة تحمل عالية في وجود فلوريد الصوديوم تليها بكتيريا البسيد وموناس لاكلريمانس والاروينيا كاروتوفورا سلالة كاروتوفورا بينما تتأثر بشدة بكتيريا الاروينيا كاروتوفورا سلالة سيتروليس .