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Malaysia's Breakthrough in Modern Actinobacteria (MOD-ACTINO) Drug Discovery Research

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Abstract: Actinobacteria are well-known producers of metabolites with medicinal value. The application of actinobacterial compounds has been expanded to other fields, including agriculture, aquaculture, and cosmeceutical. With this, the term "Modern Actinobacteria" (MOD-ACTINO) was first coined by a Malaysian researcher, Associate Professor Dr. Lee Learn Han, to define actinobacteria with modern applications. The present review aims to highlight the MOD-ACTINO research achievements in Malaysia. The Malaysian MOD-ACTINO strains are capable of exerting a wide range of bioactivities such as antimicrobial/anti-MRSA, anticancer, antioxidant, antifungal, and antimalarial. Research on MOD-ACTINO is highly encouraged to harness the benefits of actinobacteria and unravel important metabolites for various applications.

Keywords: Modern actinobacteria; actinomycetes; streptomycetes; drug discovery; anticancer; antioxidant; antimicrobial

1. Introduction

The global spread of new emerging diseases and existing diseases have been a public health burden. Researchers constantly explore Earth's natural resources to discover novel disease prevention and treatment drugs. The need for new drugs has led to the unraveling of bioactive products deriving from microorganisms found on Earth [1]. Interestingly, a study predicted that Earth houses up to one trillion microbial species and that there are as many as 99.999% microbial taxa yet to be discovered [2]. Within the Bacteria domain, the phylum Actinomycetota (synonym Actinobacteria) represents one of the largest taxonomic units

among 18 major lineages [3, 4], comprising 6 classes: *Actinomycetes* (synonym *Actinobacteria* or *Actinomycetia*), *Acidimicrobiia*, *Nitriliruptoria*, *Coriobacteriia*, *Rubrobacteria*, and *Thermoleophilia* [5]. Generally, *Actinobacteria* is the largest class, and it can be characterized into two groups, namely *Streptomyces* (dominant genus) and non-*Streptomyces*, also known as "rare *Actinobacteria*" [6, 7].

Streptomyces and rare *Actinobacteria* are important sources of novel bioactive secondary metabolites. Associate Professor Dr. Lee Learn Han coined "Modern Actinobacteria" (MOD-ACTINO) to define actinobacteria with modern applications. Briefly, this term applies to: (1) actinobacteria that synthesize natural products with new bioactivities; (2) actinobacteria that produced approved drugs and have been subjected to drug repurposing efforts; and (3) known or novel actinobacteria found from the unique environment [8]. This review aims to unravel the discovery of MOD-ACTINO from the diverse ecosystems in Malaysia. This review will also offer insights into the vast bioactive properties of Malaysia's MOD-ACTINO, such as anti-MRSA/antimicrobial, anticancer, antioxidant, antifungal, and antimalarial.

2. Actinobacteria Reservoir

Actinobacteria are the most prolific source of secondary bioactive metabolites, with diverse structural complexity [9, 10]. They demonstrate beneficial applications for humankind in agricultural, medical, and industrial fields [9][3]. Notably, around 50% of actinobacteria originate from the genus *Streptomyces*, and approximately 75% of commercially valuable antibiotics have been derived from *Streptomyces* [11]. Many bioactive compounds have been isolated from actinobacteria in recent years, particularly from the genus *Streptomyces*. *Streptomyces*-derived compounds produce bioactivities such as antimicrobial, anticancer, antioxidant, antifungal, and antimalarial [12-16]. Given the significant contribution by actinobacteria, current research focuses on exploring new metabolites from these microorganisms. Researchers are beginning to tap into underexplored locations or extreme environments to isolate actinobacteria and investigate their secondary metabolites. Actinobacteria are abundant in nature, and they are crucial soil inhabitants. The isolation source of actinobacteria was initially from terrestrial areas, which then expanded to freshwater and marine environments [1]. To date, actinobacteria are widely distributed across various underexplored areas and extreme environments, including mangroves [17], caves [18], mountain plantations [19], deserts [20], hot springs [21], and even in the Arctic and Antarctic regions [22, 23].

In Malaysia, the most notable discoveries include the identification of numerous novel and bioactive *Streptomyces* species isolated from mangrove soil. These novel *Streptomyces* species also harbored valuable bioactive molecules. Asia has one of the most extensive mangrove coverages, with a total global mangrove area of 42%. As a matter of fact, 3.7% of the global mangrove coverage is located in Malaysia [24]. The mangrove ecosystem is one of the most productive environments. It is an underexplored source of actinobacteria with a huge potential to harvest biologically active compounds [7, 25]. A study investigating actinobacteria from Tanjung Lumpur mangrove forest (City of Kuantan, Pahang State,

Malaysia) unraveled that there was indeed a high level of diversity within the phylum Actinobacteria. Out of the 87 isolates, 59.8% (n = 52) belonged to the genus *Streptomyces*. In fact, they also discovered several rare *Actinobacteria*, namely *Sinomonas*, *Streptasidiphilus*, *Leifsonia*, and *Terrabacter*, which were genera not commonly found in mangrove environments. Additionally, most actinobacteria isolates were found to possess detectable biosynthetic genes polyketide synthetase (PKS) and non-ribosomal polyketide synthetase (NRPS), which indicated the ability to produce bioactive secondary metabolites [11]. Therefore, mangrove is a good source for bioactive actinobacteria discovery.

3. MOD-ACTINO Novel Species Discoveries in Malaysia

3.1. Novel Streptomyces in Malaysia

Many novel *Streptomyces* species have been isolated and identified from Malaysia's mangrove forest. Several of the novel *Streptomyces* species that were discovered in Tanjung Lumpur (Pahang) include *Streptomyces pluripotens* (MUSC 135^T, MUSC 137) [14, 26-28] *Streptomyces malaysiense* (MUSC 136^T) [29-31], *Streptomyces gilvigriseus* (MUSC 26^T) [32, 33], *Streptomyces mangrovisoli* (MUSC 149^T) [34, 35], and *Streptomyces antioxidans* MUSC 164^T [17, 36]. There are also some novel *Streptomyces* species that were discovered in Kuching (Sarawak), such as *Streptomyces monashensis* (MUSC 1J^T) [37-39], and *Streptomyces colonosanans* (MUSC 93J^T) [40].

3.2. Novel Rare-Actinobacteria in Malaysia

Several novel rare Actinobacteria strains have been discovered in Malaysia, including *Monashia flava* (MUSC 78^T) [41], *Microbacterium mangrovi* (MUSC 115^T) [42], Sinomonas humi (MUSC 117^T) [43], and *Mumia flava* (MUSC 201^T) [44]. These novel rare- actinobacteria strains were isolated from the mangrove soil of Tanjung Lumpur, Kuantan, in the State of Pahang.

4. MOD-ACTINO in Malaysia with Important Bioactivities

4.1. Anti-Methicillin-Resistant Staphylococcus Aureus (MRSA) Activity

Streptomyces is the largest antibiotic-producing genus that can produce antibiotics of different classes, including macrolides (tylosin from *Streptomyces fradiae*), β-lactams (cephamycin and clavulanic acid from *Streptomyces clavuligerus*), aminoglycosides (streptomycin from *Streptomyces griseus*), tetracycline (tetracycline from *Streptomyces aureofaciens*), and glycopeptide (vancomycin from *Streptomyces orientalis*) [15, 45-50]. However, the improper use of antibiotics has led to an issue with multidrug-resistant pathogens, such as, carbapenem-resistant *Enterobacteriaceae* (CRE), vancomycin-resistant *Enterococcus* (VRE), and Methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is non-susceptible to conventional antibiotic therapy, which results in life-threatening infections, posing a significant challenge to the health care system [51-53].

Increased antibiotic resistance is associated with biofilm formation- a vital pathogen virulent factor. Biofilm is a slimy layer comprised of a cluster of bacterial cells encased

within a hydrated matrix of polysaccharides and proteins, which can attach to animate or inanimate surfaces [51, 54]. Biofilm acts by protecting bacteria from the host immune system and antibiotics, causing the bacterial cell encapsulated within the biofilm to be more resistant to conventional antibiotics than their planktonic counterpart [51, 55]. Biomedical device-associated infections, which are often persistent, recurrent, and hard to treat, are primarily due to biofilms formed by *Staphylococcus aureus* [51]. Hence, it is crucial to discover new compounds targeting the biofilm to halt the spread of antibiotic-resistant pathogens.

A few potent anti-MRSA compounds produced by *Streptomyces* spp., such as polyketomycin, marinopyrrole, and griseusin A, showed promising results as future clinical drugs [9, 56]. The ability of *Streptomyces* spp. to offer sources for new antibiotics against MRSA has been highlighted through actinobacteria studies in Malaysia. For example, the novel bacteria sister strains MUSC 135^T and MUSC 137, with the name *Streptomyces pluripotens, were* isolated from Tanjung Lumpur mangrove soil. Interestingly, these two strains have been confirmed to be the same species, but only *S. pluripotens* MUSC 135^T strain exhibited antimicrobial activity. The study showed that *S. pluripotens* MUSC 135^T exhibited broad-spectrum bacteriocin activity against pathogens, including MRSA strain ATCC BAA-44 (inhibition zone of 10.5mm), *Aeromonas hydrophila* ATCC 7966^T (4mm), and *Salmonella typhi* ATCC 19430^T (4mm) [26, 27]. *S. pluripotens* MUSC 135^T demonstrated the strongest antibacterial activity against MRSA; thus, it can be a valuable source of the anti-MRSA agent. Furthermore, *S. pluripotens* MUSC 135^T exhibited antioxidant properties and produced metabolites with anti-colon cancer properties [27, 57]. Additionally, the analysis of the draft genome of MUSC 135^T (7,480,269 bp) revealed 72 biosynthetic gene clusters contributing to secondary metabolite production. In particular, 5 clusters demonstrated 85% or more similarities with known gene clusters: albaflavenone (100% similarities), ectoine (100%), desferrioxamine or deferoxamine (100%), antimycin (93%), and informatipeptine (85%) [27].

The *Streptomyces* sp. MUSC 125 (genome size of 7,656,461bp), also isolated from Tanjung Lumpur mangrove soil, had shown promising anti-MRSA and anti-biofilm compounds. This strain displayed a high 16S rRNA sequence similarity with *S. pluripotens* MUSC 135^T (99.93%), *Streptomyces cinereospinus* (99.24%), and *Streptomyces mexicanus* (99.17%) [58][59]. *Streptomyces* sp. MUSC 125 methanolic extract was demonstrated to be effective in inhibiting biofilm formation by MRSA at 1/8 × MIC (equivalent to 1.5625 mg/mL), the lowest concentration tested [58]. Although the methanolic extract of *Streptomyces* sp. MUSC 125 showed anti-MRSA activity, the MIC values of methanolic extract *Streptomyces* sp. MUSC 125 were relatively high compared to other recent studies [58, 60, 61]. Besides, *Streptomyces* sp. MUSC 125 possessed the biosynthetic gene clusters encoding for polyketide synthase type 1 (PKS I) and PKS II. PKS I is the primary enzyme for the biosynthesis of macrocyclic polyketides, whereas PKS II plays a role in aromatic polyketides synthesis. In fact, there are drugs derived from *Streptomyces* that are macrocyclic polyketides (erythromycin) or aromatic polyketides (doxorubicin, tetracyclin) [58, 62]. Hence, *Streptomyces* sp. MUSC 125 is a promising producer of cryptic polyketides that can be valuable therapeutic agents [58].

Streptomyces sp. SUK 25 isolated from medicinal plants in Malay Peninsular had demonstrated to be a potential anti-MRSA agent. Results showed that *Streptomyces* sp. SUK 25 crude extracts exerted antimicrobial activity against MRSA ATCC 49476, MRSA ATCC 43300, MRSA ATCC 33591, and the inhibition zones were 30mm, 20mm, and 21mm respectively. *Streptomyces* sp. SUK 25 showed more potent inhibition activity than the vancomycin control. *Streptomyces* sp. SUK 25 exhibited strong inhibitory activity with MIC value of 1.95µg/mL, at aeration of 140rpm, but not at higher speeds [63].

Based on the above findings, it is evident that streptomycetes possess anti-MRSA activity. Particularly, *S. pluripotens* MUSC 135^T had demonstrated to be a strong broad-spectrum bacteriocin against several pathogens and exerted significant anti-MRSA activity, which strengthens its potential to be a good source of antibiotics targeting MRSA.

4.2. Anticancer and Antioxidant Properties

Anticancer and antioxidant are interrelated in the event of cancer due to the association between cancer initiation and progression with oxidative stress, characterized by increased free radicals [36, 64]. Free radicals can cause modifications or cause damage to critical cellular macromolecules, for instance, DNA, lipid, and protein. These effects consequently compromise the functioning of DNA repair system, resulting in an increased mutation rate and an increased risk of cancer [34, 40]. Antioxidants exert their free radicals scavenging ability to prevent harmful effects of excessive free radicals during oxidative stress [40]. Several common anticancer drugs have been previously derived from *Streptomyces*[65]. These drugs are pentostatin, bleomycin, mitomycin C, aclarubicin, and doxorubicin [40]. Moreover, the antioxidants that have been sourced from *Streptomyces* spp. include antiostatins $A_1$to A_4 and B_2 to B_5 [66], benthocyanins A, B, C [67, 68], carazostatin [69], and carbazoquinocins A to F [70].

In Malaysia, numerous studies have revealed the anticancer and antioxidant properties of novel *Streptomyces* and rare *Actinobacteria* species, especially those isolated from Malaysian mangroves. The Malaysian MOD-ACTINO with anticancer and antioxidant properties that will be discuss in detail are *Streptomyces colonosanans* (MUSC 93J^T) [40], *Streptomyces monashensis* (MUSC 1J^T) [37-39], *Streptomyces antioxidans* MUSC 164^T [17, 36], *Streptomyces mangrovisoli* (MUSC 149^T) [34, 35], *Streptomyces malaysiense* (MUSC 136^T) [29-31], *Streptomyces pluripotens* (MUSC 137) [28], *Monashia flava* (MUSC 78^T) [41], *Microbacterium mangrovi* (MUSC 115^T) [42], and *Sinomonas humi* (MUSC 117^T) [43].

MUSC 93J^T and MUSC 1J^T are novel *Streptomyces* strains isolated from the mangrove soil in Kuching, Sarawak [71]. MUSC 93J^T was known by its colon-healing properties, hence, the name *Streptomyces colonosanans*; while MUSC 1J^T was known by its antioxidative activity and its given name is *Streptomyces monashensis* [37-40, 72]. *S.*

colonosanans MUSC 93J^T extract had demonstrated anticancer activity against human colon cancer cell lines (HCT-116, HT-29, Caco-2, and SW480) without significant cytotoxic effect against human normal colon cells (Table 1). The MUSC 93J^T extract also exhibited potent antioxidant activity. For instance, the extract (2mg/mL) exhibited 11.80 ± 3.75% of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) **(**ABTS) radicals scavenging activity, 50.06 ± 1.95% of metal chelating activity, and 83.32% ± 2.62% superoxide dismutase(SOD)-like activity. Additionally, this strain can produce chemo-preventive related metabolites via gas chromatography-mass spectrometry (GC-MS) analysis [40]. For example, compound 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester was detected in MUSC 93J^T extract, and this compound had been reported to have potential antibacterial, antifungal, and cytotoxic activities [40, 73, 74]. Most of the chemical compounds identified via GC-MS (Table 1) are recognized for their anticancer and antioxidant activities. Thus, these compounds might contribute to the antioxidant and cytotoxic properties exerted by MUSC 93J^T. Furthermore, Antibiotics & Secondary Metabolite Analysis SHell (antiSMASH) analysis detected 23 biosynthetic gene clusters in MUSC 93J^T. Four of the biosynthetic gene clusters exhibited more than 70% similarities to known gene clusters, and 1 cluster was linked to ectoine production (75% gene similarities)- a protective molecule [75].

S. monashensis MUSC 1J^T has a genome size of 10,254,857 bp. *S. monashensis* MUSC 1J^T extract demonstrated significant antioxidative activity, as it exhibited metal chelating activity of 75.50 ± 1.44%, and exerted up to 83.80 ± 4.80% SOD-like activity. These results suggest *S. monashensis* MUSC 1J^T can produce antioxidant(s), targeting oxidative stress. In terms of its cytotoxic activity, *S. monashensis* MUSC 1J^T extract exhibited significant cytotoxic effects against the colon cancer cell lines HCT-116 and SW480. However, it demonstrated the highest cytotoxic activity against SW480 with the lowest cell viability of 81.7% ± 4.0% when tested at the highest concentration of 400µg/mL [38, 39] (Table 1). A total of 14 compounds were detected via GC-MS (Table 1). Similar to *S. colonosanans* MUSC 93J^T, compounds Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-; Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- ; Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-; and Phenol, 2,4-bis(1,1-dimethylethyl)- were detected in *S. monashensis* MUSC1J^T [38, 39]. Whole genome analysis of *S. monashensis* MUSC 1J^T revealed the presence of biosynthetic gene clusters, where more than half of them were hypothesized as protein kinase synthases and non-ribosomal protein kinase synthetases, potentially producing compounds such as micromonolactams (100% known gene cluster similarities) and indogoidine (100%) [37]. Other studies have reported Indigoidine exerted antioxidant and antibacterial activities [76, 77]. Additionally, a gene cluster linked to the biosynthesis of desferrioxamine B (83%) was also detected [37]. Therefore, the detection of

these compounds in *S. monashensis* MUSC 1J^T via GC-MS and genome analysis has increased its value as a good microbial source for drug discovery.

S. antioxidans MUSC 164^T (genome size: 9,118,065 bp) isolated from the mangrove soil at Pahang exhibited antioxidative activities [36][17]. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the antioxidant activity at 2mg/mL of methanolic crude extract was 18.31 ± 2.03%. The extract also reduced ABTS radical (up to 30.38 ± 2.27), chelated ferrous ion (up to 43.66 ± 0.98%), and exerted 53.09-79.84% for the SOD assay. Furthermore, whole genome and bioinformatic analyses of *S. antioxidans* MUSC164^T revealed the presence of biosynthetic gene clusters associated to siderophores production such as desferrioxamine B, reaffirming its antioxidative potential [17].

S. mangrovisoli MUSC 149^T is another mangrove-derived novel *Streptomyces* that showed antioxidative potential as it exhibited significant free-radical scavenging up to 36.5 ± 3.0% at the highest concentration of 2mg/mL. Notably, the compound pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- was detected in the methanolic crude extract of *S. mangrovisoli* MUSC 149^T. Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- has been reported to demonstrate a strong antioxidant activity by other studies, and thus, further strengthens the hypothesis that this could be one of the compounds contributed to MUSC 149^T antioxidative potential [34, 78, 79]. The antioxidant potential of *S. mangrovisoli* MUSC 149^T is further highlighted with the detection of siderophores biosynthetic gene clusters – desferrioxamine B [35].

S. malaysiense MUSC 136^T (genome size: 7,963,326 bp) demonstrated promising antioxidant activity and cytotoxic potential against cancer cells [30][29]. It exhibited up to 68.27 ± 3.67% SOD-like activity. *S. malaysiense* MUSC 136^T extract was also found to possessed the highest cytotoxic activity against human colon cancer HCT-116 cells with the lowest cell viability of 48.8 ± 4.1% (at 400 μg/mL concentration) (Table 1). This occurrence was postulated to be mediated via the p53-dependent apoptosis pathways, as it was reported that there was an increase in p53 protein expression and a decrease in intracellular glutathione levels in HCT-116 cells treated with the extract. This phenomenon was accompanied by morphological changes related to cell death, such as the presence of shrunken cells. Additionally, *S. malaysiense* MUSC 136^T extract demonstrated the ability to produce chemo-preventive related metabolites through the compounds identified via GC-MS [30]. (Table 1) The antiSMASH analysis based on the whole genome sequence of *S. malaysiense* MUSC 136^T detected 36 biosynthetic gene clusters, whereby 7 clusters demonstrated more than 80% similarities to known gene clusters associated to ectoine, terpene, thiopeptide, lantipeptide,

and desferrioxamine. The production of desferrioxamine by *S. malaysiense* MUSC 136^T was hypothesized to contribute to the strain's cytotoxicity against colon cancer cell lines [29]

S. pluripotens MUSC 137, the sister strain of MUSC 135^T, has antioxidative and cytotoxic potentials. The antioxidant activity of *S. pluripotens* MUSC 137 tested via DPPH free radical scavenging assay was 35.03 ± 3.74% at 2mg/mL of extract. The cytotoxicity of *S. pluripotens* MUSC 137 extract was tested against various human cancer cell lines, including colon cancer cells: HCT-116, Caco-2, SW480, and HT-29; breast cancer cell: MCF-7, lung cancer cell: A549; prostate cancer cell: DU145; and cervical cancer cell: Ca Ski. Among the listed cancer cells, breast cancer cell MCF-7 was the most susceptible with the lowest IC_{50} of 61.33 ± 17.10 μg/mL, followed by HCT-116 (83.72 ± 7.17 μg/mL), A549 (147.20 ± 19.23 μg/mL). The chemical profile of *S. pluripotens* MUSC 137 was determined by GC-MS analysis, as shown in Table 1 [28].

On the other hand, several rare *Actinobacteria* strains also have shown anticancer potentials [80]. *Monashia flava* MUSC 78^T [41], *Microbacterium mangrovi* MUSC 115^T [42, 81], and *Sinomonas humi* MUSC 117^T [43] are novel rare *Actinobacteria* strains that were isolated from the mangrove soil of Tanjung Lumpur, Kuantan, in the State of Pahang. The anticancer properties of these rare *Actinobacteria* strains have been examined against two human cancer cell lines: colon cancer HT-29 cell line and cervical carcinoma Ca Ski cell line. The methanolic crude extracts of *Monashia flava* MUSC 78^T and *Microbacterium mangrovi* MUSC 115^T demonstrated significant cytotoxicity against Ca Ski cells with a dose-dependent response. On the contrary, the extracts of *Monashia flava* MUSC 78^T and *Microbacterium mangrovi* MUSC 115^T showed a lower cytotoxic activity against HT-29 cells. For *Sinomonas humi* MUSC 117^T, the extract exhibited significant cytotoxicity towards HT-29 cells only [80]. Based on GC-MS analysis, the number of compounds detected in the crude extracts of *Monashia flava* MUSC 78^T, *Microbacterium mangrovi* MUSC 115^T, and *Sinomonas humi* MUSC 117^T were 20, 6, and 10 respectively [80] (Table 1). The bioactivities exhibited by these rare *Actinobacteria* could be due to the production of phenolic and pyrazine compounds known for their antioxidant and anticancer/antitumor activities [80].

Overall, the Malaysian MOD-ACTINO strains have good antioxidant and anticancer properties. They are also capable of producing pyrrolopyrazine and phenolic compounds which are known for their antioxidant activity [34, 36, 78, 79, 82, 83]. In particular, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- were detected in many of the strains mentioned including *S. colonosanans* MUSC 93J^T [40], *S. monashensis* MUSC 1J^T [38, 39], *Microbacterium mangrovi* MUSC 115^T [80], *Monashia flava* MUSC 78^T [80] and *Sinomonas humi* MUSC 117^T [80]. Furthermore, *S. monashensis* MUSC 1J^T [37], *S. antioxidans* MUSC 164^T [17], *S. mangrovisoli* MUSC 149^T [35], *S. malaysiense* MUSC 136^T [29, 30], and *S. pluripotens* MUSC 137 [28] were capable of producing desferrioxamine. Desferrioxamine or deferoxamine is an iron chelator that remove excess iron and could inhibit the growth of tumor cells via intracellular iron pool depletion [84, 85]. Besides, these *Streptomyces* strains appeared to exhibit higher cytotoxicity towards human colon cancer cells, particularly HCT-116 and SW480. Many other actinobacteria with antioxidative and/or anticancer activities have also been

discovered from Malaysian environments, including *Streptomyces* sp. MUSC 125 [58, 59], *Streptomyces* sp. MUM 212 [86], *Streptomyces* sp. MUM 256[73, 87], *Streptomyces* sp. MUM 265 [88], *Streptomyces* sp. MUM 292 [89], *Streptomyces* sp. MUSC 14 [90, 91], *Streptomyces* sp. MUSC 125 [58, 59], *Streptomyces* sp. MUSC 5 [92], and *Streptomyces* sp. MUSC 11 [93]. Thus, the findings highlight that these MOD-ACTINO strains are valuable sources for drug discovery.

Table 1. The cytotoxic activity of Malaysian novel actinobacteria strains (MOD-ACTINO) against cancer cells and the chemical compounds detected via Gas-Chromatography Mass Spectrometry (GC-MS).

Strain	**Source**	**Cell Lines**	**Highest cytotoxicity against cancer cell line**	**Range of activities against the most sensitive cell line**	**Compounds detected via Gas-Chromatography Mass Spectrometry (GC-MS)**
Streptomyces colonosanans MUSC 93J^T [40]	Mangrove soil, Kuching, Sarawak	Colon cancer cell lines: HCT-116, HT-29, Caco-2, and SW480	SW480	Lowest cell viability of 63.6% ± 3.0% after being treated with the highest concentration of crude extract (400µg/mL)	• 2(5H)-Furanone • 1-Nonanol • Phenol, 2,4-bis (1,1-dimethylethyl)- • Benzoic acid, 4-ethoxy-, ethyl ester • Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- • 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester

Streptomyces monashensis MUSC 1J^T [38, 39]	Mangrove soil, Kuching, Sarawak	Colon cancer cell lines: HCT-116 and SW480	SW480	Lowest cell viability of 81.7% ± 4.0% after being treated with the highest concentration of crude extract (400µg/mL)	• Pyrazine, 2,5-dimethyl- • Pyrazine, trimethyl- • 2-Pyrrolidone • 2-Piperidinone • Indolizine • Pyrazine, 3,5-dimethyl-2-propyl- • Phenol, 2,4-bis(1,1-dimethylethyl)- • Benzoic acid, 4-ethoxy-, ethyl ester • (3R,8aS)-3-Methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- • Phenol, 3,5-dimethoxy- • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- • 9H-Pyrido[3,4-b]indole • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-
Streptomyces malaysiense MUSC 136^T [30]	Mangrove soil, Tanjung Lumpur, Kuantan, Pahang	Colon cancer cell lines: HCT-116 and HT-29 Lung cancer cell line: A549 Cervical cancer cell line: Ca Ski	HCT-116	Lowest cell viability of 48.8 ± 4.1% after being treated with the highest concentration of crude extract (400 µg/mL)	• Isomeric dihydro-methyl-furanone • 1-Pentadecene • Phenol, 2,5-bis (1,1-dimethylethyl)- • (3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione • 1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane • Tetradecanoic acid, 12-methyl-, methyl ester • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- • Pentadecanoic acid, 14-methyl-, methyl ester • Deferoxamine
Streptomyces pluripotens MUSC 137 [28]	Mangrove soil, Tanjung Lumpur, Kuantan, Pahang	Colon cancer cell lines: HCT-116, Caco-2, SW480, and HT-29 Breast cancer cell line: MCF-7	MCF-7	Lowest IC_{50} of 61.33 ± 17.10 µg/mL	• 2,2-dimethoxybutane • Benzeneacetamide • Phenol, 2,5-bis(1,1-dimethylethyl)- • (3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione • 2,5-cyclohexadiene-1,4-dione

		Lung cancer cell line: A549 Cervical cancer cell line: Ca Ski Prostate cancer cell line: DU145			• Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- • 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane • Deferoxamine • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-phenylmethyl)-
Monashia flava MUSC 78^{T} [80]	Mangrove soil, Tanjung Lumpur, Kuantan, Pahang	Colon cancer cell line: HT-29 Cervical carcinoma cell line: Ca Ski	Ca Ski	Cell viability of 62% (estimated based on the graph in the study) after being treated with the highest concentration of crude extract (200 µg/mL)	• 2-Methylpyrazine • Pyrrole, 2-methyl- • Pyrazine, 2,5-dimethyl- • 2,3,4-Trithiapentane • Pyrazine, 2-ethyl-6-methyl- • Pyrazine, 2-ethyl-5-methyl- • Pyrazine, trimethyl- • Pyrazine, 3-ethyl-2,5-dimethyl- • 4H-Pyran-4-one, 3-hydroxy-2-methyl- • 1H-Indole • 2,4-di-tert-butyl phenol • 1H-Pyrrole, 2-phenyl- • 1-Naphthalenamine, N-ethyl- • 3,4-Dimethyl-2-phenyl-1H-pyrrole • (3R,8aS)-3-Methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- • Methyl 13-methyltetradecanoate • Hexadecanoic acid, methyl ester • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- • 3-benzyl-1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane
Microbacterium mangrovi MUSC 115^{T} [80]	Mangrove soil, Tanjung Lumpur, Kuantan, Pahang	Colon cancer cell line: HT-29 Cervical carcinoma cell line: Ca Ski	Ca Ski	Cell viability of 54% (estimated based on the graph in the study) after being treated with the highest concentration	• Methyllaurate • 2,4-di-tert-butyl phenol • (3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione

				of crude extract (200 µg/mL)	• Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- • 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane • 3-benzyl-1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane
Sinomonas humi MUSC 117^{T} [80]	Mangrove soil, Tanjung Lumpur, Kuantan, Pahang	Colon cancer cell line: HT-29 Cervical carcinoma cell line: Ca Ski	HT-29	Cell viability of 80% (estimated based on the graph in the study) after being treated with the highest concentration of crude extract (200 µg/mL)	• Butanoic acid, 3-methyl- • Butanoic acid, 2-methyl- • 2,4-di-tert-butyl phenol • (3R,8aS)-3-Methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- • Methyl n-pentadecanoate • 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane • 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1′,2′-d]pyrazine • Methyl 14-methylhexadecanoate • Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-

4.3. Neuroprotective Effect

The MOD-ACTINO strains isolated from Malaysia are capable of exerting a neuroprotective effect. In addition to the antioxidative effect, *S. antioxidans* MUSC 164^{T} extract exhibited potent neuroprotective activity on neuronal SH-SY5Y cells against hydrogen peroxide (H_2O_2). Hence, it resulted in the highest cell viability of 80.62 ± 2.75% when pre-treated at 400µg/mL [36]. Furthermore, *Microbacterium mangrovi* MUSC 115^{T} extract was reported to exhibit a similar neuroprotective effect against oxidative stress-induced cytotoxicity on neuronal SH-SY5Y cells. The extract protected neuronal SH-SY5Y cells against H_2O_2 challenged at a low concentration of 6.25 µg/mL, and the maximum efficacy was at 12.5µg/mL. *Microbacterium mangrovi* MUSC 115^{T} extract also demonstrated neuroprotective activity on dementia-induced cytotoxicity as the neuronal cells were protected from streptozotocin (STZ)-induced neuronal damage at extract concentrations ranging from 6.25 – 2.5µg/mL. As for *Monashia flava* MUSC 78^{T}, the extract produced a neuroprotective effect against hypoxia-induced cytotoxicity. It protected neuronal SH-SY5Y cells from cobalt (II) chloride ($CoCl_2$) insult at a concentration of 6.25 – 50µg/mL [80].

4.4. Antifungal Activity

Plants can be vulnerable to diseases, and conventional treatment using chemical fungicides may provide an absolute cure, but it may also cause environmental pollution. Hence, there is a need for an alternative solution. An increasing amount of research focuses on using antagonist microbes as biological control agents for an environmentally friendly approach. Many studies have shown that actinobacteria are capable of suppressing plant diseases [94]. Herein, this review will discuss the potential of Malaysian MOD-ACTINO strains as biological control agents against phytopathogenic fungi in plant diseases. The targeted plants include rice, oil palm, banana plantlet, and chili.

Rice is one of the staple foods, particularly in Asia, whereby the annual consumption of rice reaches >110kg per capita. Oryza sativa is one of the rice species that humans consume [95]. However, phytopathogenic infection in rice may lead to crop yield losses, and some fungi produce compounds that are potentially toxic upon consumption. Therefore, to prevent such occurrence, there is a need to discover effective biocontrol agents against these phytopathogens in rice [12]. The two main pathogens of rice, *Fusarium oxysporum* and *Pyricularia oryzae* (*P.oryzae*) are causative agents of root rot and blast in rice, respectively [96]. A study by Awla et al. found that *Streptomyces* isolate UPMRS4, from rice fields soils of Tanjung Karang, in the State of Selangor, produces bioactive antifungal compounds and exhibited good antifungal activity against *P. oryzae*. Ethyl acetate extract exhibited the highest inhibition of 98.33% towards mycelial growth of *P.oryzae* compared to the control at a 100µg/mL concentration and had an effective inhibitory concentration (EIC) of 1.562µg/mL. Besides, from the ethyl acetate crude extract, GC-MS detected 22 volatile compounds for which some of these compounds might account for direct inhibition of bacterial and fungal pathogens. Compounds detected include Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl), and ergotamine. Additionally, liquid chromatography-mass spectrophotometry (LC-MS) detected 35 different compounds, which include fungicidal compounds amicomacin, fungichromin, N-Acetyl-D, L-phenylalanine, and rapamycin. Thus, *Streptomyces* sp. isolate UPMRS4 can be a promising candidate as biocontrol agent against *P.oryzae* [97].

In regards to oil palm, two studies reported on using actinobacteria isolates to inhibit *Ganoderma boninense*, the main causal agent of basal stem rot (BSR) disease in oil palm (*Elaeis guineensis* Jacq.) [94, 98]. Rhizosphere soil samples from 15 healthy oil palms were collected from four oil palm plantations in Peninsular Malaysia with a severe history of *G. boninense* infection. Findings of the *in vitro* study showed that approximately 13.5% of isolates exhibited Percentage Inhibition of Radial Growth (PIRG) of more than 80% towards *G. boninense*. Also, 21 of these isolates exerted an antagonistic effect, causing the abnormal growth of *G. boninense*. The culture filtrates of four of these 21 isolates (AGA 043, AGA 048, AGA 347, and AGA 506) inhibited and showed potential production of metabolites against *G. boninense* [94]. The researchers then continued analyzing these 4 isolates and found all they belonged to the genus *Streptomyce*s. The isolates AGA347 and AGA506 showed 99% similarity with *S. hygroscopicus* subsp. *hygroscopicus* and *S. ahygroscopicus*,

respectively. Under glasshouse conditions, the study reported that powder formulation of AGA347 was the most effective in reducing BSR in seedlings by 73.1%. On the other hand, formulations using the known antifungal producer *Streptomyces noursei*, AGA043, AGA048, and AGA506 reduced BSR by 47.4%, 30.1%, 54.8% and 44.1%, respectively [98]. Hence, *Streptomyces* spp. has biocontrol potential against *G. boninense* in oil palm.

Getha et al. also investigated the antagonistic effect of *Streptomyces* sp. against *Fusarium oxysporum* f.sp. *cubense* (Foc), a pathogen that causes the wilt disease of bananas. The selected *Streptomyces* strain G10 was isolated from Port Dickson, in the State of Seremban. This strain G10 was assigned to the clade *Streptomyces violaceusniger,* and it exhibited a strong antagonistic activity against different pathogenic races of the *Fusarium* wilt pathogen. Strain G10 exhibited *in vitro* antibiosis as shown by inhibition zones, and the inhibited fungal colonies showed lysis of hyphal ends. Furthermore, the *in vitro* antagonistic effects against Foc were found to be due to the production of antifungal metabolite by strain G10 [99]. Results of *in vivo* experiment revealed that planting hole and roots of four -week-old tissue culture derived 'Novaria' banana plantlets treated with strain G10 suspension at 10^8 cfu/mL demonstrated a significant decrease in wilt severity in plantlets inoculated with 10^4 spores/mL Foc race 4. The plantlets treated with strain G10 showed a 47% and 53% reduction in the final disease severity for leaf symptoms and rhizome discoloration, respectively, compared to the untreated plantlets. However, strain G10 may provide better control at lower Foc inoculation concentrations. To sum up, *Streptomyces violaceusniger* strain G10 could interfere with the banana wilt disease cycle and may be a valuable biological control agent for the *Fusarium* wilt disease of bananas [100].

Another study discovered that *Streptomyces* isolated from rhizosphere soil of chili plants collected from a chili farm in Ulu Chuchoh and Sungai Burung, in the State of Selangor, exhibited a range of *in vitro* inhibitory activity against three different dominant species of *Colletotrichum* including *C. acutatum*, *C. gloeosporioides*, and *C. capsici*. *Streptomyces* strain P42 was selected as a biological control agent under greenhouse conditions as it demonstrated the highest inhibitory activity against all three fungi species and had high chitinase activity. Strain P42 was identified as belonging to the *Streptomyces rochei* clade and it could protect chili plants from anthracnose disease under greenhouse conditions. Therefore, it is a promising biocontrol agent of anthracnose disease in chili plants [101].

Thus far, *Streptomyces* isolates derived from different Malaysian environments are capable of exhibiting antifungal properties and thereby suggesting their use as potential biocontrol agents for various plants such as rice, oil palm, banana plantlet, and chili.

4.5. Antimalarial Activity

Malaria is a human parasitic disease that affects many parts of the world. According to the World Health Organization (WHO), an estimated 241 million malaria cases were recorded globally in 2020, in 85 malaria-endemic countries. The increase in drug resistance in the Greater Mekong subregion is worrisome as the parasite *Plasmodium falciparum* had

developed partial resistance to artemisinin, which is the core compound of the best antimalarial drugs. It is fortunate for Malaysia that there were no cases of non-zoonotic malaria for three consecutive years [102]. Additionally, chloroquine is one of the antimalarial drugs used worldwide in the 20th century. Currently there is an emergence of parasites with chloroquine resistance after decades of utilizing chloroquine as a treatment regime. *Plasmodium falciparum* is the most malignant of the four human malaria parasite species and has shown foci of chloroquine resistance in Southeast Asia since the late 1950s [103]. Hence, finding alternative bioactive compounds capable of combating these resistant parasites is crucial. A few studies in Malaysia also demonstrated the capability of MOD-ACTINO in producing valuable compounds with antimalarial activities, for instance, *Streptomyces* sp. H11809 [104, 105] and *Streptomyces* sp. SUK 10 [16]

A study conducted by Dahari et al. isolated *Streptomyces* sp. H11809 from a soil sample in Imbak Valley and Likas, in the State of Sabah, East Malaysia. The acetone crude extract of H11809 showed potent *in vitro* inhibition on the growth of *Plasmodium falciparum* 3D7 (*Pf* 3D7) with IC_{50} value of 0.57 ± 0.09 µg/mL. Dibutyl phthalate (DBP) produced by *Streptomyces* sp. H11809 showed active anti-plasmodial activity against *Pf* 3D7 (IC_{50} 4.87 ± 1.26 µg/mL equivalent to 17.50 µM). Hence, the study suggested that DBP is the bioactive compound exerting antimalarial activity via glycogen synthase kinase 3β (GSK-3β) inhibition [104]. Another bioactive compound known as nocardamine (desferrioxamine E) was also discovered from *Streptomyces* sp. H11809 chloroform extract. Nocardamine exhibited moderate antimalarial activity against *Pf* 3D7, with IC_{50} of 1.5 µM, which was more potent than DBP [105].

Besides that, Zin et al. discovered an endophytic *Streptomyces* sp. SUK 10 from the bark of *Shorea ovalis* tree in Malaysia that produces a bioactive compound known as Gacidin W, a potential low-toxicity antimalarial agent. Mice model experiment of Gancidin W against *Plasmodium berghei* PZZ1/100 showed an inhibition rate of almost 80% of the parasite at the concentration of 6.25 and 3.125 µg/kg body weight on the last day of the test. Furthermore, 50% (n=3) of mice treated with Gancidin W at a concentration of 3.125 µg/kg body weight survived until 291.13 ± 0.5 days after inoculation of infection, which is roughly the life span of normal mice (12-18 months). Hence, this suggests that Gancidin W is one of the metabolites contributing to the *in vivo* antimalarial activity, as demonstrated in the animal model [16].

5. Conclusions

Nature is a treasure chest for novel and biologically active actinobacteria discovery. This review presents mounting evidence of MOD-ACTINO discovery from Malaysia. MOD-ACTINO from Malaysia can produce medically valuable bioactive metabolites, including anti-MRSA/antimicrobial, anticancer, antioxidant, antifungal, and antimalaria metabolites. These metabolites could be further isolated and developed into useful drugs to help improve human health and well-being. Nature is undoubtedly a rich source for novel *Streptomyces*

discovery. It is anticipated that more novel strains await to be explored from unique environments, evidenced by their discoveries from mangrove environments. In summary, the MOD-ACTINO strains from Malaysia are valuable resources worth further investigation.

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