Development of thin layer chromatography – direct bioautography tests based on *Escherichia coli* and *Bacillus subtilis* strains

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Thin layer chromatography coupled with direct bioautography (TLC-DB) combines low cost and simplicity of microbiological methods with high sensitivity and specificity of analytical methods. TLC-DB does not only allow to separate the tested compounds; it also helps to verify their biological activity.

This work presents the research results concerning the development of the novel TLC-DB tests based on Gram-negative bacteria, *Escherichia coli* and Gram-positive bacteria, *Bacillus subtilis*. Influence of various factors on the viability of the bacteria was studied: time of pre-incubation and incubation of the microorganisms, pre-conditioning of the TLC plates, viscosity of the culture broth and the incubation time of the seeded TLC plates. The tests were checked for flumequine standards applied in various amounts at TLC plates.

Thin Layer Chromatography – Direct Bioautography

using Gram-negative bacteria, *Escherichia coli*

Preparation of the TLC plates:

1) **Sample application**
2) **Development with a proper mobile phase**
3) **Air-drying**

Microbiological conditions:

1) **Test organisms:**
   - ***Escherichia coli* (ATCC 25922)**
2) **Pre-incubation at 37 °C for 20 h**
3) **Incubation at at 37 °C for 2 h**

1) **Dipping in the bacterial suspencion (app. 4 x 10^7 c.f.u./mL) for 9 sec**
2) **Incubation of the TLC plates in a water vapour chamber at 37 °C for 5 h**
3) **Visualization by spraying the TLC plates with 0.2 % MTT aqueous solution with 0.1 % Triton X-100**
4) **Incubation in a water vapour chamber at 37 °C for 0.5 h**

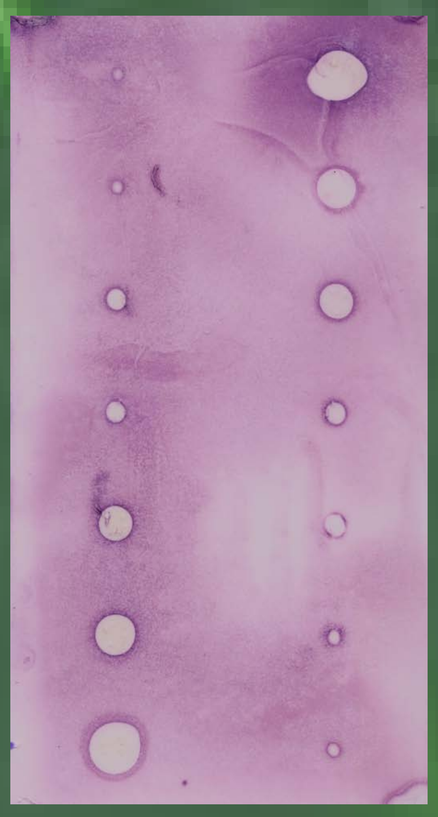

Fig. 1. TLC-DB using *Escherichia coli*. Flumequine standard solutions, TLC Si60F_{254} plate. The applied volume: 10 µl. The concentrations applied are as follows:
left track (going down) 0.005, 0.01, 0.05, 0.1, 0.5, 1.0 (µg);
right track (going down) : 1.0, 0.5, 0.1, 0.05, 0.01, 0.005 (µg).

using Gram-positive bacteria, *Bacillus subtilis*

Preparation of the TLC plates:

1) **Sample application**
2) **Development with a proper mobile phase**
3) **Air-drying**

Microbiological conditions:

1) **Test organisms:**
   - ***Bacillus subtilis* (ATCC 6633)**
2) **Pre-incubation I at 37 °C for 1 h**
3) **Pre-incubation II at 37 °C for 1 h**
4) **Incubation at at 37 °C for 6 h**

1) **Dipping in the bacterial suspencion (app. 4 x 10^7 c.f.u./mL) for 9 sec**
2) **Incubation of the TLC plates in a water vapour chamber at 37 °C for 17 h**
3) **Visualization by spraying the TLC plates with 0.2 % MTT aqueous solution with 0.1 % Triton X-100**
4) **Incubation in a water vapour chamber at 37 °C for 0.5 h**

Fig. 2. TLC-DB using *Bacillus subtilis*. Flumequine standard solutions, TLC Si60F_{254} plate. The applied volume 10 µl. The concentrations applied are as follows:
left track (going down) 0.005, 0.01, 0.05, 0.1, 0.5, 1.0 (µg);
right track (going down) : 1.0, 0.5, 0.1, 0.05, 0.01, 0.005 (µg).

Conclusions

The TLC-DB method was tested for flumequine standards. The experiments indicated that it should be possible to determine the residues of flumequine on its MRL level.

Literature:

[1] E.M. Grzelak, B. Majer-Dziedzic, I.M. Choma, *JAOAC Int.* (in press)