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Changes of metabolic activity of *Brevibacillus laterosporus* BT271 in presence of phenol and zinc

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INTRODUCTION:

The sustainable use of the natural resources and the introduction of the principles of the circular economy are two trends that are particularly relevant in the context of the European efforts for sustainable economic growth. One of the ecologically promising approaches in the restoration of contaminated soils is the bioremediation technology. The approach in which highly active biodegrading bacteria are added in bioremediation activities is a well-known one. What makes it even more attractive is the possibility to use the biodetoxification activity of some bacteria while it is combined with their abilities to promote the plants growth. An example of this is the bacteria from genus *Brevibacillus*. The presented study demonstrates an investigation of the metabolic activity of *Brevibacillus laterosporus BT271* in presence of five phenol (5-1000 mg/L) and zinc (50-1000 mg/L) concentrations.

MATERIALS AND METHODS:

Brevibacillus laterosporus BT271 was kindly provided by prof. Y. Topalova. The bacteria were isolated from phenol contaminated soil and it possesses high bioremediation potential. In these experiments they were cultured on nutrient broth at 30°C. *B. laterosporus BT271* in exponental phase were used (14 h).

The metabolic activity of the *B. laterosporus BT271* was determined by **tetrazolium salts** - 2,3,5-triphenyltetrazolium chloride (TTC) and 5-Cyano-2,3-ditolyl tetrazolium chloride (CTC). The bacteria count was monitored spectrophotometrically at 480 nm.

The **total dehydrogenase activity** (Lenhard 1956; 1964) represents the total enzymatic activity of dehydrogenases in the cells. The method is based on measuring the concentration of formazan obtained by reduction of 2,3,5-triphenyltetrazolium chloride (TTC).

The fluorescence analysis based on CTC determinates the **active and dead/fixed cells**. CTC is a dye that is reduced to the fluorescent CTC formazan in respiratory processes in cells.

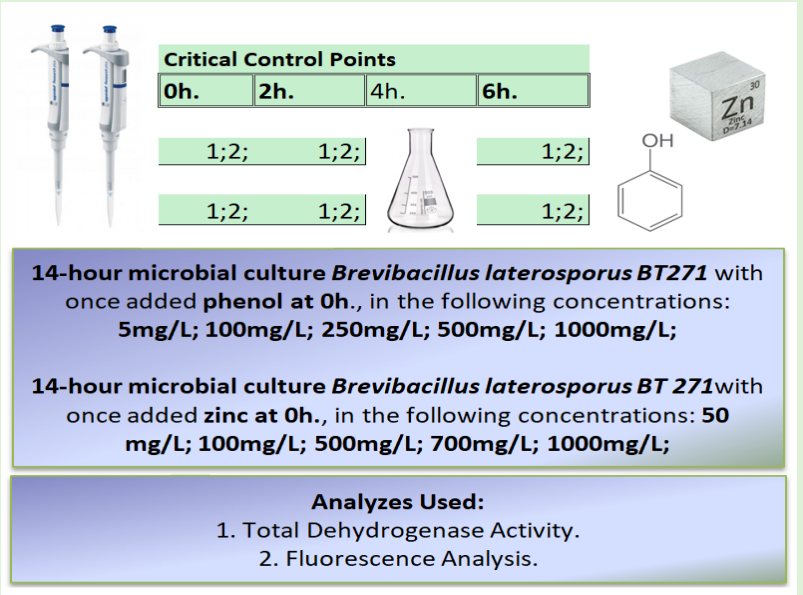

Fig.1 *Experimental design.*

Five concentrations of **phenol** or **zinc** (in the form of $ZnSO_4$) were added to the bacterial culture (0 h). The metabolic activity were studied at 0 h., 2 h. and 6 h.

RESULTS AND DISCUSSIONS:

B.laterosporus BT271 with phenol

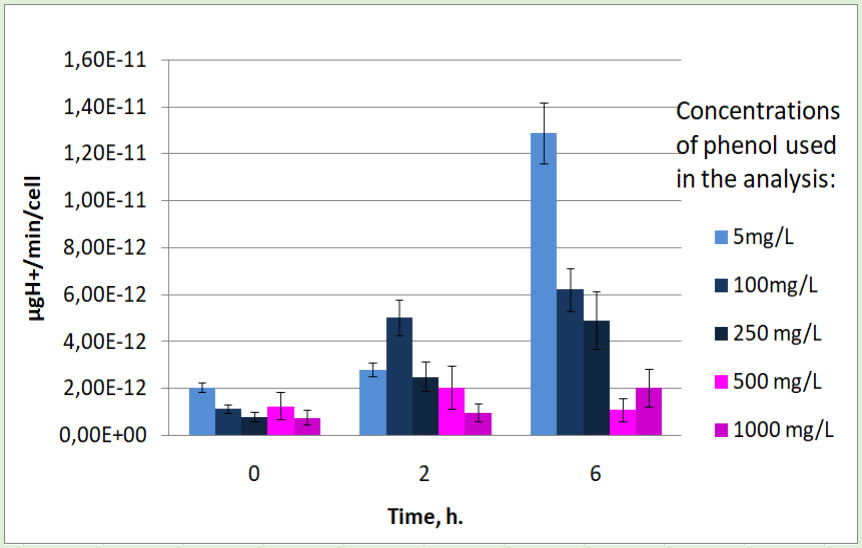

Fig. 2 *Total Dehydrogenase Activity (TDA) on B.laterosporus BT271 with phenol (5-1000 mg/L).*

As it can be seen on fig. 2, the activity of the bacteria was lower on the 0 h. At the 2 h the metabolic activity of *B.laterosporus BT271* increased with 122 %. After six hours of incubation it increased with 3.5 times. This proves that the studied bacteria are active biodegraders of phenol and phenolic compounds.

The increase of the activity of *B.laterosporus BT271* was more sign**ificant when lower phenol concentrations were applied** (5-250 mg/L). The TDA activity increased with 5 times for this concentrations, while the 500-1000 mg/L phenol raised the TDA with 54 %. This was expected and it reflects the toxic effects from the xenobiotic. Nevertheless, **an activation of the bacterial metabolism was registered when phenol was present regardless of the concentration**. This proves that *B.laterosporus BT271* were suitable for bioremediation applications.

B.laterosporus BT271 with zinc

Brevibacillus laterosporus BT271 with zinc.

Total Dehydrogenase Activity

Zn	50mg/L	100mg/L	500 mg/L	700 mg/L	1000 mg/L
0h.	0	0	0	0	0
2h.	0	0	0	0	0
6h.	0	0	0	0	0

Fig. 3 *Results of the determination of total dehydrogenase activity (Lenhard 1956; 1964) of a 14-hour culture of B. laterosporus BT271 in which five concentrations of zinc were applied.*

As it can be seen from the table that **the total dehydrogenase activity** tested by the TTC method **was not recorded at any of the zinc concentrations** applied in the experiments. This data due to the ability of *B. laterosporus BT271* to form spores when exposed to unfavorable conditions. The CTC/DAPI based fluorescence analysis showed almost complete sporulation of the bacteria (fig. 4).

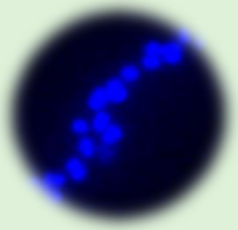

Fig. 4 *Image from the flourescence analysis that shows the sporulation of the bacteria B. laterosporus BT271.*

The fluorescent analysis demonstrated that the active bacteria reached 51 % and 63 % of the bacteria in the samples when 250 mg/L and 1000 mg/L were applied. Overall **the percentage of the active bacteria were higher after 6 hours of incubation** (33%) in the presence of phenol compared to the 0 h (12%) when only nutrient broth was in the media. This is yet another prove for the survival capabilities of *B.laterosporus BT271* in presence of phenol.

The CTC/DAPI based fluorescence analysis on *B.laterosporus BT 271* with five concentrations of phenol (5-1000 mg/L)

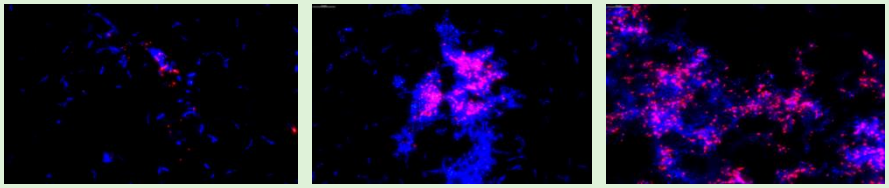

Fig. 5 *B. laterosporus BT271 + 5mg/L Phenol. 0h., 2h., 6h (left to right).*

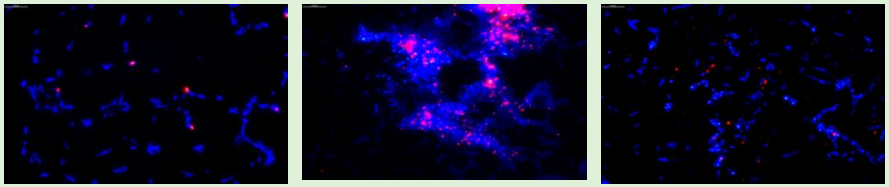

Fig. 6 *B. laterosporus BT271 + 100mg/L Phenol. 0h., 2h., 6h (left to right).*

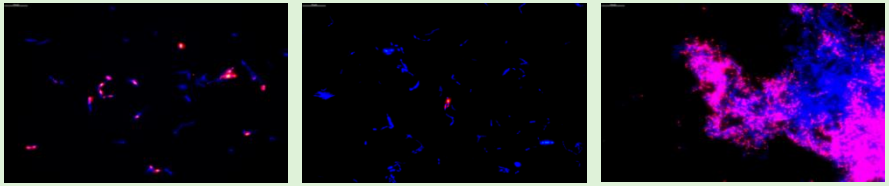

Fig. 7 *B. laterosporus BT271 + 250mg/L Phenol. 0h., 2h., 6h (left to right).*

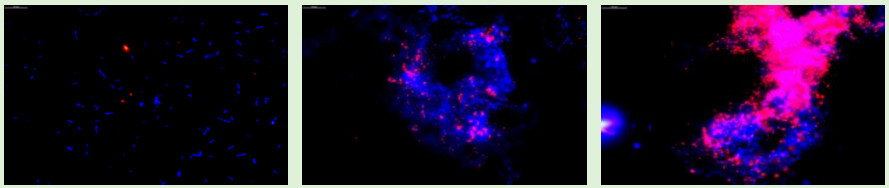

Fig. 8 *B. laterosporus BT271 + 500mg/L Phenol. 0h., 2h., 6h (left to right).*

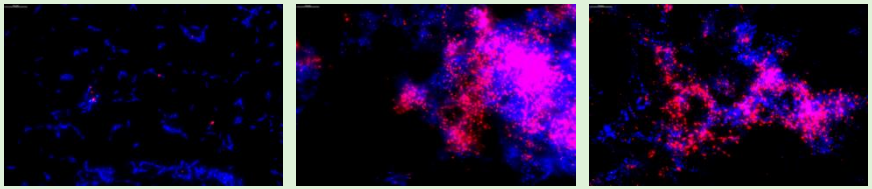

Fig. 9 *BT271 + 1000mg/L Phenol. 0h., 2h., 6h (left to right).*

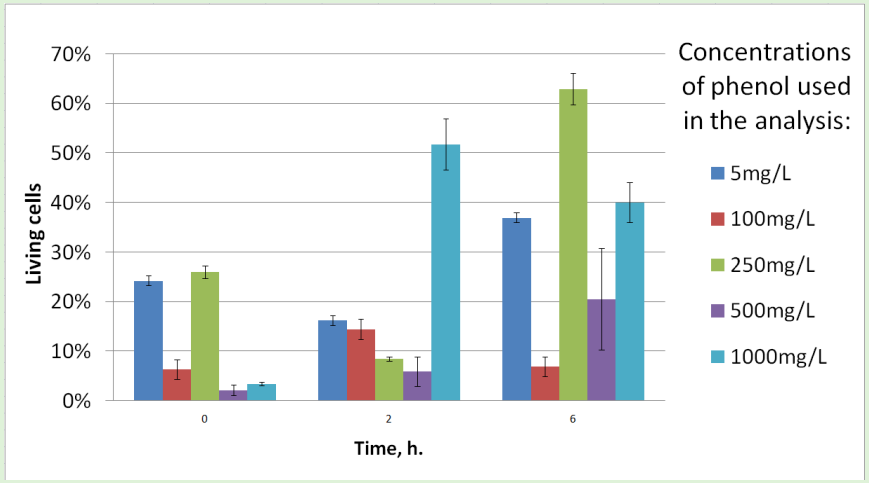

Fig. 10 *Percentage of active cells calculated on the base of the fluorescence analysis*.

The CTC/DAPI analysis (fig. 5 – 10) showed strongest effect of activation of the bacteria in the presence of phenol when higher concentrations (250-1000 mg/L) were applied. This contradicts the TDA results probably because CTC/DAPI analysis gives information on the individual level (single bacteria can be estimated) and it is more sensitive.

CONCLUSIONS:

The obtained results show that ***B.laterosporus BT271*** possesses **high activity even in presence of significant phenol concentrations** (1000 mg/L) which makes it suitable for bioremediation and phytoremediation procedures. However, the **zinc strongly inhibit the bacterial metabolic activity** as it was demonstrated in the five applied concentrations (50-1000 mg/L).

ACKNOWLEDGEMENTS

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