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Critical review of fermentation and extraction of anti-*Vibrio* compounds from *Streptomyces*

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Abstract: A single *Streptomyces* strain often have the potential to produce more than one bioactive compound. Fermentation parameters include media compositions, temperature and pH, have great impact on the secondary metabolism of *Streptomyces* and subsequently on production of different microbial products. This review aims to consolidate the studies on the cultivation parameters used to enhance the production of secondary metabolite with anti-*Vibrio* activity from a single *Streptomyces* strain. In turn, this review sheds light on the possible alterations of the cultivation parameters to obtain desired anti-*Vibrio* compounds from *Streptomyces* sp. Furthermore, the bioactive compounds with anti-*Vibrio* activity identified from *Streptomyces* sp. were demonstrated to exhibit immense values for future antibacterial agent developments.

Keywords: *Streptomyces*; *Vibrio*; fermentation; extraction; secondary metabolites

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INTRODUCTION

Fermentation is an important process for the production of various structurally-diverse bioactive substances from microorganisms, including antibiotics, anticancer, antiviral and immunosuppressants[1,2]. Given that the limited quantity of bioactive substances is usually produced by these microorganisms, fermentation is one of the feasible processes to continuously supply majority of these clinically useful drugs in the market currently. This is because the total chemical synthesis is way too complicated and costly than fermentation. For instance, antimicrobial peptides such as a novel class of antibiotics, which recently have received much attention, is not economically feasible to be synthesized chemically if involve larger or more complex peptides[3]. Furthermore, medium optimization remains one of most critical steps in fermentation technology to enhance the production of valuable bioactive compounds. To achieve maximum production of desirable compounds, the production medium containing appropriate components (e.g., carbon, nitrogen, NaCl, *etc*.) coupled with optimal fermentation conditions are required to be identified and optimized accordingly[1].

Actinobacteria have been regarded as the most prolific producers in the microbial world[4–8], especially from the genus *Streptomyces*[9,10]. The genus *Streptomyces* has responsible for the production of more than 70% of commercially important antibiotics[2,11], as well as many bioactive compounds of pharmacological and agricultural interest[12–22]. The discovery of antibiotic from *Actinobacteria* is highly dependent on the effect of growth conditions on the production of secondary metabolites[23–28]. These soil bacteria are known to have complex life cycle which is composing of different stages. Secondary metabolites are usually produced by *Streptomyces* sp. at the end of the active vegetative growth and during the dormant or reproduction stage[29]. The secondary metabolism of *Streptomyces* is based on its unique genetic make-up but the expression can be

influenced by the surrounding manipulations[30]. Therefore, the productions of secondary metabolites are often associated with the limitation of nutrients, presence of inducer or reduction of growth rate in *Streptomyces*[23]. It is well known that secondary metabolite production can be repressed by readily available carbon source, high levels of nitrogen and phosphorus, all of which keeping the bacteria at active proliferative stage. This indicated that the production of secondary metabolites can be influenced significantly by various fermentation parameters including the nutrient availability, pH, temperature, mineral salts, inducers and inhibitors[31]. Small modifications in the composition of growth media can result variation of the quantity of specific compounds, also these modifications could result in the production of a completely distinct pattern of molecules[32].

Vibrio spp. is autochthonous to various aquatic environments, including estuarine, coastal waters and sediments[33–36]. *Vibrio* spp. was known to be susceptible virtually to most of the antimicrobial agents[37,38]. However, antimicrobial resistance has emerged and evolved in many bacterial genera[39–41], including *Vibrio* spp. as a result of excessive use of antimicrobial agents in various settings[42]. For instance, applications of antibiotics in aquaculture water as prophylactics to control infectious diseases in fish and aquatic organisms. Furthermore, certain *Vibrio* species, in particular *V. parahaemolyticus* and *V. vulnificus* are significant foodborne human pathogens[43–47]. Hence, the increase in emergence of antibiotic-resistant bacterial pathogens, including *Vibrio* spp. is a major public health concern[39,40,42]. This issue not only has immense impact on human health, it is also a concern on the future ability to treat the diseases as antibiotic resistance has developed over time, from single classes of antibiotics to multidrug resistance and eventually emergence of superbug with extreme drug resistance[48,49]. Therefore, it has increased the interest of research on the search for more effective alternatives to cope with the issue of antibiotic resistant bacteria, including *Vibrio* pathogens[50,51]. The exploration of bioactive compounds sourcing from natural resources, including plant[52–56], animal[57] or microbial origins[58–64] constitute an attractive bioprospection strategy among the drug discovery scientists. In fact, numerous efforts have demonstrated that the genus *Strepto-myces* capable to synthesize various bioactive compounds against *Vibrio* pathogens, representing a valuable source for antibacterial agents with anti-*Vibrio* activities[65–67].

In the light of the promising potential of *Streptomyces* as the bioresource of anti-*Vibrio* compounds, this review provides the rationale for the designing and optimizing of fermentation medium to facilitate the process of anti-*Vibrio* metabolite production in *Streptomyces* sp. Furthermore, the importance of extraction techniques for optimum yield of the desired bioactive compounds is discussed in this review (Figure 1).

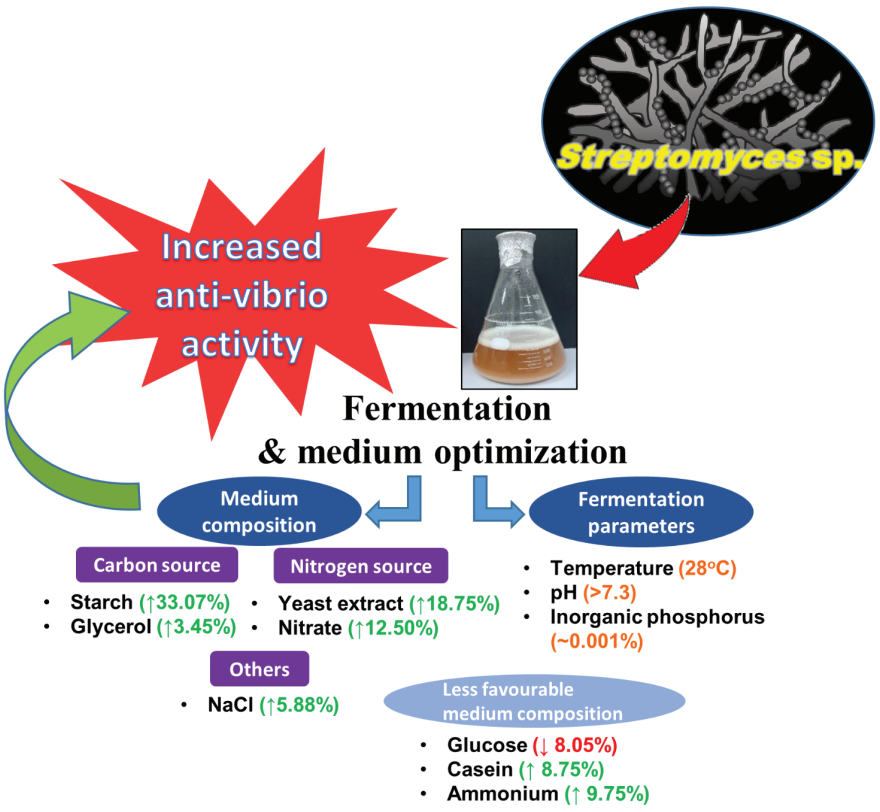

Figure 1. Fermentation and medium optimization for the production of anti-*Vibrio* compounds by *Streptomyces* sp. Fermentation is conducted to induce the production of anti-*Vibrio* active metabolites by *Streptomyces*. Starch and glycerol are both good carbon sources for the production of metabolites with better anti-*Vibrio* activity (percentage shows the changes in the anti-*Vibrio* activity when added with the indicated component in the fermentation medium). Likewise, yeast extract and nitrate are the preferable nitrogen source as compared to casein and ammonium for the production of metabolites with better anti-*Vibrio* activity by the *Streptomyces*. The fermentation parameters presented are the optimum conditions for the production of anti-*Vibrio* active metabolites.

FERMENTATION PROCESS FOR PRODUCTION OF ANTI-*VIBRIO* COMPOUNDS BY *STREPTOMYCES* SP.

Within the 64 studies analyzed in this review, a total of 38 studies conducted secondary screening of the metabolites produced by the anti-*Vibrio Streptomyces* via submerged fermentation process. This implies that 59.4% of the studies showed the anti-*Vibrio Streptomyces* strains displayed the antagonistic activities against different *Vibrio* sp. through the production of bioactive secondary metabolites. Thus, more study should perform fermentation in order to fully unravel the potential of the anti-*Vibrio Streptomyces* strains in the production of bioactive compounds against *Vibrio* sp. Solid state fermentation was reported as an alternative fermentation process to facilitate the secondary metabolites production from the anti-*Vibrio Streptomyces*[65]. The solid state fermentation involves the use of solid particles free of water or with little moisture for microbial growth and secondary metabolites production[68]. Mohana and Radhakrishnan (2014)[65] indicated that solid state fermentation process was more suitable for *Streptomyces* MA7, a strain derived from mangrove rhizosphere sediment in producing anti-*Vibrio* bioactive metabolites against *Vibrio* pathogens such as *V. cholerae* O1, *V. cholerae* O139, *V. parahaemolyticus* and *V. mimicus*. However, there is limited information on studies comparing the two different fermentation techniques in the production of secondary metabolites with anti-*Vibrio* activities. More study could be performed to investigate the optimal fermentation techniques for the production of anti-*Vibrio* compounds from *Streptomyces* at a higher yield. Nevertheless, there was study suggested that solid-state fermentation is better for antibiotic production by *Streptomyces* in the aspects of its stability and quantity[69]. For instance, solid-state fermentation of *Streptomyces* species resulted in higher yield and stability of well-known antibiotics including tetracycline[70], neomycin[71], cephamycin C[72] and oxytetracycline[73].

FERMENTATION PARAMETERS AFFECTING ANTI-*VIBRIO* COMPOUNDS PRODUCTION

Media composition

Media composition plays an important role in determining the microbial secondary metabolites as it comprises of components that may act as activators of certain signaling pathway in the production of secondary metabolites[31]. Thus, a single strain, grown under different condition may result in production of substantially different compounds. A study reported that by using a defined medium resulted in production of new metabolites which were not found in other media used to cultivate *Streptomyces* sp. C34, and exhibited antibacterial activity towards *V. parahaemolyticus*[74]. The defined medium (Table 1) containing 2 mM fluoride employed by the study was previously developed for the production of fluorinated secondary metabolites by *Streptomyces*[75]. The mechanism for the production of the novel metabolites by *Streptomyces* C34 has yet to be elucidated. Nevertheless, it was suggested that the addition of fluoride salts could have activated the unique biosynthetic genes which responsible for the production of those new compounds[75]. Therefore, other than depending on the biosynthetic potential of the microbes which determines the types of bioactive compounds, the composition of the media also plays a substantial role on the success of screening programs based on culture-dependent bioprospecting strategy. According to the 38 studies that performed submerged fermentation, different types of fermentation broths were used, including starch casein broth, soybean meal broth, potato dextrose broth, arginine glycerol broth, actinomycetes isolation broth and glycerol asparagine. Besides that, examples of fermentation broth with defined compositions used for the production of secondary metabolites from the anti-*Vibrio Streptomyces* can refer to Table 1.

Table 1. The composition of selected production media and fermentation conditions used for secondary metabolites production in the *Streptomyces* sp. displaying anti-*Vibrio* activity.

Parameters	Studies that utilized mixture of complex and simple carbon and nitrogen sources[#]													
Composition (% w/v)[*]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Glucose					1			0.4	2			0.5		0.2
Soluble starch	2	0.5	0.5	1	1	1			0.5	1	0.1	2		
Glycerol				1	1		1						1	
Myo-inositol							0.04							
Malt extract								0.4						
Soybean	2		0.5						0.5		0.1	1.5		
Casein		0.03		0.03						0.03				
Cornsteep powder					1									
Polypeptone					0.5									
Peptone						0.2			0.2					
Yeast extract					0.2	0.4		0.4	0.2			0.25		0.3
L-tyrosine													0.05	
L-asparagine													0.1	
MSG							0.5							
$CaCO_3$		0.002		0.002	0.32		0.025			0.002		0.1		0.004

NaCl		0.2	0.05	0.2	0.1		0.05			0.2			0.05	0.08
NaF							0.0084							
NH_4Cl							0.15							0.1
KBr						0.01								
KCl														0.01
K_2HPO_4			0.05	0.2			0.2		0.05	0.2			0.05	0.001
KNO_3	0.1	0.2		0.2						0.2	0.005			
$FeSO_4$		0.001		0.001		0.004	0.0025			0.001				
$MgSO_4$		0.005	0.05	0.005			0.05		0.05	0.005			0.05	0.02
$CoCl_2$							0.001							
$ZnSO_4$							0.001							
Seawater	+	+	-	-	-	-	-	+	+	+	+	+	+	-
pH	7.5	ns	ns	7.2	7.4	8	7	7.2	7.4	7.0±0.2	ns	7.5	7	7
Temperature (°C)	27	28	28	29	30	ns	28	28	28	28	27	28	28	28

* The percentage of each composition was calculated using: w/v% = (weight of solute (g)/volume of media(mL)) × 100

1 - Soybean medium[65], 2 - Starch casein broth[76], 3 - GsB broth medium[77], 4 - Casein glycerol/ starch medium[78], 5 - Production broth[79], 6 - A1BFe media[80], 7 - Defined medium[74], 8 - Fermentation broth[81], 9 - R2A medium[82], 10 - Starch casein broth[83], 11 - Soybean meal broth[84] 12 - Fermentation broth[85] 13 - Melanin production medium[86, 87],14 - Fermentation broth[88–92]

The influence of complex and simple carbon source on anti-Vibrio activity

The carbon source has significant effect on the production of antibiotic and the morphological development of *Streptomyces* sp. Several mechanisms have been described in the genus *Streptomyces* to illustrate the carbon catabolite repression effects on secondary metabolites production[93,94]. As for aim of this review, it is to consolidate and rationalize the information available on the effect of different media composition on *Streptomyces* toward the production of metabolites against *Vibrio* sp. Furthermore, major emphasis will be given towards the efficacy of the anti-*Vibrio* metabolites produced by *Streptomyces* in response to the presence of specific carbon source in the fermentation media. Based on the data of media composition presented in the reviewed studies, carbon sources such as starch, glycerol and glucose are commonly used as growth substrate in the fermentation media used to produce secondary metabolites. Majority of the studies incorporated starch (45.2%), a complex carbohydrate in the fermentation medium for the production of secondary metabolites with anti-*Vibrio* activity (Table 2).

Literatures demonstrated that the optimal production of secondary metabolites is generally achieved by culturing the microorganisms in media containing slowly assimilated nutrient sources while the readily utilized carbon source is often known to repress antibiotic production. For instance, the use of glucose as a carbon source had a negative influence on the production of nystatin as well as their morphology to a certain extent that resulted in termination of cell growth and nystatin production[95]. This is also commonly seen in other *Streptomyces* sp., such as in the production of streptomycin, chloramphenicol and cephamycin by *S. griseus*[96], *S. venezuelae*[97] and *S. clavuligerus*[97] respectively. However, previous study indicated novobiocin production by *S. niveus* is subjected to catabolite repression by citrate assimilation and not caused by glucose assimilation[98]. *Streptomyces avermitilis* was shown to assimilate glucose slowly and become the best carbon source in determining the production rate of avermec-tin[99]. Ikeda *et al.* (1988)[99] suggested that the activity of 6-phosphogluconate dehydrogenase of the pentose phosphate pathway is associated with avermectin production, in which the NADPH generated by the enzyme could be used as the intermediate for the biosynthesis of avermectin. Previous study also indicated that glucose is important for the biosynthesis of ε-rhodomycinone, an important aglycone precursor to anthracycline antibiotic in *Streptomyces*[100].

To identify the best carbon source for the production of anti-*Vibrio* metabolites by *Streptomyces*, the anti-*Vibrio* activities of the *Streptomyces* strains with or without the specific carbon source were compared based on the inhibition zones (Table 3). In Table 3, the anti-*Vibrio* activity of metabolites produced by *Streptomyces* strains increased by 33.1% in the presence of starch as carbon source. Furthermore, a ten folds increment of anti-*Vibrio* activity is demonstrated by *Streptomyces* metabolites produced in the presence starch when compared to the use of glycerol as carbon source. In contrast, the use of glucose as carbon source is shown to repress the anti-*Vibrio* activity of the *Streptomyces* metabolites by 8.1% based on the median inhibition zones. This information is in line with other studies, indicating starch is a good carbon source for anti-*Vibrio* metabolite production. The starch-based A1BFe medium (Table 1) resulted in production of twice the amount of anti-*Vibrio* compounds by *Streptomyces atrovirens* PK288-21 compare to culture in glucose-based TCG medium[80]. The study suggested that *Streptomyces atrovirens* PK288-21 utilized starch as the main carbon source that could increase the production of antibacterial compounds[80]. The continuous and gradual hydrolysis of starch could

avoid the carbon catabolite repression mechanisms that usually triggered by carbon sources that are more easily metabolized by the microorganism such as glucose[101]. In addition, the antibacterial compounds present were consisted of two benzaldehydes compounds identified from the fermented broth of *S. atrovirens* PK288-21. Both of the benzaldehyde derivatives demonstrated antibacterial activity against both *V. anguillarum* and *V. harveyi*, particularly against *V. harveyi* with lower MIC values reported as compared to ciprofloxacin (58 µg/mL). The work showed that the compound, 2-hydroxy-5-(3-methylbut-2-enyl)benzaldehyde **(9)** was as a new derivative while 2-hepta-1,5-dienyl-3,6-dihydroxy-5-(3-methylbut-2-enyl)benzaldehyde **(10)** was previously reported from fungus *Eurothium rubrum*. Similarly, another 4 studies (Table 2) also demonstrated the use of starch with concentrations ranging from 0.1 to 1% (w/v), as the sole carbon source in the fermentation medium for the production of secondary metabolites by *Streptomyces* strains, and exhibited diverse strength of antibacterial activity against *Vibrio* sp.[76,77,83,84]. Overall, starch is recommended to be a good carbon source for the production of anti-*Vibrio* metabolites from *Streptomyces*.

Table 2. The compositions of fermentation medium and the fermentation conditions extracted from the reviewed studies on *Streptomyces* with anti-*Vibrio* activity.

Parameters	Compositions	Concentration % (w/v)/ Units	Number of studies performed fermentation (n = 31)	Percentage (%)
Carbon sources	**Complex carbon source only**			
	Starch	0.1	1	
		0.5	2	
		1	2	
	Sugarcane	1	1	
	• Yeast extract	5	1	
			Total = 7	22.6
	• Glucose	0.2	5	
	• Glycerol	1	2	
	• Glycerol, myoinositol	1, 0.04	1	
			Total = 8	25.8
	Mixture of both complex and readily utilizable			
	• Starch, glucose	2, 1	1	
	• Starch & glycerol	1	1	
	• Glycerol, starch, glucose	1,1,1	2	
	• Malt extract, glucose	0.4, 0.4	1	
			Total = 5	16.1
	Media used by studies w/o specify the composition			
	• Starch casein broth	-	5	
	• Potato dextrose broth		1	
	• Arginine glycerol broth		1	
	• Glycerol asparagine broth		1	
	• ISP2		1	
	• Soybean meal medium		1	
	• Actinomycetes isolation medium		1	
			Total = 11	35.5
Nitrogen sources	**Complex nitrogen source only**			
	• Soybean	0.2	1	
		0.5	1	
	• Tryptone, yeast extract	1, 5	1	
	• Peptone, yeast extract	0.2, 0.4	1	
	• Soybean, yeast extract	1.5, 0.25	1	
	• Polypepton, yeast extract, corn steep liqour	0.5, 0.2, 0.1	1	
	• Soybean meal, peptone, yeast extract	0.5, 0.2, 0.2	1	
	• Malt extract, yeast extract	0.4, 0.4	1	

	• L-tyrosine, L-asparagine	0.05, 0.1	2	
			Total = 10	32.3
	Mixture of both complex and readily utilizable			
	• Soybean, KNO_3	0.1, 0.005	1	
	• Casein, KNO_3	0.03, 0.2	3	
	• Yeast extract, NH_4Cl	0.3, 0.1	5	
	• MSG, NH_4Cl	0.5, 0.15	1	
			Total = 10	32.3
	Media used by studies w/o specify the composition			
	• Starch casein broth	-	5	
	• Potato dextrose broth		1	
	• Arginine glycerol broth		1	
	• Glycerol asparagine broth		1	
	• ISP2		1	
	• Soybean meal medium		1	
	• Actinomycetes isolation medium		1	
			Total = 11	35.5
Phosphate	K_2HPO_4	0.001	5	
		0.05	4	
		0.2	3	
			Total = 12	38.7
Salt	NaCl	0.05	3	
		0.08	5	
		0.1	1	
		0.2	3	
		1	1	
			Total = 13	41.9
pH		7	9	
		7.2	1	
		7.4	1	
		7.5	2	
		8	1	
		Not specified	17	
			Total = 31	
Temperature (ºC)		23	1	
		25	1	
		27	2	
		28	13	
		29	1	
		30	5	
		32	1	
		35	1	
		26-30	1	
		28-32	1	
		Not specified	4	
			Total = 31	

Table 3. The effect of carbon, nitrogen and NaCl on the anti-*Vibrio* activity of *Streptomyces* metabolites.

Media composition (concentration range, w/v %)	Median of Inhibition zone (mm)		Percentage of changes in anti-*vibrio* activity (%)
	Absence	Presence	
Carbon sources			
Starch (0.32 – 2)	15.03 (n = 16)	20 (n = 10)	Increased by 33.07
Glucose (0.2 – 2)	17.40 (n = 18)	16 (n = 9)	Decreased by 8.05
Glycerol (0.12 – 1)	17.40 (n = 20)	18 (n = 8)	Increased by 3.45
Nitrogen sources			
Yeast extract (0.3 - 1)	16 (n = 16)	19 (n = 8)	Increased by 18.75
Casein (0.03 - 1)	16 (n = 15)	17.4 (n = 10)	Increased by 8.75
Ammonium salts, NH_4^+ (0.0001 - 0.12)	16.4 (n = 18)	18 (n = 5)	Increased by 9.75
Nitrate salts, NO_3^- (0.2)	16 (n = 16)	18 (n = 7)	Increased by 12.50
Others			
NaCl (0.05 - 1.2)	17 (n = 8)	18 (n = 16)	Increased by 5.88

Influence of organic and inorganic nitrogen source on anti-Vibrio activity

Nitrogen sources such as nitrate and ammonium salts which favorable for growth were shown to affect negatively on the production of secondary metabolites in *Streptomyces*. The readily utilized nitrogen sources were demonstrated to cause repression of enzymes responsible for tylosin in *Streptomyces fradiae*[102]. Complex protein source such as soybean meal and the slowly assimilated amino acid such as proline are good nitrogen source to promote high secondary metabolites production. Therefore, slow-metabolizing nitrogen sources are preferable to supply the essential nutrients to the antibiotic-producing strains. Yeast extract, corn steep liquor and soybean flour are commonly used complex organic nitrogen sources[31]. Based on the reviewed studies, soybean meal (0.2 and 0.5% w/v) was evidenced in studies[77,103] as a sole nitrogen source for the production of metabolites that exhibited anti-*Vibrio* activities by the *Streptomyces* strains (Table 2). Furthermore, the anti-*Vibrio* activity of the *Streptomyces* strains cultivated in different nitrogen sources were compared based on the median inhibition zone (Table 3). The usage of yeast extract as a complex organic nitrogen source is found to enhance the anti-*Vibrio* activity of the *Streptomyces* metabolites by 18.75%, when compared to the only 8.75% increment in the presence of casein as an organic nitrogen source. Besides that, nitrate is a more favorable inorganic nitrogen source when compared to the use of NH_4^+ in the fermentation media of the anti-*Vibrio Streptomyces*. None of the studies utilized ammonium or nitrate salts as the sole nitrogen source for the fermentation process. A total of 19 studies demonstrated the use of a mixture of readily and slowly utilizable nitrogen sources in the optimization of medium composition for the improvement of the yield of secondary metabolites (Table 1). As the readily utilizable sources such as ammonium salts and nitrate salts serve to support the exponential growth of the bacteria while the slowly used sources such as yeast extract and casein serve to sustain the production of metabolites during the stationary phase, as the rapidly assimilated sources are depleted[31]. Thus, the combination of yeast extract and nitrate salts could be used to serve as a good nitrogen sources in the production of anti-*Vibrio* metabolites in the genus *Streptomyces*.

Inorganic phosphate

Inorganic phosphorus is the common major growth-limiting nutrient in natural environments[31]. Literatures showed that high concentration of inorganic phosphate in culture media causes negative regulation on the synthesis of secondary metabolites in different *Streptomyces* sp.[104,105]. A total of 12 studies (38.7%) indicated the supplementation of dipotassium phosphate as a source of inorganic phosphate, with wide range of concentrations from 0.001 to 0.2% (w/v) (~ 0.5–115mM) in the fermentation medium for the production of anti-*Vibrio* secondary metabolites by *Streptomyces* (Table 2). None of the studies indicated the potential of inorganic phosphate that could resulted in lower production of anti-*Vibrio* compounds. Although some literatures demonstrated the supply of inorganic phosphate more than 3–5 mM are frequently inhibitory to antibiotic biosynthe-sis[105,106]. Liras *et al.* (1990)[106] indicated phosphate stimulates the expression of genes involved in the biosynthesis of macromolecules and house-keeping genes essential for growth whereas it often inhibits expression of genes encoding for biosynthesis of secondary metabolites. The *p*-ami-nobenzoic acid synthase (PABA synthase), that catalyzes the conversion of chorismic acid to *p*-aminobenzoic acid which is a precursor for candicidin (macrolide antibiotic) was found to be inhibited by potassium phosphate at 5 to 10 mM resulting in repression of candicidin biosynthesis in *Streptomyces griseus*[107]. Studies showed that the biosynthesis of several groups of antibiotic are

particularly sensitive to phosphate repression such as aminoglycosides[108], tetracyclines[109], macrolides[110] and polyenes[104]. Meanwhile, the biosynthesis of beta-lactam antibiotic and peptide secondary metabolites were poorly sensitive to high concentration of inorganic phosphate. For example, the production of cephalosporin is optimal at 25 mM phosphate but higher concentrations of phosphate resulted in 85% reduction of cephalosporin production in *S. clavuligerus*[111]. These evidences suggested that the genes encoding the enzyme for the secondary metabolites produced by the anti-*Vibrio Streptomyces* may have lower sensitivity toward phosphate repression. However, the concentration of inorganic phosphate to be used in fermentation media should be optimized to ensure maximum production of anti-*Vibrio* metabolites by the *Strep-tomyces*. By comparing the anti-*Vibrio* activity of the *Streptomyces* metabolites under different concentration of K_2HPO_4 (Table 4), based on the median of inhibition zone, it is observed that the anti-*Vibrio* activity reduced by 33.3% when the concentration of K_2HPO_4 used is increased from 0.001% to 0.2% (w/v). These data suggest that inorganic phosphate is recommended to be maintained at lower concentration such as at 0.001% (w/v) as a source of phosphorus in the fermentation media for optimal production of anti-*Vibrio* metabolites from *Streptomyces*.

Table 4. The effect of different compositions and the fermentation conditions on the anti-*Vibrio* activity of *Streptomyces* metabolite

Parameters	Concentration (w/v %) / Range	Median of inhibition zone (mm)
NaCl	0.05	15.02 (n = 2)
	0.08	30.00 (n =3)
	0.20	19.00 (n = 8)
	> 0.20	15.00 (n = 3)
K_2HPO_4	0.001	30.00 (n = 3)
	0.01 - 0.05	16.52 (n = 4)
	0.2	20.00 (n = 1)
pH	7	15.03 (n = 7)
	7.1 – 7.3	18 (n = 5)
	> 7.3	22.5 (n = 2)
Temperature (°C)	< 28	16.4 (n = 2)
	28	20 (n = 12)
	30	15 (n = 7)

Sodium chloride

The supplementation of sodium chloride in the fermentation medium is one of the non-nutritional stress factors influencing the secondary metabolites production[112,113]. Based on the reviewed studies, a total of 13 studies supplemented sodium chloride in the fermentation medium for the production of anti-*Vibrio* secondary metabolites from *Streptomyces* (Table 2). The concentration of sodium chloride used was ranging from 0.05 to 1% (w/v), showing production of anti-*Vibrio* metabolites from *Streptomyces*. In line with the literatures, the anti-*Vibrio* activity of *Streptomyces* metabolites is enhanced by 5.88% when cultivated in the presence of sodium chloride as compared to the metabolites produced in the absence of sodium chloride (Table 3). Barakat and Beltagy (2015)[114] indicated the *Streptomyces ruber* ERH2 supplemented with 1% sodium chloride (w/v) produced metabolites against *V. ordalii* fish pathogen, with high inhibition zone measured at 15mm. As indicated in Table 4, a small increase of sodium chloride concentration, such as from 0.05 to 0.08% (w/v) resulted in 99.7% increment in the anti-*Vibrio* activity, thus indicated the optimum concentration of sodium chloride for the production of anti-*Vib-rio* metabolites is at 0.08% (w/v) for *Streptomyces*. At the meantime, the further increase of sodium chloride in the fermentation media from 0.08% (w/v) to more than 0.2% (w/v) may reduce the anti-*Vibrio* activity from *Streptomyces* metabolites by 50%. Similarly, Syvitski *et al.* (2006)[115] demonstrated that the presence of salt in the growth medium could result in differential production of antibiotic by *Streptomyces*. Furthermore, this study indicated the addition of 2.5% of sodium chloride inhibited the production of actinorhodin, but activated the production of undecylprodigiosin[115]. The study also reported high salt conditions that resulted in differential expression of these genes, *act*II-ORF4 and *redD* encoding corresponding pathway specific transcriptional regulators for both actinorhodin and undecylprodigiosin biosynthesis in *Streptomyces coelicolor* A3(2)[115].

Temperature

An optimal temperature is often required for the production of secondary metabolites. Based on the reviewed studies, 28°C (41.9%) is the most common incubation temperature used for the secondary metabolite production. Slightly higher incubation temperature at 30°C is also reported in several studies (16.1%) (Table 2). There are also studies employed a lower incubation temperature ranging from 23-25°C[116,117]. The studies indicated that the optimal temperature for production of secondary metabolites can be varying considerably between the similar genera of *Actinobacteria*. Furthermore, some studies indicated that optimal temperature for production of secondary metabolites is generally lower, when compared to growth of *Streptomyces* sp. Thakur *et al.* (2009)[118] reported *Streptomyces* sp. 201 showed narrow range of incubation temperature for growth and antibiotic production, maximum mycelial growth was measured at 35°C while highest antibacterial activity was observed at 30°C. Thirumurugan and Vijayakumar (2015)[119] also reported a strain, *Streptomyces* ECR77 that produced anti-*Vibrio* secondary metabolites after cultivated at 28-30°C although this strain showed optimal growth at 35°C. Costa and Badino (2012)[120] also recommended that the reduction of temperature could be useful in increasing the production of clavulanic acid by *Streptomyces clavuligerus*. According to Table 4, repression effect could occur via increase of fermentation temperature from the optimum 28°C to 30°C, resulting in 25% reduction of anti-*Vibrio* activity (based on the median of inhibition zone) from the *Streptomyces* metabolites. Hence, these data suggest that lower incubation temperature results in lower cellular growth and substrate consumption which could minimize

the metabolite repression effects and also reduces end-product degradation, eventually increasing the yield of secondary metabolites production[120].

pH of fermentation media

The pH of the cultivation media has substantial effect on the growth of *Streptomyces* sp. and their antibiotic production ability[121,122]. Based on the reviewed studies, a narrow range of initial pH (7–8) of the fermentation media were used in cultivation of *Streptomyces* sp. for secondary metabolites production (Table 2). Kontro *et al.* (2005)[122] reported that the pH ranges for the optimal growth of *Streptomyces* sp. were species specific and strongly influenced by the nutrient compositions of the media. The use of neutral to slightly alkaline pH as described by majority studies suggested that these pH range are more preferable for developing a fermentation medium for antibiotic producing-*Streptomyces*. In agreement with others, anti-*Vibrio* activity from *Streptomyces* metabolites could be enhanced by performing the fermentation under slightly alkaline pH. As depicted in Table 4, the anti-*Vibrio* activity could be increased by 49.7% with a small increase of the fermentation media from pH 7 to 7.3. According to Guimaraes *et al.* (2004)[121] findings, the low pH level of the cultivation media (at the end of the shake flask fermentation) resulted in no detection of retamycin although the final cell concentrations of *S. olindensis* ICB20 reached 4 g/liter, indicating that the pH negatively affected the activity of the biosyn-thetic enzymes that involved in the secondary metabolism. Meanwhile, at higher pH of 8.0, it was reported to have negative effect on the excretion of the antibiotic, demonstrated by the higher intracellular content of retamycin was produced, rather than the yield of extracellular retamycin[121].

EXTRACTION OF SECONDARY METABOLITES

The extraction is a critical step to isolate the desirable secondary metabolites from the complex fermented products[123]. Solvent extraction is one of the most common extraction methods due to the high selectivity and solubility of target compositions. It has been widely utilized to extract fermentation-derived microbial products prior to the final purification of bioactive compounds by chromatography[74,123,124]. There are a wide range of approaches available for the recovery of microbial metabolites. Primarily, the types of extraction method employed is chosen depending on the compounds of interest residing whether it is excreted into the medium or produced intracellularly. Generally, direct solvent extraction is conducted if the desired product is present in the cell and the medium. However, the common practice in extraction of microbial product from the cultivation media involves the separation of the microorganism biomass by centrifugation or filtration prior to solvent extraction of the cell free medium[125,126]. Among the 31 studies that performed fermentation, 18 studies (58.1%) conducted solvent extraction method to extract and determine the antibacterial activity of the bioactive compounds present in the fermented product.

The selection of most appropriate solvent is critical in determining the successfulness of yielding the desired product. Nonpolar solvents (petroleum ether, chloroform and hexane) are useful in extracting lipophilic compounds such as alkanes, sterols, alkaloids, fatty acids, coumarins and some terpenoids. Some alkaloids and flavonoids are compounds with medium polarity can be extracted with medium polarity solvents such as ethyl acetate. Meanwhile the more polar compounds such as flavonoid glycosides, tannins and some alkaloids are extracted with the carbon-bonded oxygen-bearing extractants include alcohols, esters and ketones [123]. Table 5 shows the examples of bioactive compounds isolated from anti-*Vibrio Streptomyces* using different organic solvents.

Table 5. The bioactive compounds identified from the *Streptomyces* sp. displaying anti-*Vibrio* activities.

Source	Compounds	Antibacterial activity	References
Ethyl acetate of *Streptomyces rosa* var. *notoensis*	Nanaomycin A **(1)**	MIC: 6.3 µg/mL against *V. alginolyticus* 138-2 MIC: 3.1 µg/mL against *V. parahaemolyticus* K-1	[127]
	Nanaomycin D **(2)**	MIC: <0.05 µg/mL against *V. alginolyticus* 138-2 MIC: <0.05 µg/mL against *V. parahaemolyticus* K-1	
Methylene chloride extract of endophytic *Streptomyces* sp. NRRL30562 derived from plant, *Kennedia nigriscans*	Munumbicin B **(3)**	16 mm against *V. fischeri* PIC345	[116]
	Munumbicin C **(4)**	9 mm against *V. fischeri* PIC345	
	Munumbicin D **(5)**	12 mm against *V. fischeri* PIC345	
Methanol extract of desert soil-derived *Streptomyces* sp. C34	Chaxalactin A **(6)**	MIC: 12.5 µg/mL against *V. parahaemolyticus*	[74]
	Chaxalactin B **(7)**	MIC: 20 µg/mL against *V. parahaemolyticus*	
	Chaxalactin C **(8)**	MIC: 12.5 µg/mL against *V. parahaemolyticus*	
Acetone extract of *Streptomyces atrovirens* PK288-21 derived from marine seaweeds	2-hydroxy-5-(3-methylbut-2-enyl)benzaldehyde **(9)**	MIC: 20 µg/mL against *V. harveyi* MIC: 65 µg/mL against *V. anguillarum*	[80]

	2-hepta-1,5-dienyl-3,6-dihydroxy-5-(3-methylbut-2-enyl)benzaldehyde **(10)**	MIC: 32 µg/mL against *V. harveyi* MIC: 65 µg/mL against *V. anguillarum*	
Acetone extract of *Streptomyces* sp. K01-0509	Guadinomine B **(11)**	IC_{50}: 14 nM potent type III secretion system (TTSS) inhibitor	[128]
Ethyl acetate extract of *Streptomyces* sp. SCSIO 01689 derived from submarine sediment	Pyranosesquiterpene compound **(12)**	MIC: >100 µg/mL against *V. anguillarum*	[82]
	Cyclo(D)-Pro-(D)-Ile **(13)**	MIC: 0.05 µg/mL against *V. anguillarum*	
	Cyclo(D)-Pro-(D)-Leu **(14)**	MIC: 0.04 µg/mL against *V. anguillarum*	
	Cyclo(D)-trans-4-OH-Pro-(D)-Phe **(15)**	MIC: 0.07 µg/mL against *V. anguillarum*	

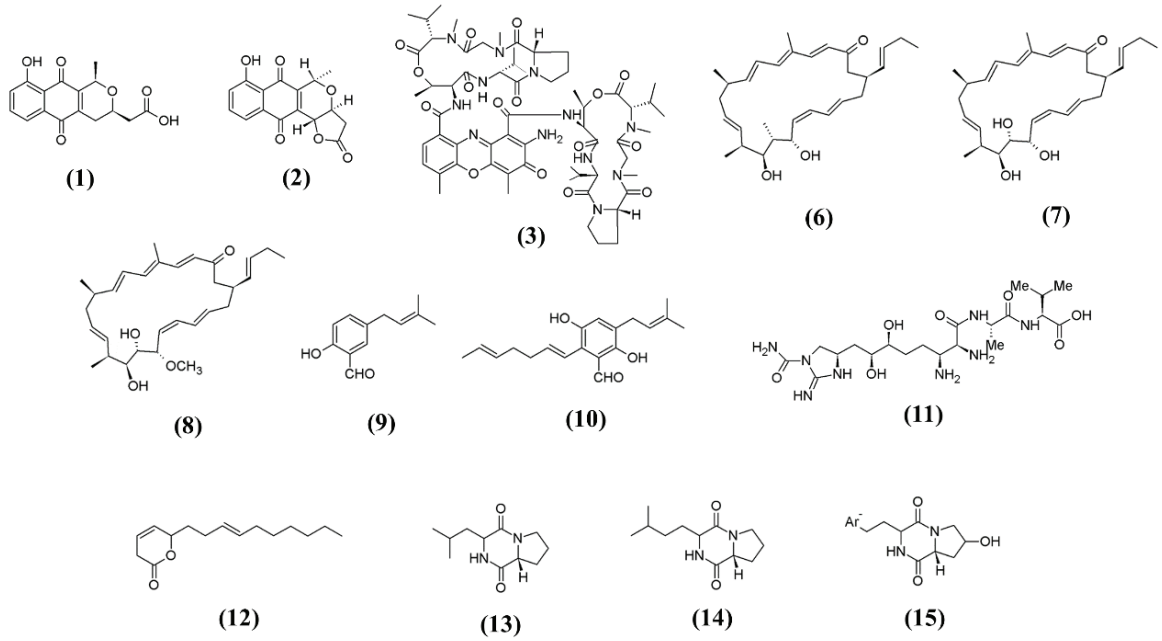

Figure 2. The chemical structures of anti-*Vibrio* secondary metabolites isolated from *Streptomyces* sp.

Based on the data, the commonly used solvents for the extraction of bioactive compounds include, methanol, acetone, chloroform, ethyl acetate, *n*-butanol, *n*-hexane and petroleum ether. From these studies, ethyl acetate (83.3%) was the most commonly used solvent. This may be due to the property of ethyl acetate which is only partially miscible with water, hence allowing easier recovery of the metabolites from the fermentation broth by liquid-liquid extraction methods. Besides that, methanol was the second (27.8%) most commonly utilized solvent among the reviewed studies. Usually, methanol is preferable for the extraction of unknown metabolites from new strains of bacteria. This is because methanol has been known to be efficient in extracting a wide range of metabolites from bacteria[129]. Eventually, the resulting extract is filtered, concentrated by vacuum evaporation before being used for bioactivity analysis. It is imperative to remove the solvent or extractant completely from the resulting extracts as their presence in the final product is undesirable and might affect the results of the bioactivity screening. Gas chromatography is a useful tool for the detection of residual solvents. This is because of the low detection limits allowing for the detection of trace organic compounds[130]. Furthermore, supercritical carbon dioxide at 200 atm and 35°C was shown to be effective in removing organic solvents from antibiotic without affecting the antibiotic activities[131].

Moreover, it is common to find that interesting compounds can be overlooked due to the presence of other molecules in a crude extract, or simply because of its low titers in an extract resulted overall low activity observed. Fractionation step after the extraction could be a way to overcome these issues. For instance, the fractionation of *Streptomyces* sp. C34 methanolic extracts with three other different solvents, *n*-hexane, dichloromethane and ethyl acetate and eventually identified the three novel macrolactones from the dichloromethane fraction with the most diverse metabolic profile[74].

Once a bioactive extract is identified, a more detailed analysis is performed, normally involving chromatography-based separation of the individual constituents, to identify the specific bioactive molecules and also structure elucidation with NMR analysis.

Subsequently, the bioactive compounds from these screening activities are tested in an *in vivo* model to examine efficacy and safety. Most of current clinically used antibiotics have been discovered using this approach. For instance, Barakat and Beltagy (2015)[114] demonstrated that the phthalic acid isolated from *S. ruber* EKH2 with antagonistic activity against *V. ordalii* is non-toxic toward *Artemia salina* (brine shrimp) up to 2800 µg/mL, suggesting that the compound is natural and minimum side effects. Furthermore, the conventional screening process also provides valuable information such as the potency of the antibiotic by determining the minimum inhibitory concentration (MIC) of the antibiotic toward specific pathogens, the spectrum of activity. Cho and Kim (2012)[80] determined the potency of benzaldehyde compounds isolated from *S. atrovirens* PK288-12, revealing a lower MIC displayed by both compounds as compared to ciprofloxacin against *V. harveyi.*

By referring to the studies which reported the isolation of *Streptomyces* with anti-*Vibrio* activity, most of them have focused on the preliminary screening and optimization of the various culture conditions. However, there is only limited number of the study that further analyzed and identified the bioactive compounds that displayed potent antibacterial activity against *Vibrio* sp. Hence, there is a need to improve the isolation and screening strategies, as the conventional methods of cultivation, extraction and bioac-tivity testing of anti-*Vibrio Streptomyces* are time consuming and prone to rediscovery of known compounds. New research strategies such as genome mining, which reveals the silence biosynthetic gene cluster, coupling with the advanced chemical separation and characterization techniques[132] have been developed to enhance the antibiotic production and discovery of new compounds in *Streptomyces*. Furthermore, more advanced extraction method could be employed to replace the conventional organic solvent extract method. For example, supercritical fluid extraction, pressurized solvent extraction and ultrasound-assisted extraction have been discussed as some of the better alternative extraction techniques to isolate bioactive natural products[133]. These advanced extraction methods are known for their higher selectivity, shorter extraction time, nontoxic organic solvents and more environmental friendly as compared to the conventional solvent extraction method[133]. Majority of these advanced extraction methods have been widely used to extract biologically active compounds with antioxidant and antimicrobial activity from plants[53,134]. Despite that, only a small portion of studies have utilized the advanced extraction methods to extract the bioactive compounds from the fermentation broth of microorganism. For instance, griseofulvin, which is one of the few examples of microbial antifungal antibiotic, was extracted with supercritical carbon dioxide extraction method[135]. Although the supercritical carbon dioxide is less effective in extracting highly polar compounds, this extraction method offers a better alternative to organic solvents because of its nontoxic property, inexpensive and most importantly can be easily removed from the final products[133]. This is because the residual organic solvent presents a major concern over the safety of food and pharmaceutical products over the years[136]. Therefore, future studies are encouraged to utilize one of these advanced extraction methods to improve the yield and purification of the biologically active compounds from *Streptomyces*.

CONCLUSION

Given the ever-increasing reports of antibiotic resistant *Vibrio* pathogens, there is a critical need to search for alternatives of major antibiotics. Numerous studies demonstrated the production of promising bioactive compounds with anti-*Vibrio* activity by *Streptomyces* sp. Fermentation parameters can have great impact on the secondary metabolism of *Streptomyces* and subsequently on production of different microbial products. The information and knowledge obtained in this review could help in the optimizing of suitable fermentation medium is important for better yield and antimicrobial activity from *Streptomyces* sp. We suggest that starch and yeast extract are both good carbon and nitrogen source for the secondary metabolites production by the anti-*Vibrio Streptomyces*. The temperature, concentrations of phosphate and sodium chloride are also important criteria should be taken into consideration when designing the fermentation medium and condition for the anti-*Vibrio* metabolite production in the genus *Streptomyces*. The limited findings on the bioactive compounds with anti-*Vibrio* activity from *Streptomyces* sp. suggesting that more studies should focus on identifying the potential bioactive compounds that specifically against *Vibrio* sp. Taken together, with optimal fermentation conditions and appropriate extraction techniques, future development of clinically important drugs is warranted from these *Streptomyces* sp. to treat infections inflicted by *Vibrio* pathogens.

Author Contributions

The literature review and manuscript writing were performed by LT-HT, L-HL and B-HG. L-HL and B-HG founded the research project.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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