IOP Conference Series: Earth and Environmental Science

PAPER • OPEN ACCESS

Aerial mycelium formation in rare thermophilic *Actinobacteria* on media solidified with agar and gellan gum

To cite this article: D C A F Sari *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **483** 012017

Aerial mycelium formation in rare thermophilic *Actinobacteria* on media solidified with agar and gellan gum

D C A F Sari[1,2], F Ningsih[1,2], A Yokota[3], S Yabe[3,4], W Sjamsuridzal[1,2], A Oetari[1,2]*

[1] Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
[2] Center of Excellence for Indigenous Biological Resources-Genome Studies, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
[3] Department of Microbial Resources, Graduate School of Agricultural Science, Faculty of Agriculture, Tohoku University, 468-1 Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-0845, Japan
[4] Hazaka Plant Research Center, Kennan Eisei Kogyo Co., Ltd., 44 Aza-Inariyama, Oaza-Ashitate, Murata-cho, Shibata-gun, Miyagi 989-1311, Japan

*E-mail: a-oetari@sci.ui.ac.id; aoetari@gmail.com

Abstract. This study investigated aerial mycelium formation in 12 isolates of rare thermophilic *Actinobacteria* from Indonesia on four different media (International *Streptomyces* Project ISP 1, ISP 2, ISP 3, and Bennett's solidified with agar and gellan gum). The results from media solidified with agar showed that aerial mycelium formation was observed on 9 isolates as follows: 3 isolates on ISP 1 agar (*Amycolatopsis* and *Microbispora*); 3 isolates on ISP 2 agar (*Amycolatopsis* and *Microbispora*); 9 isolates on ISP 3 agar (*Actinoallomurus*, *Amycolatopsis*, *Microbispora*, *Thermobispora*, and *Streptoalloteichus*); and 2 isolates of *Amycolatopsis* on Bennett's agar. Aerial mycelium formation was not observed in 3 isolates (*Microbispora* and *Nocardia*) on all media solidified with agar. The results from media solidified with gellan gum showed that aerial mycelium formation was observed in all 12 isolates as follows: 8 isolates on ISP 1 gellan gum (*Amycolatopsis*, *Microbispora*, *Nocardia* and *Thermobispora*); 5 isolates on ISP 2 gellan gum (*Amycolatopsis*, *Microbispora*, and *Nocardia*); 11 isolates on ISP 3 gellan gum (*Actinoallomurus*, *Amycolatopsis*, *Microbispora*, *Nocardia*, *Thermobispora*, and *Streptoalloteichus*); and 5 isolates on Bennett's agar (*Amycolatopsis*, *Microbispora*, *Nocardia*, and *Streptoalloeichus*). These results indicate that the media solidified with gellan gum induced aerial mycelium formation in larger number of rare thermophilic *Actinobacteria* isolates compared to media solidified with agar.

Keywords: aerial mycelium; geothermal area; solidifying agent; thermophilic *Actinobacteria*

1. Introduction

The phylum *Actinobacteria* are Gram positive filamentous bacteria with high G+C content in their DNA [1]. *Actinobacteria* are frequently found in a wide range of habitats including soil, freshwater, marine, and endophyte on plant [2]. During the last few decades, researches have focused on isolating

Actinobacteria from special habitats and extreme environments such as deep sea, salt-lake, and hot spring [3].

Actinobacteria are known as one of the most important sources of secondary metabolites such as antimicrobial, enzyme inhibitor, and biocontrol agent [1]. The genus *Streptomyces* produces almost 80 % of bioactive compounds known today [4]. Secondary metabolites of *Actinobacteria* are produced on late growth phase along with aerial mycelium production and sporulation [5]. Aerial mycelium production and sporulation is directly dependent on the quality and quantity of nutrient in the media [6].

Taxonomically, rare *Actinobacteria* are known as non-*Streptomyces Actinobacteria* and relatively difficult to isolate, cultivate, and maintain [7]. Rare *Actinobacteria* have gained attention related to their diversity and a promising source of secondary metabolites [8]. Various media and isolation techniques have been developed for the isolation, mycelium production (substrate mycelia and aerial mycelia), and sporulation of *Actinobacteria* [9]. According to Hamedi and Poorinmohammad [10], *Actinobacteria* form a substrate mycelium in both liquid and solid media, while aerial mycelia are formed specifically on solid media. Suzuki [11] reported that media with agar and gellan gum stimulated the formation of aerial mycelia as well as aerial spores of rare *Actinobacteria*.

In the previous study, Ningsih *et al.* [12] successfully isolated a novel thermophilic *Actinobacteria* genus and species, *Gandjariella thermophila* and another five genetically closely related isolates to this novel taxon from forest soil near geyser in Cisolok geothermal area. In another study, Ningsih *et al.* [13] obtained 25 isolates of thermophilic *Actinobacteria* from soil samples in the geyser of Cisolok and its vicinity. Their phylogenetic tree analyses based on 16S rRNA gene sequences showed that 15 out of 25 isolates are taxonomically regarded as rare *Actinobacteria*. In addition, we also obtained four isolates of rare *Actinobacteria, e.g.* three isolates from soil of Cisolok geyser and one isolate from litter sample of Galunggung geothermal area (unpublished data). However, the study of substrate and aerial mycelium formation of these rare thermophilic *Actinobacteria* isolates on various media solidified with agar and gellan gum has not yet been conducted.

This study investigated aerial mycelium formation in rare thermophilic *Actinobacteria* from geothermal area in Indonesia on four different media (International Streptomyces Project (ISP) 1, ISP 2, ISP 3, and Bennett's) solidified with agar and gellan gum. The results of this study will provide information about the effect of each solidifying agent on mycelium formation, in order to improve the methods of isolation, cultivation, and antimicrobial screening of rare thermophilic *Actinobacteria*.

2. Materials and Methods

2.1 Microorganisms

Twelve rare thermophilic *Actinobacteria* isolates from soil and litter samples in geothermal area of Cisolok and Galunggung, West Java were used in this study (Table 1). All isolates were maintained on ISP 1 medium at room temperature, in 20 % (v/v) glycerol as agar blocks at -80 °C, and as lyophilized cells for long-term preservation [12].

2.2 Preparation of growth media

International Streptomyces Project (ISP) 1, ISP 2, ISP 3 medium was prepared according to Shirling and Gottlieb [14]. Bennett's medium was prepared according to Jones [15]. All media were solidified with agar and gellan gum with the addition of $MgCl_2$.

2.3 Morphological observation

All isolates were transferred to media with both solidifying agents, incubated at 45 °C and observed for seven days. Observation was carried out on colony's morphology, substrate mycelia and aerial mycelia by stereo microscope (Carl Zeiss) and digital microscope (Hirox; KH-8700).

Table 1. Twelve isolates of rare thermophilic *Actinobacteria* isolated from geothermal area in Indonesia.

No.	Isolates code	Source of isolates	Species name and homology (%)	Genbank accession no.	Ref
1	SL1-2-7 FIT	soil of Cisolok geyser, West Java	*Thermobispora bispora* (94.4 %)	-	ud
2	SL3-1-R-1	soil forest of Cisolok, West Java	*Amycolatopsis methanolica* 239^T (100 %)	LC514435	[13]
3	SL3-1-R-3	soil forest of Cisolok, West Java	*Amycolatopsis methanolica* 239^T (100 %)	LC514436	[13]
4	SL3-2-R-1	soil forest of Cisolok, West Java	*Microbispora rosea* subsp. *rosea* ATCC 12950^T (99.62 %)	LC514435	[13]
5	SL3-2-R-2	soil forest of Cisolok, West Java	*Microbispora bryophytorum* NEAU-TX2-2^T (97.45 %)	LC514442	[13]
6	SL3-2-R-11	soil forest of Cisolok, West Java	*Microbispora rosea* subsp. *rosea* ATCC 12950^T (99.63 %)	LC514443	[13]
7	SL3-2-R-12	soil forest of Cisolok, West Java	*Microbispora hainanensis* 211020^T (99.39 %)	LC514446	[13]
8	SL2-2-R-20	soil of Cisolok geyser, West Java	*Nocardia farcinica* NCTC 11134 (100 %)	-	ud
9	SL2-2-R-22	soil of Cisolok geyser, West Java	*Nocardia farcinica* NCTC 11134 (100 %)	-	ud
10	GL1-12 FIT	litter sample of Galunggung, West Java	*Actinoallonurus spadix* JCM 3146^T (97.21 %)	-	ud
11	SL3-2-6	soil forest of Cisolok, West Java	*Gandjariella thermophila* SL3-2-4^T (100 %)	LC469352	[12]
12	SL3-2-10	soil forest of Cisolok, West Java	*Gandjariella thermophila* SL3-2-4^T (100 %)	LC469355	[12]

ud: unpublished data

3. Results and Discussions

The colony growth and formation of substrate mycelia as well as aerial mycelia of 12 isolates of rare thermophilic *Actinobacteria* were observed after seven days of incubation. The morphology of representative isolates on media solidified with agar and gellan gum incubated at 45 °C for five days is shown in Figure 1. The isolates showed formation of aerial mycelia as shown by the change of colony surface colour and formation of white sheath on the colony's surface, as similarly reported by Manteca *et al*. [16].

The ability of each isolate to produce substrate mycelia is shown in Table 2. All isolates were able to form substrate mycelia on media solidified with agar and gellan gum after seven days at 45 ^oC. The results indicated that all media solidified with either agar or gellan gum supported the growth of substrate mycelia. All isolates grown on all media produced substrate mycelia within two days of incubation.

The ability of isolates to produce aerial mycelia on media solidified with agar after five days of incubation is shown in Table 3. Nine isolates from five genera were able to produce aerial mycelia. Three isolates from the genera *Amycolatopsis* (2 isolates) and *Microbispora* (1 isolate) produced aerial mycelia on ISP 1 agar. Three isolates from the genera *Amycolatopsis* (2 isolates) and *Microbispora* (1 isolate) produced aerial mycelia on ISP 2 agar. Nine isolates from the genera *Thermobispora* (1 isolate), *Amycolatopsis* (2 isolates), *Microbispora* (3 isolates), *Actinoallomurus* (1 isolate), and *Gandjariella* (2 isolates) produced aerial mycelia on ISP 3 agar. Only two isolates from the genus

Amycolatopsis were able to produce aerial mycelia on Bennett's agar. Three isolates from the genus *Nocardia* (2 isolates) and the genus *Microbispora* (1 isolate) were not able to produce aerial mycelia on all media.

Table 2. The ability of isolates to produce substrate mycelia after incubation for seven days at 45 °C

No	Isolates code	Species name and similarity (%)	Medium							
			ISP 1		ISP 2		ISP 3		Bennett's	
			A	G	A	G	A	G	A	G
1	SL1-2-7 FIT	*Thermobispora bispora* (94.4 %)	+	+	+	+	+	+	+	+
2	SL3-1-R-1	*Amycolatopsis methanolica* (100 %)	+	+	+	+	+	+	+	+
3	SL3-1-R-3	*Amycolatopsis methanolica* (100 %)	+	+	+	+	+	+	+	+
4	SL3-2-R-1	*Microbispora rosea* subsp. *rosea* (99.62 %)	+	+	+	+	+	+	+	+
5	SL3-2-R-2	*Microbispora bryophytorum* (97.45 %)	+	+	+	+	+	+	+	+
6	SL3-2-R-11	*Microbispora rosea* subsp. *rosea* (99.63 %)	+	+	+	+	+	+	+	+
7	SL3-2-R-12	*Microbispora hainanensis* (99.39 %)	+	+	+	+	+	+	+	+
8	SL2-2-R-20	*Nocardia farcinica* (100 %)	+	+	+	+	+	+	+	+
9	SL2-2-R-22	*Nocardia farcinica* (100 %)	+	+	+	+	+	+	+	+
10	GL1-12 FIT	*Actinoallomurus spadix* (97.21 %)	+	+	+	+	+	+	+	+
11	SL3-2-6	*Gandjariella thermophila* (100 %)	+	+	+	+	+	+	+	+
12	SL3-2-10	*Gandjariella thermophila* (100 %)	+	+	+	+	+	+	+	+
		Total	12	12	12	12	12	12	12	12

(+): growth

The ability of isolates to produce aerial mycelia on media solidified with gellan gum after five days of incubation is shown in Table 4. All isolates were able to produce aerial mycelia on media solidified with gellan gum. Eight isolates from the genera *Thermobispora* (1 isolate), *Amycolatopsis* (2 isolates), *Microbispora* (3 isolates), and *Nocardia* (2 isolates) produced aerial mycelia on ISP 1 gellan gum. Five isolates from the genera *Amycolatopsis* (2 isolates) and *Microbispora* (2 isolates), and *Nocardia* (1 isolate) were able to produce aerial mycelia on ISP 2 media solidified with gellan gum. Eleven isolates from the genera *Thermobispora* (1 isolate), *Amycolatopsis* (2 isolates), *Microbispora* (4 isolates), *Nocardia* (1 isolate), *Actinoallomurus* (1 isolate), and *Gandjariella* (2 isolates) produced aerial mycelia on ISP 3 gellan gum. Five isolates from the genera *Amycolatopsis* (2 isolates), *Microbispora* (1 isolate), *Nocardia* (1 isolate), and *Gandjariella* (1 isolate) produced aerial mycelia on Bennett's gellan gum.

The results showed that media solidified with gellan gum induced aerial mycelium formation in larger number of rare thermophilic *Actinobacteria* isolates compared to media solidified with agar. It was reported [17] that agar has been the dominant solidifying agent in microbial research. However, at high incubation temperature, agar-based media usually lose it strength and begin to melt or soften with the presence of significant amounts of surface water escaping from the gel.

Gellan gum has been known as an alternative solidifying agent. It has a potential as an ideal solidifying agent for isolation and growth medium of thermophilic microorganisms [18]. Gellan gum was better for cultivation and stimulating spore production of slow growing actinomycetes [19]. Media with gellan gum promoted better growth of various thermophilic bacteria such as *Bacillus acidocaldarius*, *Thermus thermophilus*, and *Thermus aquaticus* [17]. Media with gellan gum best stimulated the formation of aerial mycelia, as well as aerial spores, on *Actinobispora yunnanensis* IFO 15681^T, *Sporichthya*, *Planobispora*, and *Planomonospora* [11].

This study showed that rare thermophilic *Actinobacteria* isolated from geothermal area in Indonesia were able to grow on various media solidified with agar and gellan gum. Twelve rare thermophilic *Actinobacteria* isolates showed the ability to form substrate mycelia on both media solidified with agar and gellan gum. While, larger number of isolates formed aerial mycelia on gellan gum media compared to agar media. These results indicate that the media solidified with gellan gum induced aerial mycelia formation of rare thermophilic *Actinobacteria* isolates compared to media solidified with agar.

Table 3. The ability of isolates to produce aerial mycelia on media solidified with agar after five days of incubation at 45 °C.

No.	Isolates code	Species name and similarity (%)	Media solidified with agar			
			ISP 1	ISP 2	ISP 3	Bennett's
1	SL1-2-7 FIT	*Thermobispora bispora* (94.4 %)	-	-	+	-
2	SL3-1-R-1	*Amycolatopsis methanolica* (100 %)	+	+	+	+
3	SL3-1-R-3	*Amycolatopsis methanolica* (100 %)	+	+	+	+
4	SL3-2-R-1	*Microbispora rosea* subsp. *rosea* (99.62 %)	-	-	+	-
5	SL3-2-R-2	*Microbispora bryophytorum* (97.45 %)	+	-	+	-
6	SL3-2-R-11	*Microbispora rosea* subsp. *rosea* (99.63 %)	-	+	+	-
7	SL3-2-R-12	*Microbispora hainanensis* (99.39 %)	-	-	-	-
8	SL2-2-R-20	*Nocardia farcinica* (100 %)	-	-	-	-
9	SL2-2-R-22	*Nocardia farcinica* (100 %)	-	-	-	-
10	GL1-12 FIT	*Actinoallomurus spadix* (97.21 %)	-	-	+	-
11	SL3-2-6	*Gandjariella thermophila* (100 %)	-	-	+	-
12	SL3-2-10	*Gandjariella thermophila* (100 %)	-	-	+	-
		Total	3	3	9	2

(+): aerial mycelia observed; (-): no aerial mycelia observed

Table 4. The ability of isolates to produce aerial mycelia on media solidified with gellan gum after five days of incubation at 45 °C

No.	Isolates code	Species name and similarity (%)	Media solidified with gellan gum			
			ISP 1	ISP 2	ISP 3	Bennett's
1	SL1-2-7 FIT	*Thermobispora bispora* (94.4 %)	+	-	+	-
2	SL3-1-R-1	*Amycolatopsis methanolica* (100 %)	+	+	+	+
3	SL3-1-R-3	*Amycolatopsis methanolica* (100 %)	+	+	+	+
4	SL3-2-R-1	*Microbispora rosea* subsp. *rosea* (99.62 %)	+	+	+	+
5	SL3-2-R-2	*Microbispora bryophytorum* (97.45 %)	+	+	+	-
6	SL3-2-R-11	*Microbispora rosea* subsp. *rosea* (99.63 %)	+	-	+	-
7	SL3-2-R-12	*Microbispora hainanensis* (99.39 %)	-	-	+	-
8	SL2-2-R-20	*Nocardia farcinica* (100 %)	+	-	+	-
9	SL2-2-R-22	*Nocardia farcinica* (100 %)	+	+	-	+
10	GL1-12 FIT	*Actinoallomurus spadix* (97.21 %)	-	-	+	-
11	SL3-2-6	*Gandjariella thermophila* (100 %)	-	-	+	-
12	SL3-2-10	*Gandjariella thermophila* (100 %)	-	-	+	+
		Total	8	5	11	5

(+): aerial mycelia observed; (-): no aerial mycelia observed

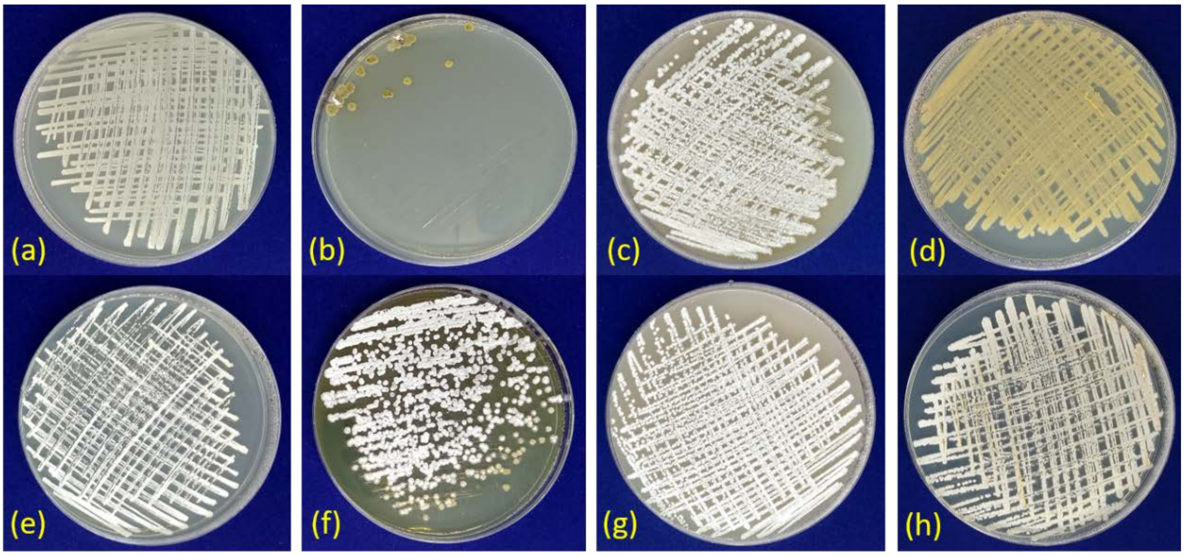

Figure 1. Morphology of representative rare thermophilic *Actinobacteria* isolates on media solidified with agar (a, b, c, d) and gellan gum (e, f, g, h), incubated at 45 °C for five days. (a, e) SL3-1-R-1; (b, f) SL3-2-R-1; (c, g) SL3-2-R-11; (d, h) SL2-2-R-22

4. Conclusions

Twelve rare thermophilic *Actinobacteria* isolates were obtained from soil and litter samples in Cisolok and Galunggung geothermal area, West Java, Indonesia. All isolates showed the ability to form substrate mycelia on both media solidified with agar and gellan gum. The results showed that aerial mycelia formation was observed on nine isolates grown on media solidified with agar, meanwhile in the remaining three isolates aerial mycelia was not observed. In media solidified with gellan gum, aerial mycelia formation was observed in all 12 isolates. Based on these results we suggested that gellan gum stimulated the formation of aerial mycelia and could be used as an alternative solidifying agent for isolation and growth medium of rare thermophilic *Actinobacteria*.

Acknowledgement

This research was supported by Hibah Publikasi Internasional Terindeks Tugas Akhir (Hibah PITTA B) Tahun Anggaran 2019 contract number NKB-0613/UN2.R3.1/HKP.05.00/2019 to A.O. and financially supported by Hibah Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT), Direktorat Jenderal Pendidikan Tinggi (DIKTI) 2018, Kementerian Riset Teknologi dan Pendidikan Tinggi Republik Indonesia, Research Grant, contract number 387/UN2.R3.1/HKP05.00/2018 to W.S.

References

[1] Verma E, Chakraborty S, Tiwari B and Mishra A K 2018 Antimicrobial compounds from *Actinobacteria*: Synthetic pathways and applications *New and Future Developments in Microbial Biotechnology and Bioengineering*, ed Singh B P, Gupta V K and Passari A K (Amsterdam: Elsevier) pp 277–295

[2] Goodfellow M and William S T 1983 Ecology of Actinomycetes *Ann. Rev. Microbiol*. **37** 189–216

[3] Bull T M 2011 *Actinobacteria* of the extremobiosphere *Extremophiles Handbook*, ed Horikoshi K (Amsterdam: Elsevier) pp 1204–40

[4] Lazzarini A, Cavaletti L, Toppo G and Marinelli F 2000 Rare genera of actinomycetes as potential producers of new antibiotics *Anton. Leeuw. Int. J. G.* **78** 399–405

[5] Barka E A, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y and van Wezel G P 2016 Taxonomy, physiology, and natural products of *Actinobacteria Microbiol. Mol. Biol. Rev.* **80** 1–43

[6] Karandikar A, Sharples G P and Hobbs G 1996 Influence of medium composition on sporulation by *Streptomyces coelicolor* A3(2) grown on defined solid media *Biotech. Tech.* **10** 79–82

[7] Azman A-S, Othma I, Velu S, Chan K-G and Lee L-H. 2015 Mangrove rare *Actinobacteria*: taxonomy, natural compound, and discovery of bioactivity *Front. Microbiol*. **6** 856

[8] Kurtboke D I 2012 Biodiscovery from rare actinomycetes: an eco-taxonomical perspective *Appl. Microbiol. Biotechnol.* **93** 1843–52

[9] Tiwari K and Gupta R K 2012 Rare actinomycetes: a potential storehouse for novel antibiotics *Cri. Rev. Biotech.* **32** 108–32

[10] Hamedi J and Poorinmohammad N 2017 The cellular structure of *Actinobacteria Biology and Biotechnology of Actinobacteria,* ed Wink J, Mohammadipanah F and Hamedi J (Switzerland: Springer International Publishing) pp 5–28

[11] Suzuki S 2001 Establishment and use of gellan gum media for selective isolation and distribution survey of specific rare Actinomycetes *Actinomycetol.* **15** 55–60

[12] Ningsih F, Yokota A, Sakai Y, Nanatani K, Yabe S, Oetari A and Sjamsuridzal W 2019 *Gandjariella thermophila* gen. nov., sp. nov., a new member of the family *Pseudonocardiaceae,* isolated from forest soil in a geothermal area *Intl. J. Syst. Evol. Microbiol.* **69** 3080–86

[13] Ningsih F, Sari D C A F, Rachmania M K, Yabe S, Yokota A, Oetari A and Sjamsuridzal W 2020 Isolation and 16S rRNA gene sequences analysis of thermophilic *Actinobacteria* isolated from soil in Cisolok geothermal area, West Java, Indonesia *IOP Conf. Ser.: Earth Environ. Sci. (In press)*

[14] Shirling E B and Gottlieb D 1966 Methods for characterization of *Streptomyces* species *Int. J. Syst. Bacteriol*. **16** 313–40

[15] Jones K L 1948 Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic *J. Bacteriol.* **57** 141–45

[16] Manteca A, Fernandez M and Sanchez J 2005 Mycelium development in *Streptomyces antibioticus* ATCC11891 occurs in an orderly pattern which determines multiphase growth curves *BMC Microbiol.* **5** 3689–97

[17] Lin C C and Casida L E 1984 GELRITE as a gelling agent in media for the growth of thermophilic microorganisms *Appl. Environ. Microbiol.* **47** 427–9

[18] Das N, Tripathi N, Basu S, Bose C, Maitra S and Khurana S 2015 Progress in the development of gelling agents for improved culturability of microorganisms *Front. Microbiol.* **6** 698

[19] Bassi C A and Benson D R 2007 Growth characteristics of the slow-growing actinobacterium *Frankia sp.* strain CcI3 on solid media *Physiol. Plant.* **130** 391–9