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SHORT COMMUNICATION

Antibacterial Screening of Secreted Compounds Produced by the Phase I Variant of *Photorhabdus luminescens*

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Abstract In this study, antibacterial activity of metabolites secreted by the phase I variant of *Photorhabdus luminescens* was investigated. Bioactivity of these metabolites was screened against 28 different bacterial species and strains. Bacterial sensitivity was determined by a modified-version of the Kirby–Bauer disk diffusion susceptibility method, whereas the phase I variant's culture permeate was utilized as the "antibacterial" agent. This investigation demonstrates that 11 of the 28 bacterial species tested were sensitive to at least one of the secreted compounds or a combination thereof.

Keywords *Photorhabdus luminescens* · Phase variation · Antimicrobials · *Heterorhabditis bacteriophora*

Photorhabdus luminescens is a Gram-negative, bioluminescent, phase-varying, enteric bacterium that is symbiotically associated with the entomoparasitic nematode *Heterorhabditis bacteriophora*. Infective juvenile nematodes of *H. bacteriophora* are able to penetrate the host insect and migrate to the hemocoel where they release phase I cells of *P. luminescens* into the hemolymph [1–5]. After release, the bacterial symbiont proliferates killing the insect within 24–48 h by the production of toxins whereas other biomolecules such as pigments and antimicrobials are produced upon death of the insect [6–10].

Li et al. [11] identified two anthraquinone-derived pigments (3,8-dimethoxy-1-hydroxy-9,10-anthraquinone and 1,3-dimethoxy-8-hydroxy-9,10-anthraquinone) and demonstrated their antimicrobial properties. The same researchers also identified a secreted antibiotic (3,5-dihydroxy-4-isopropylstilbene) which is effective against fungi. Furthermore, the authors did not describe the modes of action for any of these compounds. In the present investigation, the Kirby–Bauer disk diffusion antibiotic susceptibility method [12] was modified to observe the bioactivity of the secreted metabolites produced by *P. luminescens*. Activity was determined by testing blank paper discs impregnated with a filter-sterilized culture permeate of phase I cells against bacterial lawns of 28 species and strains.

Isolation of phase I cells was performed aseptically by dissecting infected insects that exhibited luminescence and red pigmentation as described by Inman and Holmes [13] and confirmed utilizing conventional tests [14–16]. A Sartorius Stedim Biostat® A plus bioreactor containing nutrient broth supplemented with 2.0 % trehalose was inoculated with phase I cells. Process parameters: agitation (100 rpm); air flow (1 vvm) and pH (7.20). Cultivation was ended after 24 h. A cell-free permeate was generated from tangential-flow filtration and filter-sterilized. Sterile, blank disks were impregnated with the permeate and dried.

Colonies of each microbe were suspended in tryptic soy broth and incubated at 30 °C until turbidity reached that of a 0.5 MacFarland standard. Aliquots of each culture were spread onto Muller-Hinton plates to prepare bacterial lawns. Impregnated discs and blank disks were placed onto the agar and incubated for 24 h at 30 °C. Antibacterial screening of each organism was performed in replicates of three and the diameter of the three zones was averaged.

Measurements of bacterial sensitivity are seen in Table 1. Upon analysis, 11 species of bacteria tested were sensitive to at least one of the secreted compounds. Gram-negative bacilli were not sensitive; however, all members of *Neisseriaceae* were. Further analysis suggests that Gram-positive cocci of

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Table 1 Averaged zones of sensitivity for each bacterial species

Microbe	Zone diameter (mm)
Bacillus cereus	11
B. licheniformis	0
B. megaterium	13
B. subtilis	16
B. subtilis[a]	17
B. thuringiensis	0
Citrobacter freundii	0
Enterobacter aerogenes	0
E. cloacae	0
Enterococcus faecalis	0
Escherichia coli	0
E. coli K12-wt	0
Klebsiella pneumoniae	0
Lactococcus (*Streptococcus*) *lactis*	0
Micrococcus luteus	12
Moraxella catarrhalis	19
Neisseria sicca	27
N. subflava	26
Pseudomonas aeruginosa	0
P. fluorescens	0
Salmonella enteritidis	0
S. typhimurium	0
Serratia marcescens	0
Sporosarcina ureae	18
Staphylococcus epidermidis	12
S. simulans	13
Streptococcus durans	0
S. mutans	0

[a] Antibiotic-producing strain

Micrococcaceae were sensitive; however, *Streptococcaceae* were not. As far as *Bacillus* is concerned, two species were not sensitive.

The results of this study show that one or more of the secreted compounds were effective against 39 % of the bacterial species screened. Accordingly, *P. luminescens* may be a new attractive source of antimicrobial drugs, especially for treating infections caused by Gram-negative cocci. Furthermore, more research is needed to specify the responsible compound for such sensitivity.

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