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STUDIES ON DIACEREIN BIODEGRADABILITY

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Abstract

Diacerein (DCN) is an anti-inflammatory, non-steroid drug with an analgesic effect, used in the treatment of osteoarthritis. DCN is pharmaceutically formulated as a yellow powder in capsules for oral use and it is mainly obtained from extracts of Rheum Emodi (rhubarb) and Aloe Vera. The pollution with pharmaceutical products represents one of the main interests in environmental protection. The influence of DCN was tested on different strains of bacteria and yeasts, common species that can be found in water and soil, and play an important part in decomposing chemical pollutants. The direct action of the pharmaceutical powder evenly spread or dispersed in solid specific culture media was evaluated. In liquid media in which different DCN concentrations were added, the microbial growth and DCN metabolization were analyzed using spectrophotometric methods. On solid media, DCN bioaccumulated in the microorganism colonies, giving them an orange colour depending on their specificity. In liquid media, DCN had an inhibitory effect or was an enhancer of the pigments colour naturally secreted by some strains of bacteria.

Keywords: *bacteria, bioaccumulation, biodegradation, diacerein, yeasts.*

INTRODUCTION

One of the most frequent articular affections, osteoarthritis (OA) is a degenerative disease, which occurs from the biochemical distruction of the synovial articular cartilage. OA is an idiopathic phenomenon, without having an initiation factor and it is associated to the aging process (80% of people above 60 years old present signs of OA) (Subhash et al., 2012). Diacerein is a new drug, used for the treatment of osteoarthritis, with an anti-inflammatory, analgesic and antipyretic action (Medhi et al., 2007). The drug is formulated for oral administration and presents few side effects (diarrhea, stomach ache, nausea, yellowish urine colour), due to the fact that is not totally absorbed by the digestive tract. The way DCN acts at a therapeutical level is based on the reconstruction of the articular cartilage, stimulating the production of TGF-β (growth factor), the proliferation of chondrocytes, collagen, proteoglycans and hyaluronan synthesis (McCalla, 2009; Subhash, 2012;). DCN ($C_{19}H_{12}O_8$, 4,5-Diacetyloxy-9,10-dioxo-2-anthracene-2-carboxylic acid) is a semi-synthetic derivative of anthraquinone, obtained from vegetal extracts of *Rheum emodi* and *Aloe vera* (Mahajan et al., 2006). The pharmacological properties of the product are given by the main active metabolite, rhein ($C_{15}H_{18}O_5$, 4,5-Dihydroxyanthraquinone-2-carboxylic acid) (Pelletier et al., 2000). The structural formulas of DCN and rhein are presented below (Fig. 1) (Rajesh et al., 2009).

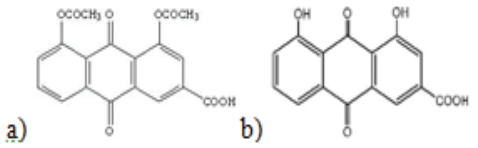

Figure 1. Structural formula of: a) DCN; b) Rhein

DCN inhibits cytokines synthesis (interleukin-1, IL-1) and metalloproteinases (collagenase, stromelysin) which are involved in articular cartilage degradation (Medhi et al., 2007).
The purpose of this paper was to evaluate the influence of DCN over some strains of bacteria

and yeasts, common species from water and soil and to study the drug's biodegradability.
The biodegradation of pharmaceutical products has a significant importance in conservation, protection and improving environmental quality.

MATERIALS AND METHODS

A. Bacterial strains (genera *Alcaligenes, Pseudomonas, Ralstonia*)and yeast strains (genera *Candida*, *Saccharomyces*, *Yarrowia*) were provided by the National Institute for Chemical Pharmaceutical Research and Development, affiliated at the WFCC-232 Collection (see Table 1).
B. Reactives: the media ingredients were purchased from Merck, Germany; dimethylsulfoxide (DMSO) from Sigma-Aldrich; Diacerein RPH (70 mg/capsule) from Rompham Company, Romania.
C. Methods
Biological method
The bacterial and yeast strains were cultivated on solid media.
The solid culture media were: *Cantacuzino Gelose* (nutritive medium for bacteria); *King B* (%g/v): proteose-peptone 2; glycerine 1; K_2HPO_4 0,15; $MgSO_4x7\ H_2O$ 0,15; Noble Agar 1,8; distilled water ad 100 ml; *YMPG* (%g/v): glucose 1; yeast extract 0,3; malt extract 0,3; peptone 0,5; Noble Agar 1,8; distilled water ad 100 ml) to which DCN was added after media sterilization (120ºC/17 minutes), dispersed on or integrated in different quantities (mg): 20, 70, 120, 165.
The liquid culture medium was *King B* (100 ml/Erlenmeyer flask) to which 1 ml stock solutions of DCN in different concentrations were added.
Stock solutions: 3,3 x 10^{-6}M; 1,1 x 10^{-5}M and 1,1 x 10^{-4}M.
The Petri dishes with selected bacteria and yeast strains were incubated for 7 days (30°C/28°C, darkness), controlling the colonies growth.
The liquid media were inoculated with 4 different bacterial strains (see Table 1) using 500 ml conical flasks. These were incubated for 96 h (30°C, darkness) using a rotary agitator (220 rpm).

Analytical methods
The bacterial cells growth was monitored by optical density (OD) using a UV-Visible spectrophotometer (Jasco Corporation V 630, Japan) at $\lambda = 550$ nm.
The free cells media were spectrophotometric analysed (270-600 nm), after centrifugation (6000 rpm/12 min) and filtration (0,45 µm syringe filter).

Table 1. Bacterial strains used in the experiment

Solid media	
Bacterial strain	Registration number
Alcaligenes latus	DSM 1123; ICCF 383
Nitrosomonas sp.	ICCF 401
Pseudomonas aeruginosa	ATCC 9027; ICCF 90
Pseudomonas aeruginosa	ICCF 389
Pseudomonas fluorescens	ICCF 392
Pseudomonas putida	ICCF 391
Pseudomonas sp.	ICCF 390
Pseudomonas sp.	ICCF 399
Pseudomonas sp.	ICCF 400
Ralstonia eutropha	DSM 545; ICCF 384
Yeast strains	Registration number
Candida albicans	ATCC 10231; ICCF 91
Candida arborea	ICCF 193
Candida boidinii	ICCF 26
Candida glabrata	ICCF 182
Candida guillermondi	ICCF 183
Candida paraffinica	ICCF 190
Candida utilis	ICCF 191
Pichia pastoris	ICCF 189
Saccharomyces cerevisiae	ICCF 225
Saccharomyces cerevisiae	ICCF 227
Yarrowia lipolytica	ATCC 16618; ICCF 214
Yarrowia lipolytica	ICCF 215
Liquid media	
Pseudomonas fluorescens	ICCF 392
Pseudomonas sp.	ICCF 390
Pseudomonas sp.	ICCF 400
Ralstonia eutropha	DSM 545; ICCF 384

RESULTS AND DISCUSSIONS

On solid media, the macroscopical aspect of microbial colonies was observed during and after the incubation and it is presented in Figures 2-5.

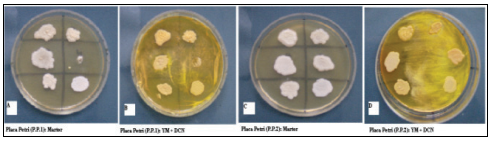

Figure 2.Yeasts on *YM* medium with DCN (165 mg)

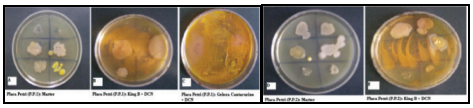

Figure 3. Bacteria on *King B* (A, B, D, E) and *Cantacuzino Gelose* (C) media with DCN (165 mg)

On solid media where on dispersed pharmaceutical powder of DCN (165 mg) was added, it was observed that bacteria and yeast strains formed yellow-orange coloured colonies (initial colour was white-beige).

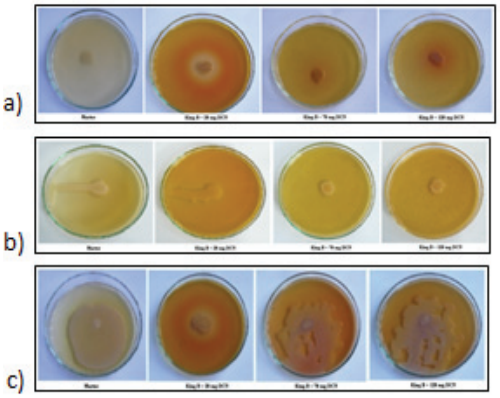

Figure 4. Bacteria on solid media with integrated DCN (20, 70, 120 mg/ Petri dish), 96 h cultivation: a) *Alcaligenes latus;* b) *Pseudomonas sp.;c) Pseudomonas putida*

On solid media in which DCN powder was integrated, besides the change of colonies colour, an accumulation of the pharmaceutical powder initially yellow coloured and then, brown-purple was noticed. Also, there was observed the formation of some pigments, possibly pyorubin (*Alcaligenes latus*) and pyoverdin (*Pseudomonas putida*), which were dependent on the added DCN quantities. On medium with the lowest amounts of DCN, clarification zones appeared (*Alcaligenes latus*; *Pseudomonas putida*) and so, the pharmaceutical powder was bioaccumulated (high concentrations in the culture medium) or it was metabolised by the microorganisms (low concentrations).

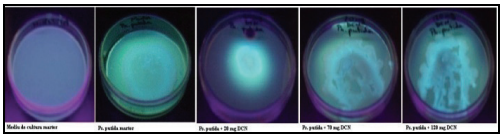

Figure 5. *Pseudomonas putida*: fluorescence on solid media with integrated DCN (0, 20, 70, 120 mg/Petri dish)

By exposure of the media with the selected strains to ultraviolet light (365 nm), DCN's noninhibitory effect on the production of the fluorescent pigment released by *Pseudomonas putida* was noticed.

In liquid inoculated with 4 selected bacterial strains, the cell growth was monitored by optical density (OD) at λ = 550 nm (dil. 1:25). The results are presented in Figure 6.

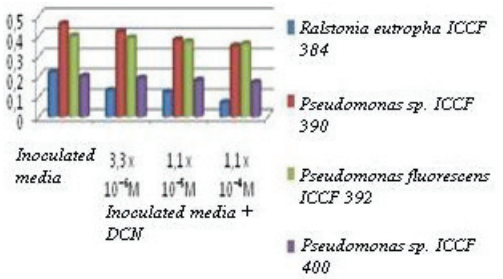

Figure 6. Dynamics of optical density (OD) values

Graphical representations of UV-Visible spectra are presented below (Figure 7-8).

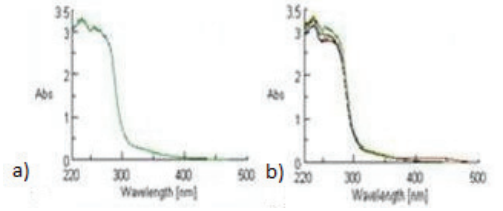

Figure 7. UV-Visible spectra of culture media before bacteria inoculation: a) initial; b) with DCN in different concentrations

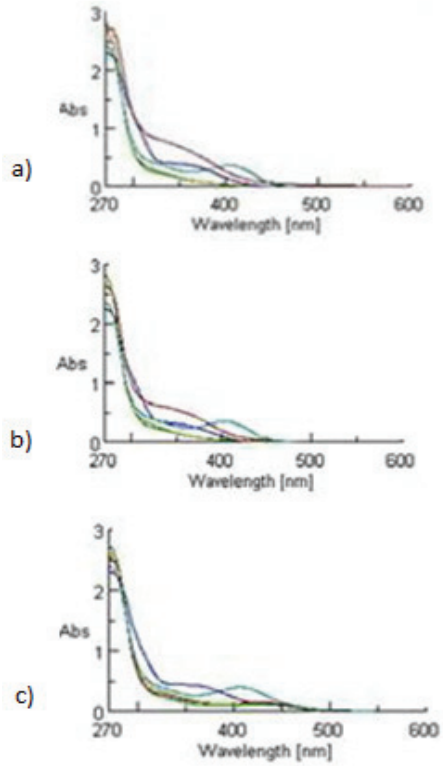

Figure 8. UV-Visible spectra of free cells media (96 h) with different concentrations of DCN (Initial medium; *Ralstonia eutropha*; *Pseudomonas sp.*; *Pseudomonas fluorescesns* ; *Pseudomonas sp.*): a) 3,3 x 10^{-6}M; b) 1,1 x 10^{-5}M; c) 1,1 x 10^{-4}M

In liquid media with DCN, the production of some new compounds was observed, which are associated with specific pigments produced by bacterial strains. The maxima of their UV-absorption were determined at the same wavelength presented in the literature: *Ralstonia eutropha* (λ = 345 nm), *Pseudomonas sp.* (λ = 340-370 nm) and *Pseudomonas fluorescenes* (λ = 408 nm) (Tourkya et al., 2011; Xiao, 1995).

CONCLUSIONS

On solid media, DCN was bioaccumulated in the bacteria and yeast colonies and it was metabolized by *Alcaligenes latus* and *Pseudomonas putida*. In small concentrations, DCN stimulated the production of an intracellular pigment (pyorubin), which has later on been excreted in the media.
DCN inhibited the fluorescent pigment production by *Pseudomonas putida*.
In liquid media with different concentrations of DCN, a higher sensitivity was shown by *Ralstonia eutropha* and *Pseudomonas fluorescens* presented a higher resistance.

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