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# *In Vitro* Nematicidal Activities of Myxobacteria from Mt. Malinao, Philippines Against *Caenorhabditis elegans*

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**Abstract**

Secondary metabolites from microorganisms are good sources of natural products which can be utilized for various medical and biotechnological purposes. Several members of the bacterial group Myxobacteria are known to be prolific producers of secondary metabolites. Philippine forest soils are rich habitats for Myxobacteria which could produce secondary metabolites with potential novel biological activities. Soils from Mt. Makiling in Laguna and from Mt. Malinao in Albay were collected for the isolation of myxobacteria which were then screened for nematicidal activities. The dung bait plate, yeast-spot plate and coli-spot plate baiting techniques were used to isolate myxobacteria from soils. Myxobacterial isolates were selected based on the presence of fruiting bodies and swarm colonies characteristic of myxobacteria. Ten representative isolates were grown in CTT medium (30°C, 150 rpm, four days) with 2% XAD-16 adsorber resin. Cell metabolites were extracted using 1:1 methanol:acetone. The crude extracts were used for the nematicidal assay against *Caenorhabditis elegans* Bristol N2 for 24 hours at 25°C. High nematicidal activities based on nematode mortality were observed from the extracts of L4D1 (90.33% +/- 3.06) and L4D9 (86.00% +/- 3.6) while the commercial preparation of mebendazole as positive control showed 100% mortality. The differences in the percentage mortalities caused by the various extracts were highly significant at $p<0.05$ based on One Way ANOVA and Tukey HSD. L4D9 and L4D1 were identified based on phenotypes and 16S rRNA sequences as strains of *Myxococcus xanthus*. This study contributes to the scarce literature and culture collection of myxobacteria in the Philippines and especially those with potential anthelminthic activities.

**Keywords:** *secondary metabolites, Mt. Makiling, Mt. Malinao, nematicide, Myxococcus*

## Introduction

There is still an intensified worldwide efforts to discover new groups of microorganisms in search for products that would provide novel mechanisms of actions on specific targets. Aside from actinomyctes and cyanobacteria, myxobacteria have been recognized as prolific producers of secondary metabolites many of which have novel chemical structures and bioactivities (Hermann *et al*., 2017). Comparative genomics of myxobacterial species reveal that this group have much more to offer in terms of the production of novel metabolites for natural product discovery (Moghaddam *et al*., 2018). Many secondary metabolites from myxobacteria are known to possess antibacterial, anticancer and antiviral activities. Fernandez and colleagues (2021) reported the potential of myxobacterial metabolites, particularly the cyclic depsipeptide chondramides, for drug development against SARS-CoV-2 using *in silico* approaches. Thus, the exploration of myxobacterial metabolites which may possess valuable pharmacologic applications is still a worthwhile endeavor.

The myxobacteria constitute a group of Gram-negative rod-shaped eubacteria under the Delta (δ) group of the Proteobacteria. Although myxobacteria are ubiquitous in many ecological habitats, these bacteria have received very little attention in the past due to their complicated life cycle which makes them difficult to isolate and cultivate. While species of myxobacteria have been discovered from Philippine soils (Garcia *et al*., 2009b; Awal *et al*., 2016), culture collections of myxobacteria in the Philippines as well

as published literature of its biosynthetic potentials are scarce. In fact, no myxobacteria is listed in the collection of microorganisms in any of the institutional members of the Philippine Network of Microbial Culture Collections (PNMCC) (Monsalud *et al*., 2006).

Myxobacteria are known to produce metabolites with new modes of action on specific targets including eukaryotic cells. This ability offers the possibility of discovering new antimicrobials to mitigate the rising cases of antibiotic resistance among pathogenic microorganisms. While many studies have shown the antifungal and antitumor potentials of some myxobacterial metabolites, literature on its possible antiparasitic activity, particularly against helminths, is quite limited. Efforts to develop new and effective anthelminthics must be given attention since only four drugs (albendazole, mebendazole, praziquantel and ivermectin) are available for the treatment of common human helminthiasis, including the parasitic nematodes, affecting mostly the resource-poor and impoverished people in developing countries (Hotez *et al*., 2008).

Majority of the myxobacterial species were discovered from soils rich in organic matter such as forest soils and dungs of herbivores (Dawid, 2000; Garcia *et al*. 2009b). The Philippines is a country with a high biodiversity available from its forest covers. Unfortunately, its immense biodiversity is being threatened or some species may have already been lost even before being discovered, mainly due to destructive anthropogenic causes. The Mt. Makiling Forest Reserve in Laguna still holds untold biodiversity even if it has already been the subject of many biodiversity assessments before. Likewise, Bicol Region is also blessed with forest covers which also hold a vast biodiversity, one of which is Mt. Malinao in Albay. Mt. Makiling is listed as one of the key biodiversity areas (KBA) in the Philippines while Mt. Malinao is a candidate KBA (Conservation International/DENR/ Haribon, 2006). These two mountains are potentially good sources of myxobacteria. There is, thus, a need to conduct more studies on Philippine isolates to tap the potential of these important bacterial resources. This study was conducted to isolate, identify and characterize myxobacteria from Philippine forest soil samples and to screen these isolates for possible nematicidal activities.

## Materials and Methods

### *Isolation, Purification and Maintenance of Putative Myxobacteria*

Fruiting bodies from the dung bait plates were carefully picked from the pellet using a 0.1 mm sterile syringe and transferred to fresh sterile dung bait plates. This process was repeated several times until only one type of fruiting body, devoid of contaminating molds and nematodes, was seen growing on the dung pellets. Some fruiting bodies were also transferred to VY/2 agar plates (per liter: Baker's yeast, 0.50 % (w/v); $CaCl_2 \cdot H_2O$, 0.10 % (w/v); cyanocobalamin, 0.50mg/uL ; pH 7.2) to induce germination and swarming of the myxospores and to further purify the isolates. From the coli-spot plates and yeast-spot plates from which only swarming colonies were observed, a portion of the edge of a discernible swarming colony was removed to make an agar block by cutting through the agar with 0.1 mm sterile syringe. The agar blocks were placed on respective fresh plates with two parallel streaks of *E. coli* or yeast suspension until only one type of swarm was seen. The swarms were further purified by cutting a portion of the agar medium containing the most distal advancing edge of the farthest swarm and subsequently transferred to fresh VY/2 medium. The agar blocks were positioned at the center of each plate to provide enough space for swarming. This purification method was performed several times depending on the degree of contamination until a pure culture was obtained. The isolates were maintained on VY/2 plates and periodically transferred every three weeks (Shimkets *et al*., 2006). Pieces of VY/2 agar with fruiting bodies were sterile filter paper strips in screw-capped tubes and later dried in a desiccator. All stock cultures were kept at room temperature.

### *Phenotypic characterization of myxobacteria*

The morphological descriptions of fruiting bodies and swarm colonies of the putative myxobacterial isolates were based on the taxonomic keys provided in *The Prokaryotes* (Shimkets *et al*., 2006) and *Bergey's Manual of Systematic Bacteriology* (Reichenbach, 2005). Fruiting bodies on the surface of dung pellets and agar plates were observed with a dissecting microscope for the color, shape, surface structure, formation, presence or absence of stalk and consistency. Swarm colonies of pure cultures after five days of incubation were observed under the dissecting microscope with oblique illumination. The morphology of the advancing or spreading swarm edge, color and of the slime sheets

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were also recorded. The swarm colonies and fruiting bodies were also observed for fluorescence upon exposure to long-wave ultraviolet light (366 nm) from a hand-held UV lamp model UVGL-25 (UVP, Inc., San Gabriel, CA) in a dark room. Vegetative cells taken from the advancing edge of the swarm colonies were Gram-stained using Hucker's Gram stain modified protocol and confirmed with the Gregersen's method using 3% potassium hydroxide (KOH) (Gregersen, 1978). Myxospores from crushed fruiting bodies were observed for their shape and size under 1000x magnification. Catalase test (3% $H_2O_2$) and Congo Red Adsorption test (0.01%) were also performed.

*Molecular identification of putative myxobacterial isolates*

Myxobacteria were grown on CTT medium (per liter: casitone, 10.00 g; 0.8 M $MgSO_4$, 10.00 ml; 1 M Tris-HCl, 10.00 ml; pH 7.6) at 30°C for 48 hours with shaking at 150 rpm. The reference strain, *Myxococcus xanthus* Beebee (ATCC 25232) was cultivated in Medium No. 219 (per liter: BactoCasitone (Difco), 20.00 g; $MgSO_4\cdot 7H_20$, 1.00 g; 0.01 M $KPO_4$ buffer; pH 7.2). Genomic DNA was extracted from the cultures using the DNeasy Blood and Tissue Kit (Qiagen, Germany) according to manufacturer's protocol. Gel electrophoresis (1% agarose gel with 0.5X TAE buffer) was conducted using a Mupid mini-agarose gel electrophoresis apparatus. The gel was stained with 5 mg/ml ethidium bromide and visualized via the BioDoc-H gel imaging system (UVP, Inc., San Gabriel, CA). Successfully extracted genomic DNA were checked for purity ($A_{260}/A_{280}$) using a nanodrop spectrophotometer. All genomic DNA samples were stored at -20°C until further processed.

Amplification of the 16S rRNA gene sequence was done using a universal bacterial primer pair pA (forward: 5'-GAGTTTGATCCTGGCTCAGGA-3') and pH (reverse: 5'-AAGGAGGTGATCCAGCCGCA-3') (Edwards *et al*, 1989; Weisburg *et al*, 1991). The PCR profile used was: one cycle of initial denaturation at 94°C for 1 min, 40 cycles of denaturation at 95°C for 1 min, annealing at 56.5°C for 1 min, extension at 72°C for 1.5 min, additional extension at 72°C for 2.0 min and holding at 4°C. The final concentrations of the components of the PCR mixture in a 50 µL reaction were 10 µg/µL DNA template, 1X buffer, 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.2 µM each of forward and reverse primers, and 1.25 units of *Taq* DNA polymerase. The amplicons were determined by gel electrophoresis in the same manner as that mentioned for genomic DNA. The amplicons were sent to Macrogen, South Korea for sequencing under the BigDye™ terminator cycling conditions and using Automatic Sequencer 3730XL. Internal primers (800R and 518F) were used for sequencing in addition to the pA and pH primers. The sequences were compared to type strains of myxobacteria in the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) nucleotide algorithm search (www.ncbi.nlm.nih.gov). Sequence alignment was done using the CLUSTAL_W algorithm of the Molecular Evolutionary Genetics Analysis (MEGA 5.0) (Tamura *et al*., 2011).The phylogenetic tree was also constructed using the MEGA 5.0.

*Extraction of Myxobacterial Metabolites*

Seed culture was prepared by inoculating three pieces of agar blocks (approximately 10 mm$^2$ per piece), containing the swarming cells of the pure culture of a myxobacterial isolate, into a 250 ml Erlenmeyer flask containing 50 ml of liquid CTT medium. The cultures were shaken at 30°C, 150 rpm, for four days until the medium became turbid. The seed culture was inoculated (10% v/v) into 500 ml of CTT fermentation medium. The culture medium was supplemented with 2% (w/v) adsorber resin (Amberlite XAD-16, Sigma) to which the metabolites were expected to adhere. Fermentation was allowed to proceed at 30°C, 150 rpm, for another four days (Garcia *et al*., 2009a).

After fermentation, the cells and the resin were collected by centrifugation at 10,000 rpm, 4°C, for 15 min (Sorvall Ultracentrifuge from Thermo Fischer Scientific, Inc., USA). The cell pellets and resin were stirred for 15 min in 50 ml equal volumes of methanol and acetone; this was done three times (Garcia *et al*., 2009a). The mixtures were filtered using Whatman No. 1 filter paper. The extraction solvents were removed from the filtrate using a rotary evaporator (Heidolph, Germany) at 40°C and the extracts were subsequently placed under the fume hood and later in the oven at 37°C to dry.

*Nematicidal Assay*

The wild type strain of *Caenorhabditis elegans* Bristol N2 and the feed bacterium *Escherichia coli* OP50 obtained from the Caenorhabditis Genetics Center at the University of Minessota were maintained according to the protocol by Stiernagle (2006). The nematodes were grown in nematode growth medium

(NGM Agar blocks; per liter: NaCl, 3.00 g; agar, 17.00 g; peptone, 2.50 g; 1 M $CaCl_2$, 1.00 ml; 5 mg/ml cholesterol in 95% ethanol, 1.00 ml, 1 M $MgSO_4$, 1.00 ml; 1 M $KPO_4$ buffer (pH 6.0), 25.00 ml) seeded with *E. coli* OP50 by inoculating each surface-dried NGM plate with 0.1 ml of the feed bacterium previously grown on Luria Bertani (LB) broth at 37ºC for 24 hours. The feed bacteria were allowed to grow on the NGM plates by incubating the plates also at 37ºC for another 24 hours. Chunks of NGM agar with actively growing *C. elegans* were transferred to the seeded NGM plates and maintained at 25ºC.

The nematodes were allowed to feed on *E. coli* OP50 seeded on NGM plates for six days prior to the assay, and washed off the plates three times, each with 2 ml of sterile M9 buffer (per liter: $KH_2PO_4$, 3.00 g; $Na_2HPO_4$, 6.00 g; NaCl, 5.00 g; 1M $MgSO_4$ , 1.00 ml) (Moy *et al*., 2006; Breger *et al*., 2007; Lei *et al*., 2010). The concentration of the nematode suspension obtained was adjusted to a titer of 200-300 nematodes per ml by adding more of the M9 buffer. To each well of a 24-well microtiter plate were distributed 0.9 ml of the nematode suspension and 0.1 ml of the test sample (consisting of 50 mg of a myxobacterial metabolite extract/ ml M9 buffer). The negative control consisted of worm suspension with M9 buffer while the positive control was a worm suspension with mebendazole (20 mg/ml). After 24 hours of incubation at 25ºC, the percentage of dead worms was determined from each well. Percentage nematode mortality was computed as the number of dead worms counted in a total population of 100 worms at various life stages (Schaeffer *et al*., 1989). Two trials were conducted for the nematicidal assay with three replicates for each trial.

Results were analysed for statistical significance using Oneway Analysis of Variance (ANOVA) and post-hoc test (Tukey HSD) in the SPSS® v26 software. Values lesser than $p \leq 0.05$ were considered statistically significant.

## Results and Discussions

Myxobacteria are usually found in soil rich in organic matter and can be isolated using various baiting methods where the fruiting bodies and swarm colonies can be easily observed (Figure 1). The rich forest soils of Mt. Makiling and Mt. Malinao yielded 21 and 11 isolates, respectively. In this study, the fruiting bodies developed mostly from the dung bait plates (69%) while swarm colonies were observed more from the coli-spot plates (28%) and yeast-spot plates (3%). This was also observed by Gaspari and co-workers (2005) who noted that the use of dung pellets selectively allowed the growth of *Myxococcus* and some *Archangium* strains with the formation of fruiting bodies. This is because the dung pellets mimicked the natural growth substance used as a source of nutrients for the myxobacteria.

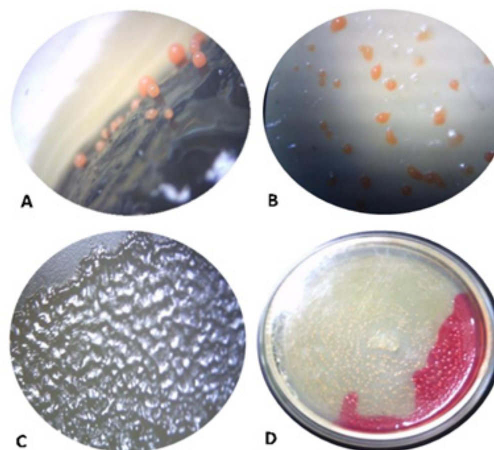


**Fig 1** Myxobacterial isolates were characterized based on the morphology of fruiting bodies on goat dung (A), VY8 agar (B), swarm colonies (C) and adsorption of Congo Red (D).

Only the 16S rRNA sequences of L4D5 and L4D9 were successfully amplified and compared to the NCBI database. L4D5 had a 100% similarity with *M. fulvus* ATCC 25199$^T$ while L4D9 had a 100% similarity with *M. virescens* DSM 2660$^T$ and 99.93% similarity with *M. xanthus* ATCC 25232$^T$. Phylogenetic evaluation of these isolates with the type strains of Myxobacteria showed that L4D5 clustered with *M. fulvus* ATCC 25199$^T$ while L4D9 clustered with *M. virescens* DSM 2660$^T$ and *M. xanthus* ATCC 25232$^T$ (Figure 2). *Myxococcus virescens* and *M. xanthus* are closely related species (Lang and Stackebrandt, 2009; Chambers *et al*., 2020).

All of the isolates were initially grown on CTT medium to determine which of them will be further fermented for metabolite extraction based on homogeneous growth. Attempts to cultivate many of the isolates in the seed and fermentation media proved futile - some grew only in the seed medium but not in the fermentation medium while other isolates did not even grow in the seed medium even after continuous incubation for several days. Some isolates may need longer time to incubate. Members of the suborder Sorangiineae are reported to require a longer time of incubation than other myxobacteria (Garcia *et*

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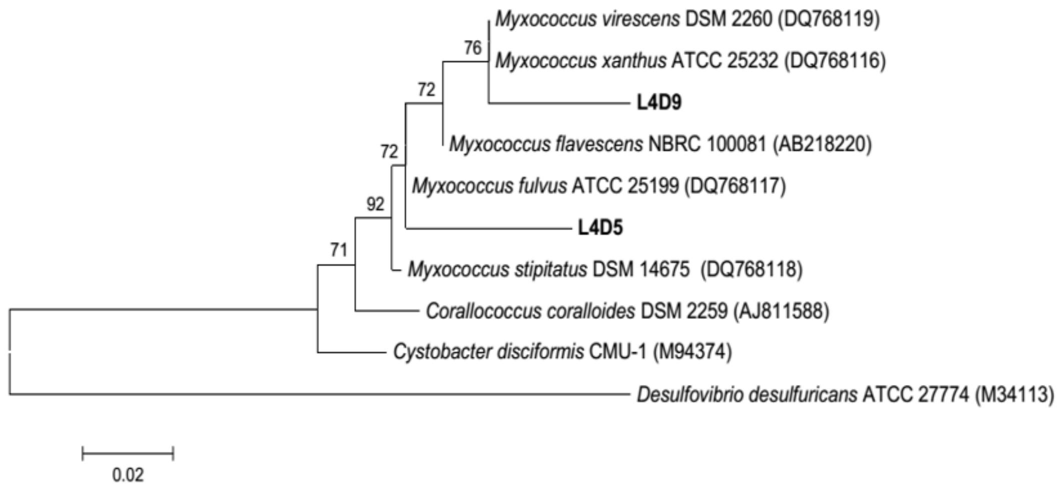


**Fig 2** A phylogenetic tree of the partial 16S rRNA sequences of L4D9 and L4D5 and type strains of Myxococcus. The numbers at branch nodes indicate bootstrap percentages derived from 1000 replications. The evolutionary distances were computed using the Maximum Likelihood method based on the Kimura 2-parameter model included in the Mega 5.0 software. Bar, 0.02 substitutions per single nucleotide position. *Desulfovibrio desulfuricans* ATCC 27774 was used as the outgroup. Accession numbers of the sequences are in parenthesis.

*al*., 2009a). Only ten isolates exhibited homogeneous growth while the other isolates either formed clumps or did not grow at all even after extended incubation. The extraction of myxobacterial metabolites was carried out only for the ten isolates which yielded crude powdered extracts, that were either brown or yellow, recovered after fermentation and evaporation of the methanol-acetone solvent.

Growth and productivity in liquid medium can be limited by several factors such as the production of ammonia and the production of extracellular matrix (Shimkets *et al*., 2006). The nonpolar Amberlite XAD-16 adsorber resin was added to the cultures during fermentation since the use of this resin increases the possibility of recovering much of the excreted metabolites, therefore, eliminating the need to test the culture supernatant for bioactivity. Secondary metabolites excreted by myxobacteria are adsorbed to the resin and eluted with methanol while acetone breaks the cells and extracts the cell-associated metabolites from the cell mass. In this study, acetone and methanol in a 1:1 ratio were used to widen and ensure the extraction of metabolites both from the resin and from the harvested cell mass.

The nematicidal toxicity assay showed that the metabolite extracts from the 10 isolates had varying killing effects on the test nematodes (Figures 3 and 4). Seven extracts showed a relatively high nematode mortality of more than 50%: L4D1 (90.33% $\pm$ 3.06), L4D9 (86.00% $\pm$ 3.61), K8D2 (76.33% $\pm$ 9.02), L5C1 (71.67% $\pm$ 5.51), K3D1 (70.67% $\pm$ 9.07), K6D3 (66.00% $\pm$ 6.93) and K4C2 (57.33% $\pm$ 4.73). The positive control, mebendazole at 20 mg/ml, was very efficient in killing the nematodes (100% nematode mortality). Only three extracts (L8D1, L4C3 and L4D7) exhibited nematode mortality of less than 50% which can be considered low (19.67% $\pm$ 5.86, 22.00% $\pm$ 4.36 and 38.00% $\pm$ 10.15, respectively), almost similar to that of the negative control (12.67% $\pm$ 1.15).

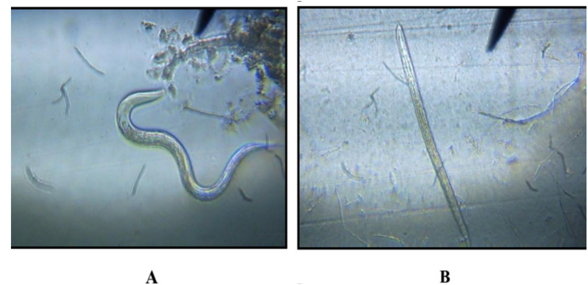


**Fig 3** Determination of live and dead nematodes. Scoring of live and dead worms was based on body configuration being sinusoidal for live worms (A) and straight for dead worms (B) as seen under the microscope (100x). Independent movement was also observed for live worms while dead worms showed no movement even when prodded with a fine needle.

The differences in mortalities caused by the 10 myxobacterial extracts were highly significant at $p<0.05$ based on the One way ANOVA. However, many authors and statisticians have emphasized the limits of declaring observations simply as either statistically significant or not. The use of the simultaneous 95% Confidence Interval (CI) based on post hoc test is an alternative and more meaningful interpretation of significance; a CI which does not include a zero is

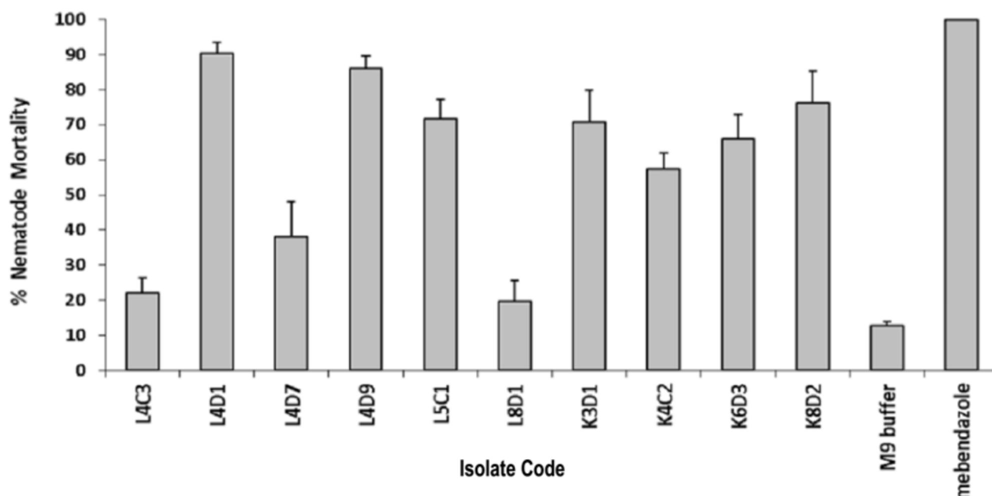


**Fig 4** Nematicidal activity of the metabolite extracts from myxobacterial isolates against *Caenorhabditis elegans* Bristol N2. Negative control was the vehicle buffer M9 while positive control was mebendazole. Values are expressed as means +/- standard deviation (SD).

considered as statistically significant (Lovell, 2013). Based on the CI values from the Tukey HSD test of the five highest mortalities as compared to the positive control, only the difference in the percentage mortalities of L4D9 and the positive control as well as between L4D1 and positive control were not statistically significant (Figure 5). This suggests that the mortalities caused by these two isolates were comparable to that of the commercial nematicide and thus the extracts may possess potent nematicidal compounds.

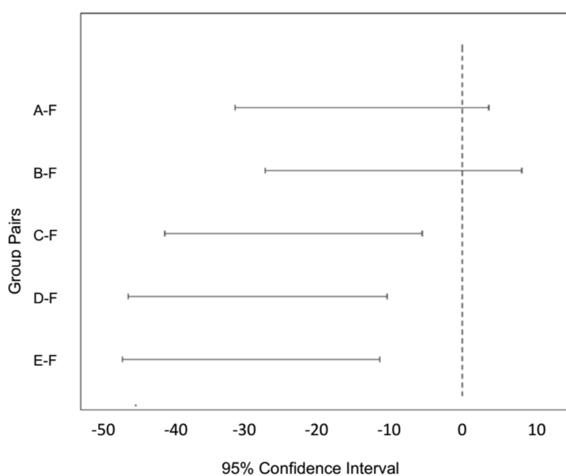


**Fig 5** Confidence Interval of the difference in group means of L4D9 (A), L4D1 (B), K8D2 (C), L5C1 (D), K3D1 (E) and positive control (F). Corresponding difference in means of CI with no zero is statistically significant (Tukey's HSD, $p < 0.05$).

Although myxobacteria are known to be prolific producers of secondary metabolites of antibacterial, antifungal and cytotoxic activities, its potential to produce antiparasitic, especially nematicidal, has not gained much attention. It has been reported by Reichenbach and Hofle (1993) that in rare instances, insecticidal activities and substances acting on animal cells have been seen in myxobacteria. Saframycin Mx1 was isolated from *Myxococcus xanthus* Mx x48; saframycins are also know to be active on animal cells, specifically as antitumor compounds (Irschik *et al*., 1988). Likewise, rhizopodin from culture extracts of *M. stipitatus* can induce mouse fibroblasts to produce rhizopodium-like extensions eventually leading to the death of the animal cell line (Sasse *et al.*1993). A patent evaluation published by the Current Opinion in Therapeutic Patents in September 1992 showed the claim for thiangazole isolated from *Polyangium* DSM 6267 as an antiparasitic agent against the nematodes *Haemonchus* sp. and *Trichostrongylus* sp., the tapeworms *Moniezia benedeni* and the liver fluke *Fasciola hepatica.* Eliamid which is a respiratory chain inhibitor (Complex I) produced by *Sorangium cellulosum* So ce439 and Soce241 were found to be lethal to the brine shrimp *Artemia salina* at 0.2–0.3 µg/mL and to the soil nematode *Panagrellus* at 5 µg/mL (Hofle *et al*., 2012). In this study, L4D1 which is identified as *M. virescens*, had consistently shown the highest nematicidal activity with an average of 93% nematode mortality. This was followed by another putative *M. virescens* isolate, L4D9, with 84% nematode mortality. The putative *M. stipitatus* isolate, K3D1, exhibited a nematode mortality of 76%.

According to Holden-Dye and Walker (2014), there are two mechanisms by which anthelminthic drugs can reach the tissues of intact *C. elegans* and these are either

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by ingestion or by diffusion across the cuticle. Known anthelminthic drugs have varied modes of action elucidated at the molecular level. While the data in this study clearly show the nematicidal activity of the tested metabolite extracts from 10 isolates, no further attempt was done to define the possible mechanism by which the metabolites caused the death of the nematodes.

The nematicidal activity of the isolates' extracts needs further evaluation specifically in terms of the type of active compound present and their toxicity levels to both the worms in human or animal hosts. Nevertheless, the promise of previously unexplored anthelminthic compounds from myxobacteria may offer a new prospect regarding the pressing concern on the need to discover and produce new lines of anthelmintics (Holden-Dye and Walker, 2014).

Traditionally, myxobacteria are grouped into two based on their ability to degrade macromolecules. The first group is composed of species which are of the proteolytic-bacteriolytic nutritional type which can lyse live and whole cells of bacteria, yeasts and other microorganisms while the other group is composed of species which are able to degrade cellulose. L4D1 and L4D9 which were both isolated from Mt. Malinao and identified as *M. virescens* belong to the proteolytic-bacteriolytic type which could present a possible mechanisms for their nematicidal activities as observed in this study.

New species and strains of myxobacteria are being discovered at a fast rate worldwide. Similarly, exploration of its metabolites is still gaining interests resulting to the elucidation of its structures and modes of actions. Genomic data showed that there are several biosynthetic gene clusters in the order Myxococcales that await further investigation for possible new metabolites (Gregory *et al.*, 2019). These metabolites could serve as molecular scaffolds for natural product discovery. The possibility of strains of known myxobacterial species to exhibit new biological activities is still immense. The potential of these unknown metabolites as antiparasitic, particularly as nematicidal of pharmaceutical and agricultural prospects, is not fully explored since drug discovery for myxobacteria is more focused on its effects against bacteria, fungi and human cancer cell lines. Moreover, the possibility of discovering new myxobacterial species in various ecological habitats in the Philippines is far from being remote.

## Conclusions

The *Myxococcus virescens* isolates L4D1 and L4D9 from Mt. Malinao showed nematicidal activities against *Caenorhabditis elegans* as the model organism. This observation can be further validated by using purified fractions from the extracts to determine its active nematicidal compounds and its effects on medically and agriculturally important parasitic worms. Profiling of the active compounds using Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and other analytical techniques is also recommended. Further, this study shows that the Philippine soils could harbor many myxobacterial species, some of which could be novel. To the best of our knowledge, no literature so far has been published documenting the nematicidal potential of myxobacteria from Philippine isolates. This study then would contribute to the still growing knowledge on myxobacterial diversity and its potential for natural product discovery towards anthelminthic and antiparasitic drugs.

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## References

(1992). Patent Evaluation: thiangazole as an antiparasitic agent, Current Opinion in Therapeutic Patents, 2(9): 1404-1405. doi: 10.1517/13543776.2.9.1404.

Awal, R.P., Garcia, R., & Muller, R. (2016). *Racemicystis crocea* gen. nov., sp. nov., a soil myxobacterium in the family Polyangiaceae. Int. J. Syst. Evol. Microbiol., 66: 2389–2395.

Breger, J., Fuchs, B. B., Aperis, G., Moy, T.I., Ausubel, F.M. & Ylonakis, E. (2007). Antifungal chemical compounds identified using a *C. elegans* pathogenecity assay. PLoS Pathogens. 3(2):0168- 0178.

Chambers, J., Sparks, N., Sydney, N., Livingstone, PG, Cookson, A.R. & Whitworth, D.E. (2020). Comparative genomics and pan-genomics of the Myxococcaceae,

including a description of five novel species: *Myxococcus eversor* sp. nov., *Myxococcus llanfairpwllgwyngyllgogerychwyrndrobwllllantysiliogogogochensiss* sp. nov., *Myxococcus vastator* sp. nov., and *Pyxidicoccus trucidator* sp. nov. Genome Biology and Evolution, 12(12): 2289-2302. doi: 10.1093/gbe/evaa212.

Conservation International/Department of Environment and Natural Resources/Haribon Foundation. (2006). Priority sites for conservation in the Philippines: key biodiversity areas. Diliman, Quezon City: Critical Ecosystem Partnership Fund.

Dawid, W. (2000). Biology and global distribution of myxobacteria in soils.FEMS Microbiology Reviews, 24:403-427.

Edwards, U., Rogall, T., Blocker H., Emde, M. & Bottger, E.C. (1989). Isolation and direct complete nucleotide determination of entire genes: characterization of a gene coding for 16S ribosomal RNA. Nucleic Acid Res, 17:7843-7853.

Fernandez, R.A., Quimque, M.T., Notarte, K.I., Manzano, J.A., Pilapil, D.Y., de Leon, V.N., Jose, J.J., Villalobos, O. & Macabeo, A.P. (2021). Myxobacterial depsipeptide chondramides interrupt SARS-CoV-2 entry by targeting its broad, cell tropic spike protein: Antagonistic prospects for anti-COVID-19 drug discovery. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.14043641.v1.

Garcia, R.O., Krug, D. & Muller, R. (2009a). Chapter 3: Discovering natural products from Myxobacteria with emphasis on rare producer strains in combination with improved analytical methods. In: Methods in Enzymology, 458:59-91.

Garcia, R.O., Reichenbach, H., Ring, M. W. & Muller, R. (2009b). *Phaselicystis flava* gen.nov.,sp.nov., an arachidonic acid-containing soil myxobacterium, and the description of *Phaselicystidaceae* fam.nov. Int. J. Syst. Evol. Microbiol, 59:1524-1530.

Gaspari, F., Paitan, Y., Manini, M., Losi, D. Ron E.Z. & Marinelli, F. (2005). Myxobacteria isolated in Israel as potential source of new anti-infectives. Journal of Applied Microbiology, 98(2):429-439. doi: 10.1111/j.1365-2672.2004.02477.x.

Gregory, K. L. A., Salvador, S., Akbar, Adaikpoh, B. I. & Cole Stevens, D. (2019). Survey of biosynthetic gene clusters from sequenced myxobacteria reveals unexplored biosynthetic potential. Microorganisms, 7(6):181. doi. 10.3390/microorganisms7060181.

Gregersen, T. (1978). Rapid method for distinction of gram-negative from gram-positive bacteria. European J. Appl. Microbiol. Biotechnol, 5:123–127. doi.org/10.1007/BF00498806.

Hermann, J., Fayadab, A. & Muller, R. (2017). Natural products from myxobacteria: novel metabolites and bioactivities. Nat. Prod. Rep, 34(2):135-160.doi: 10.1039/c6np00106h.

Hofle, G., Gerth, K., Reichenbach, H., Kunze, B., Sasse, F., Forche, E. & Prusov, E. V. (2012). Isolation, biological activity evaluation, structure elucidation, and total synthesis of eliamid: a novel Complex I Inhibitor. Chem. Eur. J, 18(36):11362-11370. doi: 10.1002/chem .201201879.

Holden-Dye, L. & Walker, R. J. (2014). Anthelminthic drugs and nematicides: studies in *Caenorhabditis elegans*. In: WormBook: The Online Review of *C. elegans* Biology (Internet). Pasadena (CA): WormBook; 2005-2018.

Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J. & Jacobson, J. (2008). Helminth infections: the great neglected tropical diseases. J Clin Invest, 118(4): 1311-1321. doi: 10.1172/JCI34261.

Irschik H., Trowitzsch-Kienast, W., Gerth, K., Hofle, G. & Reichenbach, H. (1988). Saframycin Mx1, a new natural saframycin isolated from a myxobacterium. J. Antibiot, 41 993–998. doi: 10.7164/antibiotics.41.993

Lang, E. & Stackebrandt, E. (2009). Emended descriptions of the genera *Myxococcus* and *Corallococcus*, typification of the species *Myxococcus stipitatus* and *Myxococcus macrosporus* and a proposal that they be represented by neotype strains. Request for an Opinion. Int. J. Syst. Evol. Microbiol, 59: 2122–2128.

Lei, J., Leser, M. & Enan, E. (2010). Nematicidal activity of two monoterpenoids and SER-2 tyramine receptor of *Caenorhabditis elegans*. Biochemical Pharmacology. 79:1062-1071.

Lovell, DP. (2013). Biological importance and statistical significance. J. Agric. Food Chem, 61: 8340–8348. doi:10.1021/jf401124y.

Monsalud, R.G., Siringan, M.A.T., Castillo, E.P., de la Cruz, T.E.E., Ontengco, D.D.C., Dedeles, G.R., Sabino, N.G., Mercado, B.B., V Argayosa, B., Bool, M.B., Creencia, A.R., Bulaong, S.S. & Tan Gana, N.H. (eds). (2006). Directory of culture collections in the Philippines. 2nd ed. Philippine Network of Microbial Culture Collection, Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology, UP Los Baños, College, Laguna, Philippines.

Moghaddam, J.A., Crüsemann, M., Alanjary, M., Harms, H., Dávila-Céspedes, A., Blom, J., Poehlein, A., Ziemert, N., König, G. M. & Schäberle, T.F. (2018). Analysis of the genome and metabolome of marine myxobacteria reveals high potential for biosynthesis of novel specialized metabolites. Scientific Reports, 8:16600. doi: 10.1038/s41598-018-34954-y.

Moy, T.I., Ball, A.R., Anklesaria, Z., Casadei, G., Lewis, K. & Ausubel, F.M. (2006). Identification of novel antimicrobials using a live-animal infection model. PNAS, 103(27):10414-10419. doi:10.1073/pnas.0604055103.

Reichenbach, H. (2005). The Myxococcales. *In*: Bergey's Manual of Systematic Bacteriology, 2nd ed., Part 3: The Beta-, Delta-, and Epsilonproteobacteria. G.M. Garrity (ed). NY: Springer-Verlag.

Reichenbach, H. & Hofle, G. (1993). Chapter 16: Production of bioactive secondary metabolites. *In:*Myxobacteria II. M. Dworkin and D. Kaiser (eds). Washington, DC.: American Society for Microbiology.

Sasse, F., Steinmetz, H., Hofle, G. & Reichenbach, H. (1993). Rhizopodin: a new compound from *Myxococcus stipitatus* (Myxobacteria) causes formation of rhizopodia-like structures in animal cell cultures. Production, isolation, physicochemical and biological properties. J Antibiot (Tokyo), 46(5): 741-748. doi: 10.7164/antibiotics.46.741.

Schaeffer, J.M., Bergstorm, A.R. & Turner, M.J. (1989). MK-801 is a potent nematicidal agent. Characterization of MK-801 binding sites in *Caenorhabditis elegans.* Biochem. J. 260:923-926.

Shimkets, L.J., Dworkin, M. & Reichenbach, H. (2006). The Myxobacteria. In The Prokaryotes.3rd ed. Singapore: Springer Science + Business Media, Inc.

Stiernagle, T. (2006). Maintenance of *Caenorhabditis elegans*. WormBook, ed. The *C. elegans* Research Community, WormBook. doi:10.1895/wormbook.1.101, http://www.wormbook.org.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution. 28: 2731-2739.

Weisburg, W.G., Barus, S.M., Pelletier, D.A. & Lane, D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol, 173: 697-703.

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