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IDENTIFICATION OF THREE STRAINS OF *Entomopathogenic bacteria Isolated FROM Culex spp.* LARVAE AND THEIR POTENCY ON *Culex pipiens* LARVAE.

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ABSTRACT

Biological control alone or as a part of integrated vector management stands to be a better alternative to the chemical controls aimed against pest mosquitoes. Here, A screening for microorganisms that can be utilized as new host cells for mosquito larvicides was carried out. As persistence in the environment is required of host cells, the bacterial populations in mosquitoes collected from natural breeding ponds around Mansoura University was examined. Bacterial sequences were isolated using standard 16S rRNA gene sequencing. Bacillus species, especially *Lysinibacillus sphaericus* were found to be the dominant species and toxic to *Culex* larvae, *Bacillus amyloliquefaciens* was the second highly effect against culex larvae, *B. amyloliquefaciens* was isolated from *Culex* larvae for the first time in Egypt. *Cellulosimicrobium cellulans* also isolated and had the lowest effect. Detection and isolation of these bacteria offer a potential biocontrol mechanism as they possess high potential as novel host cells for application in mosquito control.

INTRODUCTION

Due to environmental conditions and mosquito resistance to several insecticides it is now paramount to find new control methods to control mosquito and mosquito borne diseases. So , at the present time, for the comprehensive control of insects, environmentally friendly pesticides together with biological control agents are strongly recommended (Federici *et al.,* 2007 and Lacey, 2007). *Lysinibacillus sphaericus* (Meyer and Neide) is an aerobic mesophilic spore-forming bacterium that has been used with great success in mosquito control programs worldwide (Charles *et al.,* 1996). Mosquitocidal activity of *L. sphaericus* and the persistence of its spores in the environment would make this species a suitable candidate for inoculation of mosquito breeding sites as an effective and ecologically friendly biological control agent (Mulla *et al.,* 1984 and Siegel *et al.,* 2001).

The mosquitocidal metabolite produced by the *Bacillus amyloliquefaciens* was found to kill both larval and pupal stages of mosquitoes. Hence, the inventory of mosquito biocontrol agents is now augmented with yet another bacterial agent that can serve as a potent candidate in the wake of resistance development to the already available bacterial mosquitocides (Geetha *et al.,* 2011).

We decided to work in a more bio-control approach and assess what larvicide bacteria where present in Egyptian mosquito larvae. Identification of such bacteria, naturally surviving and affecting these populations would be the start of new engineering projects to minimise mosquito nesting, proliferation and would therefore impact of mosquito-borne diseases.

MATERIAL AND METHODS

Samples collection

Mosquito larvae samples were collected from two different ponds around Mansoura University during June, July, August and September of 2011, samples were collected weekly during the four successive months. Samples were putted in plastic jars and transferred to the laboratory in plastic containers with water from the same site to separate larvae of *Culex* spp. Mosquito species were identified by their morphological characteristics, the dead larvae were sterilized by first placing them in sterile distilled water for 20-s followed by two 20-s washes with 70% (v/v) aqueous alcohol and a final 20-s wash with sterile distilled water. They were then homogenized in 10 ml sterile physiological saline solution according to Días *et al*. (1992).

Isolation of entomopathogenic bacteria from mosquito larvae samples

The larvae were manually crushed with 10 ml of sterile water. The suspension was shacked well for 10 min to homogenise and then serially diluted in sterile water to 10^{-6} . Extracts were plated on nutrient agar media. After incubation at 30±1^{o}C for 2 days, plates were examined and the developed colonies were subcultured on nutrient agar plates for purification. After incubation for 2 days, the purified bacterial colonies were transferred into nutrient agar slants for maintenance until subsequent tests. Nutrient agar medium was (g/l) : peptone 5.0; beef extract 3.0 agar 20.0 and pH was 7.0. This medium was used for maintenance of all isolates. All cultures were cultivated on nutrient agar for 48 h at 28^{o}C. A heat fixed smear was stained with malachite green and counter stained with fuchsin. The slide was observed under bright field microscopy with a 100 x oil immersion objective (Holt *et al*., 1994).

Evaluation of the pathogenic potentials of the bacterial isolates on *C. pipiens*

1- Rearing of *Culex pipiens* under laboratory conditions

Rearing of *C. pipiens* in the laboratory was carried by the method described by Singh *et al.* (1972), Shams El-Dean (1982) and Abou Bakr (1984).

2- Preparation of the bacterial inoculums

The bacterial growths on the nutrient agar slants were scraped, using 5 ml sterile tap water, then transferred to a flask containing 50 ml sterile nutrient broth (Elcin, 1995). The resulting cell suspensions was calculated using plate count agar method according to Post (1988). The inocula were prepared using this suspension and were adjusted to10^{6}, 10^{5} and 10^{4} CFU/ml (colony forming unit) by dilution with sterile tap water (Collins *et al.*, 2003).

3- Pathogenicity test

Ten larvae of each larval instars (2^{nd}, 3^{rd}, and 4^{th}) of reared *C. pipiens* were placed in each concentration (10^{6}, 10^{5} and10^{4}) of the prepared bacterial suspension in rearing units (20 x 9 x 7 cm). Each treatment was replicated four times. Additional four rearing units of each mosquito larval instars were used without bacterial inoculum as a control. The mortality of the mosquito larvae were assayed after 12, 24, 36, 48, 60 and 72 hours.

4- Identification of the entomopathogenic bacteria

A molecular approach based on the amplification and sequencing of the 16S rRNA gene was used for identifying the bacteria selected from the larvae as follow:

DNA extraction and amplification

DNA was extracted from the cultures using the GenElute Bacterial Genomic DNA kit (Sigma Aldrich, UK) as per the manufacturer's instructions, with the addition of lysozyme.

Approximately 10–15 ng of template DNA was used in a 25 µL PCR reaction mixture containing: 2U of Taq DNA polymerase (New England BioLabs, UK), 1X PCR ThermoPol buffer (2.0mM Tris– HCl, 1.0mM (NH4) SO4, 1.0mM KCL) (New England BioLabs, Uk), 2.5mM deoxynucleoside triphosphate mix (Biogene, Cambs, UK), 0.25 mM of both universal bacterial primers 27F and 1525r.

Amplified products were purified using the GenElute PCR purification kits (Sigma Aldrich), according to manufacturer's instructions. Cleaned PCR fragments were sequenced in both directions (GTAC Biotech, UK) and subsequently reassembled for full gene coverage.

16S rRNA gene sequencing analyses

16S rRNA gene sequence were aligned manually with corresponding sequences derived from a BLAST search, retrieved from the DDBJ/EMBL/GenBank databases, using the PHYDIT program (http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred using the least-squares (Fitch and Margoliash 1967), maximum-likelihood (Felsenstein 1981) and neighbour-joining (Saitou and Nei, 1987) tree-making algorithms from the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices were generated for the least-squares and neighbour-joining methods, as described by Jukes and Cantor (1969). The resultant unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resampling's of the neighbour-joining dataset, using the SEQBOOT and CONSENSE options from the PHYLIP package.

Statistical analysis

For selecing the most potant strain and suceptable larval stage proc ANOVA in SAS was used (Anonymous, 1988). Mean separation of the obtained results was conducted using Duncan's multiple range in the same program.

The effectiveness of the different treatments was conducted using Propit analysis of obtained data. Results were using LDP line program expressed in terms of LT_{50} values at 95 % fiducial limits. Slopes of regression lines were represented. Statistical analysis of the obtained data were made based on Probit analysis of Finney (1971).

RESULTS AND DISCUSSION

Identification of the entomopathogenic bacteria:

In this study , three species of bacteria from *culex* larvae, *Bacillus amyloliquefaciens*, *Lysinibacillus sphaericus* and *Cellulosimicrobium cellulans* were isolated and identified (Figures1&2). The entomopathogenic

bacteria, *Bacillus amyloliquefaciens* was isolated from *Culex* larvae for the first time in Egypt.

These results are similar to those of Wraight *et al.* (1987) for *L. sphaericus* and different target species of *Culex* . In india, Geetha *et al.,* 2011 listed *Bacillus amyloliquefaciens* for the first time as a mosquito larvicidal activity against larvae of *Anopheles stephensi,Culex quinquefasciatus* and *Aedes aegypti.*

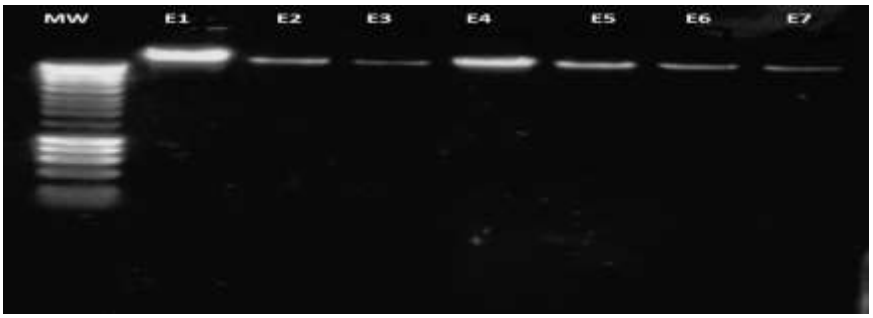


Figure 1. Genomic DNA extraction of the seven bacterial cultures isolated from mosquitos collected from natural breeding ponds around Mansoura University, Egypt.

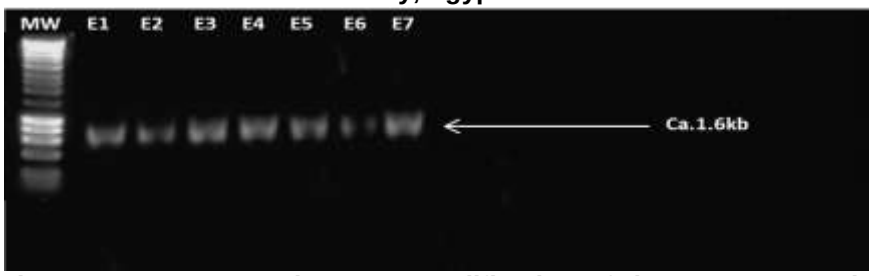


Figure 2. Representative PCR amplification of the seven genomic extracts, targeting the 27F-1525r region of the 16S rRNA gene sequence. The arrow indicates the size of the fragment being approximately 1.6kb.

Pathogenicity test:

Results of applying dose-mortality as log-probit model is presented in tables (1, 2 & 3) and graphically illustrated in figures(3, 4 & 5).

After 12, 24, 36, 48, 60 and 72 hours the calculated LT_{50} values were shown in table 1, 2 & 3 and graphically illustrated in fig.3, 4 & 5. The mortality of the *Culex pipiens* (2^{nd}, 3^{rd} & 4^{th} instar larvae) exposed to the different concentrations of bacerial strain showed that the most active bacerial was *Lysinibacillus sphaericus*. The *Culex pipiens* was found susceptible to *L. sphaericus* infection. Besides, as the concentrations of the bacerial increased, the *Culex pipiens* morality was also increased. This is due to smaller slope for the most active bacerial used compared with other ones. This means that speed of effect is concentration dependant but efficacy is not concentration dependant. These results are in complete accordance with those previously.

Statistical analysis showed that there was signifecant differeance in the effecincy between [investigated three strains (Bacillus species, especially *Lysinibacillus sphaericus* were found to be the dominant species and toxic to *Culex pipiens*, *Bacillus amyloliquefaciens* was the second highly effect and *Cellulosimicrobium cellulans* also isolated and had the lowest effect), larval stage (the third instar larval was more suseptable than the senod and fourth instar larvae), concentrations (the 10^6 concentration was more toxic effect than 10^5 and 10^4 on larvae stage and time mortality.

Table (1): Values of LT_{50} , LT_{90} and slopes for the tested *Lysinibacillus sphaericus* versus 2nd instar larvae of *C. pipiens.*

Conc.	LT_{50}	Lower limit	Upper limit	1	2	3	Index	RR	Slope	LT_{90}
10^6	24.107						100	1	2.458	80.09
10^5	37.56						64.183	1.558	2.23	141.088
10^4	46.323						52.041	1.922	2.224	174.603

Index compared with 221 Resistance Ratio (RR) compared with 221

Table (2): Values of LT_{50} , LT_{90} and slopes for the tested *Lysinibacillus sphaericus* versus 3rd instar larvae of *Culex pipiens.*

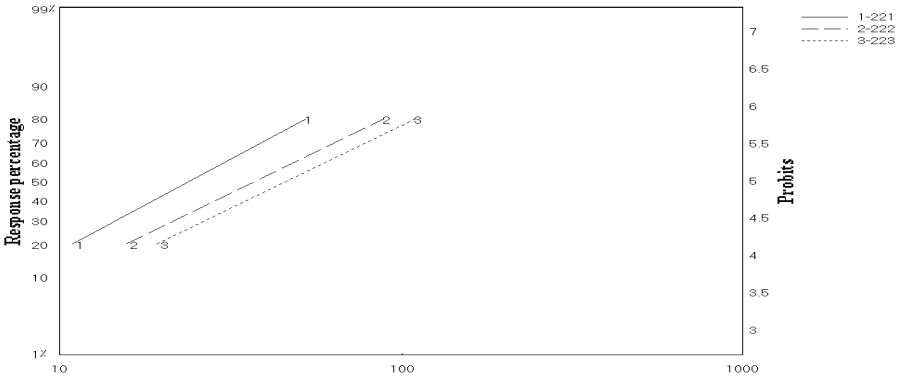
Conc.	LT50	Lower limit	Upper limit	1	2	3	Index	RR	Slope	LT90
10^6	19.904						100	1	2.218	75.285
10^5	22.254	18.023	26.063		*		89.44	1.118	1.712	124.805
10^4	36.888	32.141	42.298			*	53.958	1.853	1.799	190.295

Index compared with 321 Resistance Ratio (RR) compared with 321

Table (3): Values of LT_{50} , LT_{90} and slopes for the tested *Lysinibacillus sphaericus* versus 4th instar larvae of *Culex pipiens.*

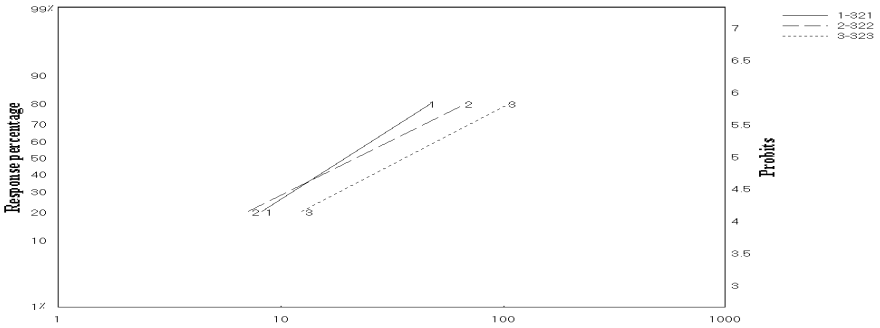
Conc.	Line name	LT_{50}	Lower limit	Upper limit	1	2	3	Index	RR	Slope	LT_{90}
10^6	421	58.933	46.9	115.945	*		*	100	1	2.09	241.797
10^5	422	95.439						61.749	1.619	1.566	628.014
10^4	423	124.012	92.373	212.143	*		*	47.522	2.104	1.67	725.8

Index compared with 421 Resistance Ratio (RR) compared with 421



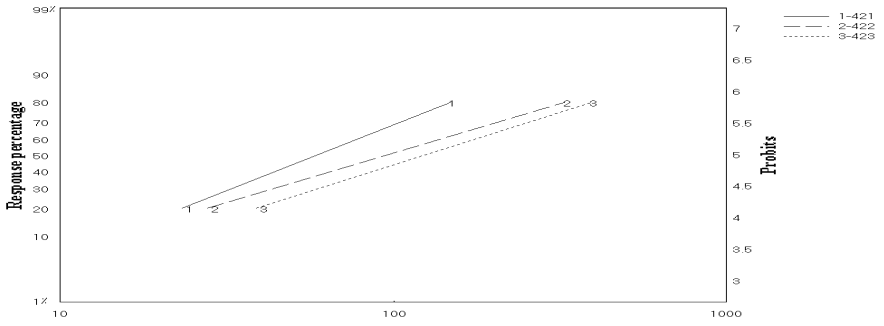
Log time (Days)

Figure 3. LT lines for the tested *Lysinibacillus sphaericus* concentrations versus 2nd instar larvae of *C. pipiens*



Log time (Days)

Figure 4. LT lines for the tested *Lysinibacillus sphaericus concentrations* versus 3rd instar larvae of *C. pipiens.*



Log time (Days)

Figure 5. LT lines for the tested *Lysinibacillus sphaericus concentrations* versus 4th instar larvae of *C. pipiens*

These results are similar to those of Porter *et al*., 1993 and Vaidyanathan and Scott (2007) who recorded that some strains of *L. sphaericus* are toxic towards mosquito larvae and are used in the biological control of the vectors of malaria, filariasis, dengue fever and West Nile fever. Also, Federici *et al.* (2006 and 2007) regarded *L. sphaericus* as a toxic species against *Anopheles* immature stages. Tranchida *et al*., 2011 reported that the toxicity of *L. sphaericus* against immature stages of mosquitos results from the expression of the binary toxins (41.9 and 51.4 kDa) encoded by the binA and binB genes, which are loci expressed at the start of sporulation. The presence of both gene products comprising this toxin is necessary to produce mortality in mosquito larvae (Broadwell *et al*., 1990; Thanabalu *et al.,* 1992 and Davidson *et al*.,1990). Another toxin of 100 kDa, called Mtx, is present in *L. sphaericus* strains of both low and high toxicity. The study of 16S rRNA gene sequences by Nakamura (2000) indicated that the *L. sphaericus* species is a heterogeneous taxon formed by seven groups. The high larval toxicity found in some *L. sphaericus* strains, such as 2362 (Weiser, 1984) and IAB59 (de Barjac *et al*., 1988), has led to the commercial use of these strains as biopesticides against populations of mosquitos.

The effects of bacteria, *Bacillus amyloliquefaciens* on mosquito larvae was recorded in india by Geetha *et al.,* 2011 who reported that hemosquitocidal metabolite produced by this bacteria was found to kill both larval and pupal stages of mosquitoes. *B. amyloliquefaciens* is known to occupy a variety of terrestrial sites due to the versatility of the enzymes it secretes. Strains of *B. amyloliquefaciens* have been known for the production of cellulase and restriction enzyme BamH1 (Dubinina *et al*., 1983 and Lee *et al*., 2008). Strains of *B. amyloliquefaciens* are reported for the production of cyclic lipopeptides viz., surfactin, fengycin and bacillomycin D (Lee *et al.,* 2007; Ramarathnam *et al.,* 2007 and Chen *et al*., 2009a, 2009b). The cyclic lipopeptide, surfactin which is a powerful biosurfactant, produced by *B. subtilis* subsp*. subtilis* has been reported to be mosquitocidal (Geetha *et al*., 2010 and Geetha & Manonmani, 2010).

Cellulosimicrobium cellulans had very small effect on mosquito larvae because it infest mosquito larvae accidentally, this bacteria also isolated from males of *Aedes albopictus* as reported by Moro *et al*., 2013.

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REFERENCES

Abou Bakr, H.E. (1984). Studies on certain biological control agents of mosquitoes in Egypt. Ph.D Thesis, Fac. Agric., Cairo Univ. 243 pp.

Anonymous (1988) SAS/STAT User`s Guide, Ver. 6.03. SAS Institute Inc., Cary, North Carolina, 22 pp.

Broadwell, A.H., Baumann, L. and Baumann, P. (1990). The 42- and 51-kilodalton mosquitocidal proteins of *Bacillus sphaericus* 2362: construction of recombinants with enhanced expression and in vivo studies of processing and toxicity. J. Bacteriol., 172(5):2217–2223.

Charles, J.F., Nielsen-LeRoux, C. and Delécluse, A. (1996). *Bacillus sphaericus* toxins: molecular biology and mode of action. Annu. Rev. Entomol., 41:451–472.

Chen, X.H., Koumoutsi, A., Scholz, R. and Borriss, R. (2009_b). More than anticipated production of antibiotics and other secondary metabolites by *Bacillus amyloliquefaciens* FZB42. J. Mol. Microbiol. Biotechnol., 16:14–24.

Chen, X.H., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Sussmuth, R., Piel, J. and Borriss, R. (2009_a). Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. J. Biotechnol., 140: 27–37.

Collins, D.P., Jacobsen, B.J. and Maxwell, B. (2003). Spatial and temporal population dynamics of a phyllosphere colonizing *Bacillus subtilis* biological control agent of sugar beet *Cercospora* leaf spot. Biological control, 26: 224-232.

Davidson, E.W., Oei, C., Meyer, M., Bieber, A.L., Hindley, J. and Berry, C. (1990). Interaction of the *Bacillus sphaericus* mosquito larvicidal proteins.Can J Microbiol., 36: 870–878.

de Barjac, H., Thiery, I., Cosmao-Dumanoir, V., Frachon, E., Laurent, P., Charles, J.F., Hamon, S. and Ofori, J. (1988). Another *Bacillus sphaericus* serotype harbouring strains very toxic to mosquito larvae: serotype H6. Ann Inst Pasteur Microbiol., 139(3):363–377.

Días, J.M.C.S., Gomes Monnerat, R. and Silva-Werneck, J.O. (1992). Aulas prácticas de bacteriología. In: Segundo curso de Controle Microbiano de Insetos. Brasilia. Brasil. pp 23.

Dubinina, G.P., Basnak'ian, I.A., Tsvetkova, N.V., Semina, I.E. and Tarasov, A.V., (1983). Isolation of the specific endonuclease BamH1 in the multicyclic cultivation of *Bacillus amyloliquefaciens*. Zh. Mikrobiol. Epidemiol. Immunobiol., 12–17.

Elcin, Y.M. (1995). *Bacillus sphaericus* 2362 – Calcium alginate microcapsules for mosquito control. Enzyme Microbiol Technol., 17: 587-591.

Federici, B.A., Park, H.W. and Sakano, Y. (2006). Insecticidal protein crystals of *Bacillus thuringiensis*. In: Shively JM (ed) Microbiology monographs series, Volume I. Inclusions in Prokaryotes. Springer, Berlin, 195–236.

Federici, B.A., Park, H.W., Bideschi, D.K., Wirth, M.C., Johson, J.J., Sakano, Y. and Tang, M. (2007). Developing recombinant bacteria for control of mosquito larvae. In: Floore TG (ed) Biorational control of mosquitoes, vol 7. American Mosquito Control Association, 164–175.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol., 17:368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39:783–791.

Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package), version 3.5c Department of Genetics. University of Washington, Seattle, USA.

Finney, D.J. (1971). Probit Analysis. *(3rd edition, Cambridge University Press, Cambridge, 318 pp).*

Fitch, W.M. and Margoliash, E. (1967). Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome c sequences is one of general applicability. Science 155:279–284.

Geetha, I. and Manonmani, A.M. (2010). Surfactin: a novel mosquitocidal biosurfactant produced by *Bacillus subtilis* ssp. *subtilis* (VCRC B471) and influence of abiotic factors on its pupicidal efficacy. Lett. Appl. Microbiol., 51: 406–412.

Geetha, I., Manonmani, A.M. and Paily, K.P. (2010). Identification and characterization of a mosquito pupicidal metabolite of a *Bacillus subtilis* subsp. *subtilis* strain. Appl. Microbiol. Biotechnol., 86: 1737–1744.

Geetha, I., Manonmani, A.M. and Prabakaran, G. (2011). *Bacillus amyloliquefaciens*: A mosquitocidal bacterium from mangrove forests of Andaman & Nicobar islands, India. Acta Tropica, 120: 155– 159.

Holt, J.G., Murray, R.G.E., don Brenner, J., Bryant, M.P., Krieg, N.R., Moulder, J.W., Pfennig, N., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology, 6th edn., Journal of Endocrinology, 142(1): 171-179.

Jukes, T.H and Cantor, C.R. (1969). Evolution of Protein Molecules. New York: Academic Press, 21–132.

Lacey, L.A. (2007). *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. In: Floore TG (ed) Biorational control of mosquitoes, Bull. Nº 7. J Am Mosq Control Assoc., 23(2):133–163.

Lee, S.C., Kim, S.H., Park, I.H., Chung, S.Y. and Choi, Y.L. (2007). Isolation and structural analysis of bamylocin A, novel lipopeptide from *Bacillus amyloliquefaciens* LP03 having antagonistic and crude oil-emulsifying activity. Arch. Microbiol., 188: 307–312.

Lee, Y.J., Kim, B.K., Lee, B.H., Jo, K.I., Lee, N.K., Chung, C.H., Lee, Y.C. and Lee, J.W. (2008). Purification and characterization of cellulase produced by *Bacillus amyoliquefaciens* DL-3 utilizing rice hull. Bioresour. Technol., 99: 378–386.

Moro, C.V., Florence, H.T., Fara, N.R., Pierre, R. and Patrick, M. (2013). Diversity of culturable bacteria including Pantoea in wild mosquito *Aedes albopictus*. BMC Microbiology, 13:70.

Mulla, M.S., Darwazeh, H.A., Davidson, E.W. and Dulmage, H.T. (1984). Efficacy and persistence of the microbial agent *Bacillus sphaericus* againts mosquito larvae in organically enriched habitats. Mosq News, 44:166–173.

Nakamura, L.K. (2000). Phylogeny of *Bacillus sphaericus*-like organisms. Int J Syst Evol Microbiol., 50:1715–1722.

Porter, A.G., Davidson, E.W. and Liu, J.W. (1993). Mosquitocidal toxins of bacilli and their genetic manipulation for effective biological control of mosquitoes. Microbiol Rev., 57:838–861.

Post, F.J. (1988). . A laboratory manual for food microbiology and biotechnology. Star Publishing Co., Belmont, CA.

Ramarathnam, R., Bo, S., Chen, Y., Fernando, W.G., Xuewen, G. and de Kievit, T. (2007). Molecular and biochemical detection of fengycin- and bacillomycin D-producing *Bacillus* spp., antagonistic to fungal pathogens of canola and wheat. Can. J. Microbiol., 53: 901–911.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for constructing phylogenetic trees. Mol Biol Evol., 4:406–425.

Shams El-Dean, M.M. (1982). Utilization of the nematode, *Romanomermis culicivorax* in the biological control of some mosquito species. M.Sc. thesis, Fac.Agric., Cairo Univ. 150pp.

Siegel, J.P., Smith, A.R. and Novak, R.J. (2001). Recovery of commercially produced *Bacillus thuringiensis* var. *Israelensis* and *Bacillus sphaericus* from tires and prevalence of bacilli in artificial and natural containers. J Am Mosq Control Assoc., 17:33–41.

Singh, K.R.P., Patterson, R.S., LaBrecque, G.C. and Razdan, R.K. (1972). Mass rearing of *Culex fatigans*. WHO/VBC/72.386, 26 pp.

Thanabalu, T., Hindley, J. and Berry, C. (1992). Proteolytic processing of the mosquitocidal toxin from *Bacillus sphaericus* SSII-1. J Bacteriol., 174(15):5051–5056.

Vaidyanathan, R. and Scott, T.W. (2007). Geographic variation in vector competence for West Nile virus in the *Culex pipiens* (Diptera: Culicidae) complex in California. Vector Borne Zoonotic Dis., 7(2):193–198. doi:10.1089/vbz.2006.0589.

Wraight, S.P., Molloy, D.P. and Singer, S. (1987). Studies on the culicine mosquito host range of *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* with Notes on the Effects of temperature and instar on bacterial efficacy. J Invertebr Pathol., 49:291–302.

تعريف ثلاث سلالات بكتيرية تم عزلها من يرقات بعوض الكيولكس وقياس فاعليتها ضد يرقات البعوض

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يعتبر استخدام المكافحة الحيوية منفردا أو بالتكامل مع طرق مكافحة ناقلات الأمراض الأخرى من أهم البدائل لتجنب استخدام المكافحة الكيميائية ضد يرقات البعوض. خلال هذه الدراسة تم تجميع عينات يرقات البعوض من بعض البرك المتواجدة بجوار جامعة المنصورة-مصر. حيث تم نقل العينات للمعمل وعزل يرقات بعوض الكيولكس ثم تم غسل وتعقيم تللك اليرقات ثم عزل البكتيريا المتواجده بها. حيت تم تعريف 3 سلالات بكتيرية وتم اختبارها ضد يرقات البعوض المرباة بالمعمل. حيث أظهرت النتائج أن بكتيريا *Lysinibacillus sphaericus* هى أكثر السلالات تأثيرا على يرقات البعوض، كذلك تم عزل السلالة *Bacillus amyloliquefaciens* لأول مرة من يرقات البعوض فى مصر وكذلك وجد لهذه السلاة تأثير على يرقات البعوض. كذلك تم عزل السلالة *Cellulosimicrobium cellulans* *ولكنها ذات تأثير ضعيف على يرقات بعوض الكيولكس.*

قام بتحكيم البحث

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