Applied Biochemistry and Biotechnology (2020) 192:1224–1237
https://doi.org/10.1007/s12010-020-03361-3

Awakening the Secondary Metabolite Pathways of *Promicromonospora kermanensis* Using Physicochemical and Biological Elicitors

Fatemeh Mohammadipanah[1] · Fatemeh Kermani[1] · Fatemeh Salimi[2]

Received: 2 April 2020 / Accepted: 22 June 2020 / Published online: 27 July 2020

Abstract

The drug discovery rate is dramatically decreasing due to the rediscovery of known compounds. Genome mining approaches have revealed that a large portion of the actinobacterial genome that encodes bioactive metabolites is cryptic and not expressed under standard lab conditions. In the present study, we aimed to induce antibiotic encoding biosynthetic genes in a member of *Micrococcales* as a new species of *Promicromonospora*, *Promicromonospora kermanensis*, by chemical and biological elicitors as it was considered to produce numerous valuable bioactive metabolites based on the whole genome results. Induction has been done via chemical (antibiotics, histone deacetylase inhibitors (HDAIs), rare earth elements (REEs), fatty acid synthesis inhibitors, and extreme pH changes) and biological elicitors (live and dead Gram-positive and negative bacteria). The results showed that valproic acid (as HDAIs), DMSO, lanthanum chloride (as REE), triclosan (as fatty acid synthesis inhibitors), alkaline pH, and supernatant of *Pseudomonas aeruginosa* UTMC 1404 culture could act as stimuli to provoke antibacterial synthetic pathways in *Promicromonospora kermanensis* DSM 45485. Moreover, it was revealed that eliciting agents in cell filtrated of *P. aeruginosa* culture is resistant to detergent, acidic, and basic condition and have amphipathic nature. The inducing effect of alkaline pH on metabolite induction of *Actinobacteria* was first reported in this study. In the follow-up studies, the induced antibacterial producing clusters can be subjected to the characterization, and the implemented approach in this study can be used for metabolites induction in other selected species.

Keywords *Actinobacteria* · Antibiotics · Bioactive secondary metabolites · Cryptic pathways · Elicitors · *Promicromonospora kermanensis*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12010-020-03361-3) contains supplementary material, which is available to authorized users.

✉ Fatemeh Mohammadipanah
fmohammadipanah@ut.ac.ir

Extended author information available on the last page of the article

Introduction

It is predicted that the antibiotic-resistant strains lead to 10 million deaths by 2050 and impose an economic cost of $100 trillion [1, 2]. Many antibiotics have been discovered from *Actinobacteria* as a rich source of natural compounds, and mining rare *Actinobacteria* are one of the most promising strategies to find new and structurally diverse microbial-derived compounds [3].

The time-consuming and labor-intensive process of purification and structure elucidation of isolated antibiotics and, in particular, the high percentage of the rediscovery of previously known compounds has dramatically declined the efficiency of new compound discovery over time [4]. Due to the high rate of rediscovery, major pharmaceutical companies have abandoned screening for novel antibiotics and have employed other alternative strategies such as bioinformatics-based approaches to reinvigorate antibiotic discoveries.

The genome analyses revealed that a large portion of the biosynthetic gene clusters (BGCs) of *Actinobacteria* is unexplored. It was envisaged that the currently available data on BGCs in microbial genomes would revolutionize the rate of discovery through genome-mining based approaches; however, it could not make acceleration as it was predicted [5].

For instance, genome mining of *Actinobacteria* shows that they have metabolite-encoding genes for thousands of unknown compounds in their genomes, which remain undiscovered since they are not transcribed under general laboratory conditions [6].

This mismatch between biosynthetic potential and isolable bioactive compounds in the fermentation process may result from week expression and/or translation of BGCs and disability of available detection methods.

These cryptic BGCs represent an enormous reservoir of new druggable leads to produce novel antimicrobials and other bioactive compounds. Inducing production of silent secondary metabolites can be encouraged through several techniques including, molecular (genetic manipulation and expression of BGC of interest in heterologous hosts or ribosome engineering), cultivation-based approaches (modifications in chemical constituents of culture media and cultivation parameters like pH, temperature, or aeration), and elicitation approach (using elicitors to induce the expression) [7–9].

The production of secondary metabolites can be increased in culture-based methods by altering the levels of substrates or activity of the involved enzymes affecting gene regulation, metabolism, and growth parameters. Although medium optimization can alter the metabolic profile of various microorganisms, it has disadvantages like laborious and resource-intensive need that limit its application in transcription induction of new metabolites [10].

The elicitation approach has been developed because the products of cryptic gene clusters may involve in competitiveness and responsiveness to environmental stress in a natural ecosystem. Therefore, in an alternative approach, microbial cells, which are secondary metabolite production prone, can be subjected to biological, physical, or chemical elicitation. These agents may mimic ecological and environmental stimuli.

In this regard, chemicals, including antibiotics (at sub-inhibitory levels) [11], polysaccharides or their monomers [12], bacterial or plant hormones [13, 14], exposure to metals (at low-level) [15], rare earth elements (REEs) (scandium, yttrium, lanthanides, lanthanum, lutetium) [16], inorganic or organic compounds (dimethyl sulfoxide (DMSO) and ethanol), molecules with modulating ability of fatty acid biosynthesis [14], and physical (various temperatures and irradiation) approaches can be applied to trigger the secondary metabolite pathways [17, 18].

It has been shown that elicitors can modify the transcription of clusters of secondary metabolite genes in *Actinobacteria*. Among these chemicals, inhibitors of histone deacetylases (HDAC) are well known to be involved in the transcriptional regulation of biosynthetic pathways in *Streptomyces coelicolor* A3(2) through modifying the nucleoid structure [19]. REEs can activate the expression of silent or poorly expressed genes in bacteria and consequently enhance the production of bioactive compounds, including antibiotics, enzymes, pigments, mycotoxins, and phytotoxins [20]. Ethanol and DMSO have also been applied to elicit biosynthesis of microbial compounds, perhaps by mistranslation or inducing the stress response [21, 22].

The presence of polysaccharides, antibiotics, and bacterial hormones (at low concentrations) in the culture medium can also be used to stimulate the production of bioactive metabolites [23, 24]. Hormones, e.g., gamma-butyrolactones play a critical role in bacterial communication, known as quorum sensing promote antibiotic production in many *Streptomyces* spp. [25]. In this communication, bacteria synchronize their gene expression via producing and detecting the autoinducing agents [26].

It has been revealed that a family of molecules that modulate fatty acid production referred to as antibiotic remodeling compounds (ARCs) can elicit cryptic genes via changing fatty acid pool by inhibition of key biosynthetic enzymes like FabI. Since there is a competition among a synthesis of fatty acid and secondary metabolism for the common substrate, acetyl-CoA, these compounds may act through partial inhibition of FabI and therefore make a preferential flow of acetyl-CoA for the synthesis of antibiotic. Remarkably, triclosan, a well-known biocide and inhibitor of fatty acid biosynthesis, can stimulate polyketide antibiotic synthesis by this mechanism [27].

According to these findings, several studies have been conducted on the elicitation of cryptic genes of *Streptomyces,* while the activation, production, and identification of natural products from rare *Actinobacteria* are less investigated. Here, we aimed to de-silence the hidden secondary metabolite biosynthetic cluster of a soil-driven rare Actinobacterium*, Promicromonospora kermanensis* [28], which shows no antimicrobial activity under reasonable lab condition by chemical (DMSO, triclosan, antibiotics, lanthanum chloride, HDAC inhibitors, alkaline pH) and biological (live and dead cell mass) approaches.

Material and Methods

Chemicals and Bacterial Strains

All chemicals were of analytical grade. Sodium butyrate, valproic acid, lanthanum chloride, benzamide, triclosan, and DMSO were obtained from Merck (Germany). Streptomycin, bacitracin, and vancomycin were purchased from Sigma-Aldrich (USA). *Promicromonospora kermanensis* DSM 45485, *Nocardia* sp. UTMC 557, *Pseudomonas aeruginosa* UTMC 1404, and Methicillin-resistant *Staphylococcus aureus* (MRSA) UTMC 1401 were provided from University of Tehran Microorganisms Collection (UTMC).

Treatments with Antibiotics as the Inducing Agents

To evaluate the efficiency of antibiotics on provoking the expression of silent pathways, ISP2 broth medium (4 g L^{-1} of glucose, 4 g L^{-1} yeast extract, 10 g L^{-1} malt extract, pH 7.2 ± 0.2) was prepared and autoclaved at 121 °C with 1.2 bar. After cooling, antibiotics including

vancomycin (5 and 10 mg mL^{-1}), bacitracin (5 and 10 mg mL^{-1}), and streptomycin (25 and 50 mg/mL) were added to the medium following 0.45 μm after filtration of the stock solutions.

Evaluation of Chemical Factors as the Inducing Agents

To assign the provoking ability of chemical inducers, compounds, including sodium butyrate (25 and 50 mM), valproic acid (0.05 and 1 mM), triclosan (1 and 5 μM), DMSO (1 mM and 2 mM), benzamide (2.5 mM and 5 mM), lanthanum chloride (50 and 100 μM), were supplemented to the ISP2 broth medium. To analyze the inducing activity of alkaline condition, the pH of ISP2 broth medium was adjusted in pH 9 using 5 M NaOH.

Microbial Inoculation and Incubation Condition

The antibiotic and chemical supplemented media, and also alkaline ISP2 medium, were inoculated by 10^7–10^8 spores mL^{-1} and were incubated at 28 °C and 170 rpm for 5 days. The uninoculated ISP2 broth medium and ISP2 broth medium without inducing agents (pH 7.2 ± 0.2) were used as the control to evaluate the antimicrobial activity of medium and *Promicromonospora kermanensis*, respectively. The growth of *Promicromonospora kermanensis* in supplemented and control media was assessed via the colony counting.

Elicitation via Filtrated Supernatant of Bacterial Culture

Pseudomonas aeruginosa UTMC 1404 and *Nocardia* sp. UTMC 557 were cultured in liquid ISP2 medium and incubated at 28 °C, at 170 rpm for 4 days. At the end of the incubation period, the fermentation broth was centrifuged at 2500 *g* for 15 min. Supernatants were autoclaved at 121 °C with 1.2 bar and then used as a stimulant for *Promicromonospora kermanensis* metabolite production. The flasks were incubated at 28 °C, shaken at 170 rpm for 5 days.

Evaluation of Dead Bacteria Debris as Induction Agents

Suspensions of *Nocardia* sp. UTMC 557 (10^8 cell mL^{-1}, 2.5% v/v) and *Pseudomonas aeruginosa* UTMC 1404 (10^8 cells ml^{-1}, 5% v/v) were separately added to ISP2 medium and then were autoclaved at 121°C with 1.2 bar. The media were inoculated with *Promicromonospora kermanensis* and incubated at 28 °C, shaken at 170 rpm for 5 days.

Elicitation Using Cell Wall Component

The N-acetylglucosamine-supplemented (2 mg/mL) ISP2 broth medium was inoculated with *Promicromonospora kermanensis* and incubated at 28 °C, shaken at 170 rpm for 5 days.

Evaluating the Chemical Characteristics of Elicitors in the Supernatant of *Pseudomonas aeruginosa* Culture

The stimulating activity of compounds in the supernatant of *Pseudomonas aeruginosa* UTMC 1404 culture was evaluated after their exposure to different types of stress agents, including H_2O_2, SDS, and acidic (pH 4) and basic pH (pH 9) conditions. Treated supernatants were added to *Promicromonospora kermanensis*–inoculated ISP2 broth media and incubated at 28

°C, at 170 rpm for 5 days. Untreated supernatant of *Pseudomonas aeruginosa* UTMC 1404 culture and unelicited *Promicromonospora kermanensis* were used as negative controls.

Evaluation of the Hydrophobicity of Eliciting Agents in the Supernatant of *Pseudomonas aeruginosa* Culture

To evaluate the polarity or hydrophobicity of eliciting agents in the supernatant of *Pseudomonas aeruginosa* UTMC 1404 culture, the supernatant was passed through the solid state RP column and silica gel columns. The eluted samples were added to fermentation ISP2 broth of *Promicromonospora kermanensis* and incubated at 28 °C, 170 rpm for 5 days.

Extraction of Secondary Metabolites

The fermented broth was centrifuged at 2500 *g* for 10 min to remove the biomass and cell debris. The extracellular metabolites were extracted from supernatant using an equal volume of ethyl acetate by vigorous shaking for 1 h. The resultant organic phase was separated and concentrated at low pressure and temperature (Heidolph, Germany). The fermentation broth extracts of *Promicromonospora kermanensis* were stored at − 20 °C until use.

Qualitative Evaluation of the Extracts Using Thin-Layer Chromatography

The obtained extracts were qualitatively analyzed using the thin-layer chromatography (TLC). The extracts (10 μL with a concentration of 5 mg mL^{-1}) were spotted on TLC silica gel 60F254 (Macherey-Nagel) and developed using the solvent system of dichloromethane/methanol (93:7 v/v). The developed TLC plates were observed under UV illumination of 254 nm and 366 nm and reacted by anisaldehyde reagent.

Antibacterial Activity Evaluation of the Extracts

The antibacterial activity of extracts was determined using the disc diffusion assay in triplicate experiments. In brief, the extracts were loaded on blank discs (50 and 150 μg per disc). The loaded discs were placed on inoculated Mueller-Hinton agar plates with Methicillin-resistant *Staphylococcus aureus* (MRSA) UTMC 1401. The vancomycin disc (30 μg/per disc) was used as a positive control. The plates were left at 4 °C for 3 h for diffusion before the bacterial growth. Then, the growth inhibition zones were measured after incubated at 37 °C for 18–24 h. The process of triggering and evaluation are summarized in Fig. 1.

Results

The Ability of Tested Antibiotics on Improving Antimicrobial Activity

The spiked vancomycin, streptomycin, and bacitracin had no inducing effect on antibacterial metabolite production of *Promicromonospora kermanensis* at two levels of sub-MIC concentrations as summarized in Table 1, and no growth inhibition zone was observed around loaded discs with extracts of *P. kermanensis.* The streptomycin at the applied concentration even inhibited the growth of *P. kermanensis.*

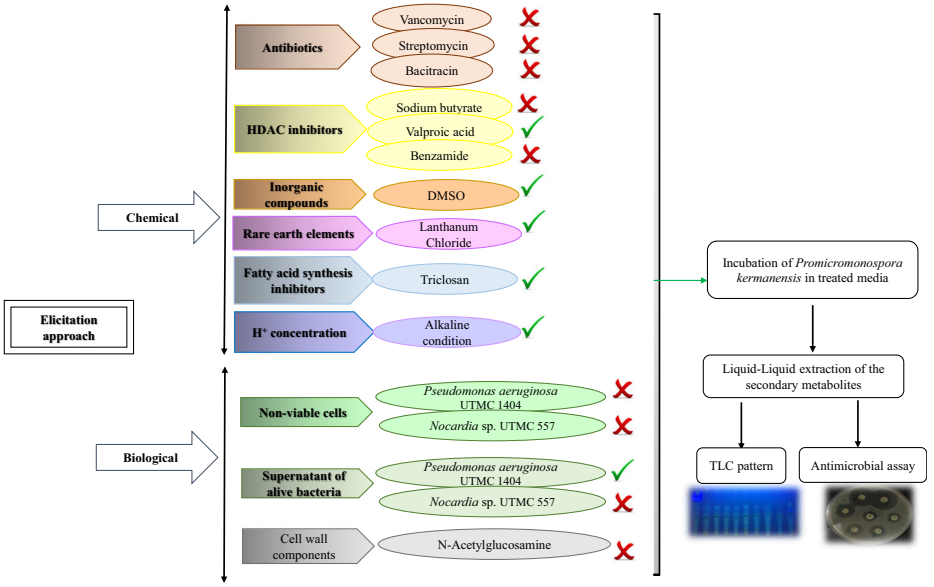

Fig. 1 Schematic presentation of the secondary metabolite induction of *Promicromonospora kermanensis* using chemical and biological elicitors

Evaluating the Effect of Chemical Agents as Inducers

The categories of compounds, including HDACs inhibitors (sodium butyrate, valproic acid, benzamide), rare earth element (lanthanum chloride), fatty acid synthesis inhibitors (triclosan)and miscellaneous (DMSO and high hydroxide concentration) were applied as chemical-inducing factors. All applied HDACs inhibitors except benzamide induced the expression of antimicrobial genes of *P. kermanensis*. It was observed that the extract of supplemented medium with 5 μM triclosan had higher antibacterial activity than that of supplemented medium with 1 μM triclosan. In contrast, lanthanum chloride showed its evoking activity at the lower applied concentration (50 μM), and the inducing activity was not observed at a higher concentration of 100 μM.

The extracts of sodium butyrate containing ISP2 broth media showed antibacterial activity. According to the low growth rate of *P. kermanensis* in these media, it is deduced that the antibacterial growth is due to the antimicrobial activity of sodium butyrate, not its eliciting activity (Table 2). The results showed that the production of the antibacterial secondary metabolite of *Promicromonospora kermanensis* could be stimulated in the alkaline condition of the growth medium in liquid ISP2. The appearance of anti-MRSA activities (17 and 16 mm) is attributed to the induction of the expression of antibacterial compound encoding genes.

Biological Induction Approach

Induction of cryptic or low yield secondary metabolite biosynthetic gene clusters of *P. kermanensis* via biotic elicitors was also effective. The result of this investigation is summarized in Table 3. Among the applied biological inducers, just live *P. aeruginosa* cells could induce the antibacterial activity against MRSA, while its culture supernatant had no antibacterial activity on the tested MRSA (Fig. S1). According to TLC patterns, new metabolite bands were appeared in elicitation

Table 1 Antibacterial activity of *Promicromonospora kermanensis* extracts following the stimulation using sub-MIC concentration of antibiotics

Type of inducing agent	Inducers	Mode of action/goal	Concentration	50 μg per disc	150 μg per disc	Growth
Negative controls	Not elicited fermentation broth	Investigation of antibacterial activity under normal cultivation condition	-	NA	NA	G
	Not inoculated medium	Investigation of antibacterial activity of uninoculated medium	-	NA	NA	G
Antibiotics	Streptomycin	Disruption of the protein synthesis	25 μg/mL	NA	NA	NG
			50 μg/mL	NA	NA	NG
	Vancomycin	Inhibition of cell wall synthesis	5 μg/mL	NA	NA	G
			10 μg/mL	NA	NA	G
	Bacitracin	Interference in peptidoglycan synthesis	5 μg/mL	NA	NA	G
			10 μg/mL	NA	NA	G

NA, no antimicrobial activity; NG, no growth; and G, growth

Table 2 Antibacterial activity of the extracts of *Promicromonospora kermanensis* induced by HDAC inhibitors, rare earth elements, inhibitors of fatty acids synthesis, DMSO and high concentration of hydroxide ion

Category of eliciting agent	Inducers	Concentration	50 μg per disc	150 μg per disc	Growth
HDAC inhibitors	Sodium butyrate	25 mM	24.5 ± 0.5 mm	27 ± 1.0 mm	NG
		50 mM	15.5 ± 0.5 mm	18 ± 0.5 mm	NG
	Valproic acid	1 mM	16 ± 1.0 mm	17 ± 1.5 mm	G
		0.5 mM	-	-	G
	Benzamide	2.5 mM	-	-	G
		5 mM	-	-	G
	DMSO	1 mM	18.5 ± 0.5 mm	17 ± 2.5 mm	G
		2 mM	19 mm ± 1.0	17 mm ± 0.5 mm	G
Rare earth elements	Lanthanum chloride	50 μM	10.5 ± 0.5 mm	11 mm ± 0.5 mm	G
		100 μM	-	-	G
Fatty acid synthesis inhibitors	Triclosan	1 μM	14 ± 2 mm	18.5 ± 0.5 mm	G
		5 μM	21.5 ± 0.5 mm	28. ±0.5 mm	G
H^+ concentration	Alkaline condition	pH 9	17.5 ± 0.5 mm	16.5 ± 0.5 mm	G

G, growth and NG, negligible growth

using dead cells, alive cells, and cell wall component; however, only induced metabolite by the alive cells of *P. aeruginosa* had antibacterial activity (Fig. S2).

Chemical Stability of *Pseudomonas aeruginosa* Elicitating Agent

According to the stability experiments, metabolites of *Pseudomonas aeruginosa* with provoking activity on *P. kermanensis* metabolites were sensitive to oxidative stress as they lost their inducing ability in the presence of H_2O_2, at the same time they were resistant to SDS, acidic, and basic pH (Table 4).

Hydrophobicity Features of Eliciting Factors in Supernatants of *Pseudomonas aeruginosa*

Eluted samples from RP and silica gel columns could not induce the antibacterial metabolites of *Promicromonospora kermanensis* with anti-MRSA activity. Therefore,

Table 3 Biotic elicitation of cryptic or low-expressing antimicrobial encoding genes of *Promicromonospora kermanensis* via dead and supernatant of a Gram-positive (*Nocardia* sp. UTMC 557) and Gram-negative (*Pseudomonas aeruginosa* UTMC 1404)

Type of biological inducers	Bacteria / Cell wall component	Concentration	50 μg per disc (mm)	150 μg per disc (mm)
Non-viable cells	*Pseudomonas aeruginosa* UTMC 1404	5% (v/v) 10^8 cells/mL	0	0
	Nocardia sp. UTMC 557	2.5% (v/v) 10^8 cells/mL	0	0
Supernatant of alive bacteria	*Pseudomonas aeruginosa* UTMC 1404	-	23 ± 0.5	25 ± 0.5
	Nocardia sp. UTMC 557	-	0	0
Cell wall component	N-Acetylglucosamine	2 mg/mL	0	0
No elicitation (negative control)	-	-	0	0
Positive control	Vancomycin	30 μg/μL	18 ± 0.5	

Table 4 The provoking activity of treated metabolites of *Pseudomonas aeruginosa* UTMC 1404 compared to the controls.

Type of exposed stress on the supernatant of *Pseudomonas aeruginosa*	Agent	Concentration	50 μg per disc (mm)	150 μg per disc (mm)
Oxidative stress	H_2O_2	3% (v/v)	--	-
Surfactant	SDS	10 μg/mL	15 ± 0.5	15 ± 0.5
Acidic pH	4	-	9 ± 0.5	17 ± 0.5
Basic pH	9	-	11 ± 0.5	14 ± 0.5
Elicited *Promicromonospora kermanensis* via supernatant of *Pseudomonas aeruginosa* culture	-	-	17 ± 1.0	21 ± 0.5
Unelicited *Promicromonospora kermanensis*	-	-	-	-
Negative control Supernatant of *Pseudomonas aeruginosa* culture (non-autoclaved)	-	-	17 ± 0.5	18 ± 0.5
Positive control	Vancomycin	30 μg/μL	19 ± 0.5	

it can be concluded that eliciting agents have both nonpolar and polar identity (Table 5).

Discussion

Drug discovery is entering a new era using the available data on genome sequences of various microorganisms. The discovery of new microbial small molecules can be contributed by the induction of metabolite production. Increased synthesis of rare metabolites is another critical usage of this procedure, and also increased production of well-known metabolites via elicitors has been reported.

The knowledge of the existence of new biosynthetic pathways in the bacterial genomes enables us to work on activating the silent or poorly expressed genes via various stimulants that act as transcription activators and reinforce their expression [20].

The expression of cryptic metabolites in fungi and bacteria can be successfully elicited by small exogenous compounds, like growth-inhibitory molecules, e.g., antibiotics. It seems that

Table 5 The antibacterial activity of the fractions derived from the biological inducer

Category of the treatment	Treatment	50 μgr per disk (mm)	150 μgr per disk (mm)	Growth
Induction effect	*Promicromonospora kermanensis*	-	-	G
	Promicromonospora + *Pseudomonas aeruginosa* UTMC 1404	10 ± 0.5	15 ± 0.5	G
Negative controls	Non-Autoclaved *Pseudomonas aeruginosa* UTMC 1404	-	10 ± 0.5	G
	Autoclaved *Pseudomonas aeruginosa* UTMC 1404	-	-	G
Fractions of the inducer metabolic broth	Eluted from RP column	-	-	G
	Eluted from silica gel column	-	-	NG
Positive control	Vancomycin	30 μg/μL		18 ± 0.5

G, growth and NG, negligible growth

these antibiotics at sub-inhibitory concentrations are involved in inter- and intra-species interactions, probably via stress response mechanisms, which can modify the activity of the ribosome and thus evoke the silence genes. Goadsporin, bacitracin, kanamycin, streptomycin, thiostrepton, paromomycin, tetracycline, and gramicidin D are some antibiotics from which eliciting activities at sub-inhibitory levels are reported. Some of these antibiotics exhibit hormonal characteristics at low concentrations and can play a stimulatory role to induce synthesis of antibiotic while at high concentrations exhibit their inherent antibacterial properties. Trimethoprim is known as a general activator of secondary metabolism in *Burkholderia thailandensis* [29]. However, none of the applied antibiotics in this study could exhibit eliciting activity on antibiotic encoding genes of *Promicromonospora kermanensis*.

Stimulatory activity of triclosan, a potent synthetic antibiotic via inhibiting lipid synthesis, was observed at low concentration (1 and 5 μM). Moreover, triclosan (1 μM) has efficiently acted as an eliciting agent in increasing the production of actinorhodin and salinomycin by *S. coelicolor* A3(2) and *S. albus* likely via reinforcement of substrates like acetyl-CoA and malonyl-CoA [30, 31].

Transcriptional inhibition of secondary metabolites could be a strategy to preserve bacteria from autotoxicity. Production of metabolically pricey and potentially autotoxic secondary metabolites is likely only provoked whenever a suitable environmental trigger is present [32, 33]. The use of small-molecule elicitors, in some cases, non-random targeted elicitors such as methyltransferase and histone deacetylase inhibitors, allows researchers to dominate the transcriptional inhibition of considerable gene clusters in the lab.

According to the results, *Promicromonospora kermanensis* DSM 45485 showed significant anti-MRSA activity when inoculated in an ISP2 broth medium containing valproic acid and DMSO (Fig. 2). They are agents that probably affect histone acetylation and subsequently

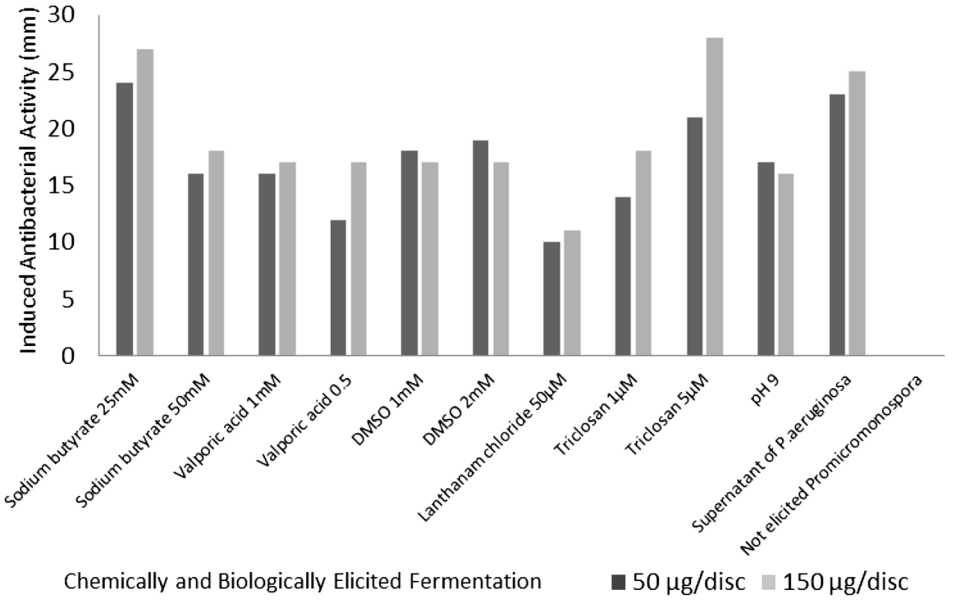

Fig. 2 The anti-MRSA activity of the elicitated *Promicromonospora kermanensis*. Two concentrations of the extracted metabolites (50-μg/disc and 150-μg/disc) of each elicitation treatment were assessed. Only inducible elicitors are shown. Benzamide, antibiotics, the supernatant of *Nocardia*, *P. aeruginosa*, and *Nocardia* debris could not induce the antibacterial metabolites of *Promicromonospora kermanensis* in the applied concentrations

modify the structure of chromatin and may lead to the activation of biosynthetic clusters for natural compounds [34]. HDAC proteins are rather extensive, and a lot of them exist in bacteria, e.g., *S. coelicolor* A3(2) possesses three HDAC-like genes [19]. Although there are significant differences in structural DNA organization of eukaryotes and *Actinobacteria*, inhibitors of HDAC like valproic acid and suberanilohydroxamic acid can provoke antibiotic production in several *Streptomyces* strains [35]. Another investigated HDAC inhibitor, benzamide, could not stimulate the expression of antimicrobial encoding biosynthetic gene clusters in *Promicromonospora kermanensis*.

There are some reports on the eliciting activity of sodium butyrate as an HDAC inhibitor. For instance, the production of actinorhodin by *S. coelicolor* A3(2) strain M145 was stimulated in the presence of sodium butyrate (25 mM sodium butyrate) while inhibited on R5 medium (rich medium) [19]. Also, sodium butyrate (25 mM) induced five recognized cryptic pathways with end products of deoxysugar synthetase, sesquiterpene cyclase, hopanoids, germicidin, and coelibactin in *S. coelicolor* A3(2) [36]. However, sodium butyrate imposed inhibitory effect on *Promicromonospora kermanensis*, and the observed antimicrobial activities might be due to its inhibitory effect on MRSA growth, not eliciting its antimicrobial encoding biosynthetic gene clusters. It is also possible that sodium butyrate can impose its eliciting activity on minimal media. It has been reported that the eliciting ability of HDAC inhibitors like sodium butyrate and valproic acid is due to a created general stress response via these molecules [19, 37].

The eliciting activity of DMSO on various *Actinobacteria* (*S. venezuelae* and *S. azureus*) has been reported. DMSO enhanced the production of thiostrepton (by *S. azureus*) [38], chloramphenicol (by *S. venezuelae*), and the polyketide tetracenomycin C (by *S. glaucescens*) [22]. The eliciting activity of a derivate of DMSO, dimethyl sulfone, has also been reported [38]. Accordingly, DMSO exhibited eliciting activity on the expression of antibiotic coding gene clusters in *Promicromonospora kermanensis* in this study. Its promising eliciting ability could be as the consequences of translation perturbation or creation of stress responses.

Another most important eliciting agents involved in expression or overproduction of silent or poorly expressed secondary metabolites coding gene clusters are rare earth elements. It has been shown that scandium (10–100 μM) enhances the production of actinorhodin, actinomycin, and streptomycin by *S. coelicolor, S. antibioticus*, and *Streptomyces* sp. [39]. Scandium imposes its effect at the transcription level of regulatory genes in *S. coelicolor*. Scandium or lanthanum promoted the expression of nine secondary metabolite biosynthetic clusters of *S. coelicolor* A3(2) at low concentrations [40]. Also, Xu reported that the anti-microbial activity of 15 actinobacterial strains were induced or enhanced by the addition of $LaCl_3$ [16]. Similarly, the eliciting activity of lanthanum (50 μM) was observed on *Promicromonospora kermanensis* by vanishing the inducing activity at the higher applied concentration (100 μM).

In addition to chemical elicitors, extreme changes in the acidity of culture media can provoke the expression of cryptic or poorly expressed biosynthetic gene clusters. Eliciting activity of alkaline pH can be due to lowering the concentrations of the proton or increasing the level of the common stress sigma factors [17]. Hayes et al. showed that *S. coelicolor* A3(2) produces more methylenomycin when it was influenced by an acidic condition [41]. According to our results, alkaline pH also plays an eliciting impact on genes in *Promicromonospora kermanensis* which are involved in the production of antibacterial agents.

The external stimuli can trigger the involved regulators in signal transduction networks [42]. Small molecule metabolites or hormone-like compounds of the bacteria can have a regulatory effect on gene expression of other bacteria or even eukaryotes [43].

The secondary metabolite pathway of *Promicromonospora kermanensis* was triggered by the diffusible metabolites produced by *Pseudomonas aeruginosa*. This probable signal or hormone-like molecule had amphipathic and oxidation sensitive nature, while it was stable in pH 4–9.

The monomers of polysaccharides and alkaline conditions were first applied for their elicitation effect on *Actinobacteria* in this study. This triggering effect on *Promicromonospora* can be investigated on other *Actinobacteria* for the same purpose.

In conclusion, eliciting the expression of cryptic or poorly expressed biosynthetic gene clusters via biological and chemical elicitors is considered as a promising approach in the enhancement of drug discovery yield due to some of its advantages, including feasibility, cost-effective, and lack of requirement to comprehensive knowledge on physiological and genomic information of the strains of interest. Since the complicated secondary metabolism of *Actinobacteria* challenges the favorable changes in the whole metabolic system through genetic manipulation, stimulating the expression of such silent BGCs can make it possible to significantly enhance the chemical diversity of microbial-derived origin compounds and improve the yield of drug discovery.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Zaman, S. B., Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T., & Hossain, N. (2017). A review on antibiotic resistance: alarm bells are ringing. *Cureus, 9*(6), 1403.
2. O'Neil, J. (2014). Antimicrobial resistance: tackling a crisis for the health and wealth of nations. *Review on Antimicrobial Resistance, 20*, 1–16.
3. Wink, J., Mohammadipanah, F., & Hamedi, J (2017). *Biology and biotechnology of actinobacteria*. Springer, Berlin.
4. Genilloud, O. (2019). Natural products discovery and potential for new antibiotics. *Current Opinion in Microbiology, 51*, 81–87.
5. Mohamed, A., Nguyen, C. H., & Mamitsuka, H. (2015). Current status and prospects of computational resources for natural product dereplication: a review. *Briefings in Bioinformatics, 17*(2), 309–321.
6. Seyedsayamdost, M. R. (2014). High-throughput platform for the discovery of elicitors of silent bacterial gene clusters. *Proceedings of the National Academy of Sciences, 111*(20), 7266–7271.
7. Romano, S., Jackson, S. A., Patry, S., & Dobson, A. D. (2018). Extending the "one strain many compounds"(OSMAC) principle to marine microorganisms. *Marine Drugs, 16*(7), 244.
8. Baltz, R. H. (2016). Genetic manipulation of secondary metabolite biosynthesis for improved production in Streptomyces and other actinomycetes. *Journal of Industrial Microbiology & Biotechnology, 43*(2-3), 343–370.
9. Hug, J. J., Bader, C. D., Remškar, M., Cirnski, K., & Müller, R. (2018). Concepts and methods to access novel antibiotics from actinomycetes. *Antibiotics, 7*(2), 44.
10. Bode, H. B., Bethe, B., Höfs, R., & Zeeck, A. (2002). Big effects from small changes: possible ways to explore nature's chemical diversity. *ChemBioChem, 3*(7), 619–627.
11. Covington, B. C., Spraggins, J. M., Ynigez-Gutierrez, A. E., Hylton, Z. B., & Bachmann, B. O. (2018). Response of hypogean actinobacterial genera secondary metabolism to chemical and biological stimuli. *Applied and Environmental Microbiology, 84*(19), e01125–18.
12. Tawfike, A., Attia, E. Z., Desoukey, S. Y., Hajjar, D., Makki, A. A., Schupp, P. J., Edrada-Ebel, R., & Abdelmohsen, U. R. (2019). New bioactive metabolites from the elicited marine sponge-derived bacterium Actinokineospora spheciospongiae sp. nov. *AMB Express, 9*(1), 12.
13. Van der Meij, A., Willemse, J., Schneijderberg, M. A., Geurts, R., Raaijmakers, J. M., & van Wezel, G. P. (2018). Inter-and intracellular colonization of Arabidopsis roots by endophytic actinobacteria and the impact of plant hormones on their antimicrobial activity. *Antonie Van Leeuwenhoek, 111*(5), 679–690.

14. Takano, E. (2006). γ-Butyrolactones: Streptomyces signalling molecules regulating antibiotic production and differentiation. *Current Opinion in Microbiology, 9*(3), 287–294.
15. Haferburg, G., & Kothe, E. (2013). *Activation of silent genes in actinobacteria by exploiting metal stress, Actinobacteria. Application in bioremediation and production of industrial enzymes* (pp. 56–73). *Boca Raton*: *CRC Press*.
16. Xu, D., Han, L., Li, C., Cao, Q., Zhu, D., Barrett, N. H., Harmody, D., Chen, J., Zhu, H., & McCarthy, P. J. (2018). Bioprospecting deep-sea actinobacteria for novel anti-infective natural products. *Frontiers in Microbiology, 9*, 787.
17. Kim, Y. J., Song, J. Y., Moon, M. H., Smith, C. P., Hong, S.-K., & Chang, Y. K. (2007). pH shock induces overexpression of regulatory and biosynthetic genes for actinorhodin production in Streptomyces coelicolor A3 (2). *Applied Microbiology and Biotechnology, 76*(5), 1119–1130.
18. Hassan, S. S. u., Jin, H.-Z., Abu-Izneid, T., Rauf, A., Ishaq, M., & Suleria, H. A. R. (2019). Stress-driven discovery in the natural products: A gateway towards new drugs. *Biomedicine & Pharmacotherapy, 109*, 459–467.
19. Moore, J. M., Bradshaw, E., Seipke, R. F., Hutchings, M. I., & McArthur, M. (2012). Use and discovery of chemical elicitors that stimulate biosynthetic gene clusters in Streptomyces bacteria. *Methods in Enzymology*. Elsevier, 517, 367–385.
20. Ochi, K., & Hosaka, T. (2013). New strategies for drug discovery: Activation of silent or weakly expressed microbial gene clusters. *Applied Microbiology and Biotechnology, 97*(1), 87–98.
21. So, A. G., & Davie, E. W. (1964). The effects of organic solvents on protein biosynthesis and their influence on the amino acid code. *Biochemistry, 3*(8), 1165–1169.
22. Chen, G., Li, X., Waters, B., & Davies, J. (2000). Enhanced production of microbial metabolites in the presence of dimethyl sulfoxide. *The Journal of Antibiotics, 53*(10), 1145–1153.
23. Zhu, L.-W., Zhong, J.-J., & Tang, Y.-J. (2008). Significance of fungal elicitors on the production of ganoderic acid and Ganoderma polysaccharides by the submerged culture of medicinal mushroom Ganoderma lucidum. *Process Biochemistry, 43*(12), 1359–1370.
24. Antoraz, S., Santamaría, R. I., Díaz, M., Sanz, D., & Rodríguez, H. (2015). Toward a new focus in antibiotic and drug discovery from the Streptomyces arsenal. *Frontiers in Microbiology, 6*, 461.
25. Sidda, J. D., & Corre, C. (2012). Gamma-butyrolactone and furan signaling systems in Streptomyces. *Methods in Enzymology*. Elsevier, 517, 71–87.
26. Garg, N., Manchanda, G., & Kumar, A. (2014). Bacterial quorum sensing: circuits and applications. *Antonie Van Leeuwenhoek, 105*(2), 289–305.
27. Tanaka, Y., Izawa, M., Hiraga, Y., Misaki, Y., Watanabe, T., & Ochi, K. (2017). Metabolic perturbation to enhance polyketide and nonribosomal peptide antibiotic production using triclosan and ribosome-targeting drugs. *Applied Microbiology and Biotechnology, 101*(11), 4417–4431.
28. Mohammadipanah, F., del Carmen Montero-Calasanz, M., Schumann, P., Spröer, C., Rohde, M., & Klenk, H.-P. (2017). Promicromonospora kermanensis sp. nov., an actinobacterium isolated from soil. *International Journal of Systematic and Evolutionary Microbiology, 67*(2), 262–267.
29. Okada, B. K., Wu, Y., Mao, D., Bushin, L. B., & Seyedsayamdost, M. R. (2016). Mapping the trimethoprim-induced secondary metabolome of Burkholderia thailandensis. *ACS Chemical Biology, 11*(8), 2124–2130.
30. Craney, A., Ozimok, C., Pimentel-Elardo, S. M., Capretta, A., & Nodwell, J. R. (2012). Chemical perturbation of secondary metabolism demonstrates important links to primary metabolism. *Chemistry & Biology, 19*(8), 1020–1027.
31. Olano, C., Lombo, F., Méndez, C., & Salas, J. A. (2008). Improving production of bioactive secondary metabolites in actinomycetes by metabolic engineering. *Metabolic Engineering, 10*(5), 281–292.
32. Patterson, G. M., & Bolis, C. M. (1997). Fungal cell wall polysaccharides elicit an antifungal secondary metabolite (phytoalexin) in the cyanobacterium scytonema ocelutum 2. *Journal of Phycology, 33*(1), 54–60.
33. Fisch, K., Gillaspy, A., Gipson, M., Henrikson, J., Hoover, A., Jackson, L., Najar, F., Wägele, H., & Cichewicz, R. (2009). Chemical induction of silent biosynthetic pathway transcription in Aspergillus niger. *Journal of Industrial Microbiology & Biotechnology, 36*(9), 1199–1213.
34. Bok, J. W., Chiang, Y.-M., Szewczyk, E., Reyes-Dominguez, Y., Davidson, A. D., Sanchez, J. F., Lo, H.-C., Watanabe, K., Strauss, J., & Oakley, B. R. (2009). Chromatin-level regulation of biosynthetic gene clusters. *Nature Chemical Biology, 5*(7), 462–464.
35. Tyurin, A. P., Alferova, V. A., & Korshun, V. A. (2018). Chemical elicitors of antibiotic biosynthesis in actinomycetes. *Microorganisms, 6*(2), 52.
36. Song, L., Barona-Gomez, F., Corre, C., Xiang, L., Udwary, D. W., Austin, M. B., Noel, J. P., Moore, B. S., & Challis, G. L. (2006). Type III polyketide synthase β-ketoacyl-ACP starter unit and ethylmalonyl-CoA extender unit selectivity discovered by Streptomyces coelicolor genome mining. *Journal of the American Chemical Society, 128*(46), 0.14754–0.14755.

37. Bentley, S. D., Chater, K. F., Cerdeño-Tárraga, A.-M., Challis, G. L., Thomson, N., James, K. D., Harris, D. E., Quail, M. A., Kieser, H., & Harper, D. (2002). Complete genome sequence of the model actinomycete Streptomyces coelicolor A3 (2). *Nature, 417*(6885), 141–147.
38. Pettit, R. K. (2011). Small-molecule elicitation of microbial secondary metabolites. *Microbial Biotechnology, 4*(4), 471–478.
39. Kawai, K., Wang, G., Okamoto, S., & Ochi, K. (2007). The rare earth, scandium, causes antibiotic overproduction in Streptomyces spp. *FEMS Microbiology Letters, 274*(2), 311–315.
40. Tanaka, Y., Hosaka, T., & Ochi, K. (2010). Rare earth elements activate the secondary metabolite–biosynthetic gene clusters in Streptomyces coelicolor A3 (2). *The Journal of Antibiotics, 63*(8), 477–481.
41. Hayes, A., Hobbs, G., Smith, C. P., Oliver, S. G., & Butler, P. R. (1997). Environmental signals triggering methylenomycin production by Streptomyces coelicolor A3 (2). *Journal of Bacteriology, 179*(17), 5511–5515.
42. Horinouchi, S. (2003). AfsR as an integrator of signals that are sensed by multiple serine/threonine kinases in Streptomyces coelicolor A3 (2). *Journal of Industrial Microbiology and Biotechnology, 30*(8), 462–467.
43. Mohammadipanah, F, & Zamanzadeh, M. (2019). *Bacterial mechanisms promoting the tolerance to drought stress in plants, In secondary metabolites of plant growth promoting Rhizomicroorganisms* (Harikesh Bahadur Singh H. B., Keswani C., Reddy M. S., Sansinenea E. & García-Estrada C.), Springer, Singapore, pp.185–224.

Affiliations

Fatemeh Mohammadipanah[1] · Fatemeh Kermani[1] · Fatemeh Salimi[2]

[1] Pharmaceutical Biotechnology Lab, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran 14155-6455, Iran

[2] Department of Cellular and Molecular Biology, School of Biology, Damghan University, Damghan, Iran