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Impact of Temperature and pH on Anti-Fungal Secondary Metabolites Production Ability of *Streptomyces* sp.

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Abstract: ***Seven types of bioactive anti-fungal secondary metabolites were isolated from Streptomyces tendae during present research work. Highest level of production of all seven types of secondary metabolites by Streptomyces tendae were achieved at either 30ºC or 37ºC temperature. Median and low level of secondary metabolite production was observed at 20ºC, 25ºC, and 40ºC temperature during culture. Best suitable pH for highest level of secondary metabolites production by Streptomyces tendae was observed as 7.0 or 7.5. Median or low level of secondary metabolite production by this bacteria was observed at 8.0 pH. But pH level of 5.0, 5.5, 9.0 and 10.0 were not remained suitable for secondary metabolite production by this bacteria.30ºC & 37ºC temperature and 7.0 & 7.5 pH remained suitable for production of anti-fungal secondary metabolites at highest level by Streptomyces tendae.***

Keywords: *Temperature, pH, Secondary Metabolites, Streptomyces tendae*

1. Introduction

The filamentous actinomycetes bacteria remain as an important source of bioactive metabolites useful for control of pathogenic bacteria and fungi. They are known for production of several types of antibiotics, anti-fungal secondary metabolites, hydrolytic enzymes as well as growth promoting substances (Oskay, 2009). But, only 3% of these naturally occurring substances were isolated from *Streptomyces* sp. and immense number of anti-fungal compounds remain to be discovered (Bordy, 2005).

Streptomyces generally horbour soil and identified as mycelium forming Gram positive bacteria. They naturally conduct mineralization process in soil by producing different types of secondary metabolites. These microbes remain as heterotrophic filamentous prokaryotes which forms hypha during their life cycle. *Streptomyces* are now commercially used for manufacture of active anti-fungal pharmaceutical compounds by fermentation. But up-till-now, the *Streptomyces* sp. naturally available in the soil of Gaya district was not evaluated for their anti-fungal secondary metabolite production ability. In this perspective, present research work was conducted.

2. Materials and Method

Soil samples were collected from different locations of Gaya district and transported to laboratory. *Streptomyces* sp. was isolated from soil samples by serial dilution method and identified on the basis of morphological, physiological, biochemical as well as genomic characteristics. The isolated actinomycetes was identified as *Streptomyces tendae*. Extraction, separation and identification of secondary metabolites obtained was carried out by TLC-GC adopting proper protocol for this purpose. The production of different secondary metabolites from this species of *Streptomyces* was observed under different temperature such as 20ºC, 25ºC, 30ºC, 37ºC, 40ºC and pH such as 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0 and 10.0. Observed information were tabulated and presented in Table-1 and 2.

Seven types of anti-fungal secondary metabolites were isolated and purified from *Streptomyces* sp. (*S. tendae*) while extracted in chloroform and ethyl acetate mixture (1: 9, V/V). These bioactive compounds are Acetic acid phenyl ester, 1-H-indole-3 carboxylic acid, 2-D, hydnoxy-5 methyl benzaldehyde, hexadeconic acid (methyl ester), hexahydro-3-2-methyl propyle-2H-1, 4-diazepin, 4-(4-hydroxy phenyl)-2-butanone and Octa deconoic acid.

The most suitable temperature for secondary metabolites production was observed as 30ºC and 37ºC. Highest production of Acetic acid and Octa-deconoic acid was observed at 37ºC temperature. Highest production of 2-dihydroxy-5-methyl benzaldehyde, hexahydro-3-2-methyl propyl-2, H-1, 4-diazepin and 4-(4-hydroxy phenyl)-2-butanon was observed at 30ºC temperature.1-H-indole-3-carboxylic acid and hexadeconic acid (methy ester) were produced at high rate during both 30ºC and 37ºC temperature of the culture medium (Table-1).

The most suitable pH for secondary metabolites production by *Streptomyces tendae* was observed at 7.0 and 7.5 pH level of culture medium. Highest level of the production of acetic acid phenyl ester, 1-H-indole-3-carboxylic acid and hexahydro-3-2-methyl phenyl-2H-1, 4 diazepin were observed at 7.0 pH.2-dihydroxy-5-methyl benzaldehyde, 4-(4-hydroxy phenyl)-2-butanon and Octa-deconoic acid was produced at highest level during pH level of 7.5. Highest level of production of hexadeconoic acid (methyl ester) was observed at both 7.0 and 7.5 pH. Low or median levels of secondary metabolite production by *Streptomyces tendae* was observed at 8.0 pH level. Not a single type of secondary metabolites was recovered when *Streptomyces* was cultured at 5.0, 5.5, 9.0 and 10.0 pH level (Table-2).

Table 1: Impact of Temperature on Antifungal secondary metabolites production from *Streptomyce* sp. (pH=7.5, incubation time= 96 hrs, Glucose with Casein)

S. No.	Antifungal compounds	Temperature ºC				
		20º	25º	30º	37º	40º
1.	Acetic Acid phenyl ester	Low	Low	Medium	Highest	Low
2.	1-H-indole-3-Carboxylic Acid	Nil	Low	Highest	Highest	Low
3.	2-dihydroxy-5-methyl benzaldehyde	Nil	Low	Highest	Median	Nil
4.	Hexadeconic Acid (methyl ester)	Low	Low	Highest	Highest	Low
5.	Hexahydro-3-2-methyl propyl-2, H-1, 4-diazepin	Nil	Nil	Highest	Median	Nil
6.	4-(4-hydroxy phenyl)-2-butanon	Low	Low	Highest	Median	Low
7.	Octa-deconoic Acid	Nil	Low	Low	Highest	Low

Table 2: Antifungal secondary metabolites production from *Streptomyces* sp. at different pH level (Temp. =37ºC incubation time=96hrs. Glucose with Casein)

S. No.	Antifungal compounds	pH level								
		5.0	5.5	6.0	6.5	7.0	7.5	8.0	9.0	10.0
1.	Acetic Acid phenyl ester	Nil	Nil	Low	Low	Highest	Median	Low	Nil	Nil
2.	1-H-indole-3-Carboxylic Acid	Nil	Nil	Nil	Low	Highest	Median	Median	Nil	Nil
3.	2-dihydroxy-5-methyl benzaldehyde	Nil	`Nil	Nil	Nil	Median	Highest	Low	Nil	Nil
4.	Hexadeconic Acid (methyl ester)	Nil	Nil	Nil	Low	Highest	Highest	Low	Nil	Nil
5.	Hexahydro-3-2-methyl phenyl-2H-1, 4-diazepin	Nil	Nil	Low	Low	Highest	Median	Median	Nil	Nil
6.	4-(4-hydroxy phenyl)-2-butanon	Nil	Nil	Nil	Low	Median	Highest	Low	Nil	Nil
7.	Octa-deconoic Acid	Nil	Nil	Nil	Low	Median	Highest	Low	Nil	Nil

Singh *et al*. (2017) observed during their research work that *Streptomyces cholikensis* obtained from soil of Gwalior exhibited maximum growth and highest level of antimicrobial compound production at 30ºC temperature, followed by 35ºC, 40ºC, 25ºC, 20ºC and 45ºC. Thus, it became evident that optimization of the culture conditions is necessary to achieve high level of secondary metabolite production by *Streptomyces* sps. Pavani *et al*., (2014) observed during their research work that 28ºC temperature remain as optimum for maximum growth and secondary metabolite production by *Streptomyces malaysiensis.* Best antibiotic production at 31ºC temperature by *Streptomyces spororaveus* was reported by Askar *et al*. (2011). Hitit *et al*. (2022) observed during their research work that *Stroptomyces* sp. culture grows suitably between 27 to 37ºC temperature. Thus, they concluded that antifungal activity of *Streptomyces* sp. decreases at increased temperature.

Antifungal metabolite production by *Streptomyces* sp. was examined at different temperature during present research work to determine the impact of temperature on the metabolite production ability of these bacteria. It was observed that antifungal secondary metabolite production decreased at increased temperature. This result remains in conformity with results obtained by Augustine *et al*. (2005) and Oskay (2009), who also observed decreased secondary metabolite production at increased temperature by *Streptomyces* sp. Askar *et al*. (2011) observed that *Streptomyces spororaveus* shows good growth at 31ºC temperature Oskay (2009) observed maximum secondary metabolite production at 30ºC temperature by *Streptomyces* sp. examined by them. Ayari *et al*. (2012) observed that *Streptomyces* sp. shows optimum growth at 28ºC temperature They also pointed out that this microbe did not grow at 45ºC and 50ºC. Temperature of 30ºC and 37ºC remained most suitable for production of different types of secondary metabolites by *Streptomyces tendae* (Table-1). Thus, results of present research remained in conformity with results obtained by Singh *et al*. (2017), Askar *et al*. (2011) and El-Raheem (2022).

Singh *et al*. (2017) observed most suitable pH level as 7-7.5 for optimum antibiotic production by *Streptomyces* sp. Adverse effect on growth and antibiotic production was observed by them at higher pH level of culture medium. Highest level of anti-fungal metabolite production by *Streptomyces spororaveus* was observed at 7.5 pH during research study conducted by Askar *et al*. (2011). Oskay (2007) also observed that initial pH of 7 remain best suitable for optimum level of growth and secondary metabolite production for *Streptomyces* sp. But Balachandar *et al*. (2018) observed during their research work that pH 7 remain suitable for optimum growth of *Streptomyces* sp.

During present research work it was observed that pH 7.0 and 7.5 remain most suitable for production of different types of secondary metabolites by *Streptomyces tendae* (Table-2). The results of present research show similiarity with results obtained by Singh *et al*. (2017), Askar *et al*. (2011), Oskay (207) and Balachandar *et al*. (2018).

3. Conclusion

Thus, it became evident that 30ºC & 37ºC temperature and 7.0 & 7.5 pH remain most suitable for production of different types of antifungal secondary metabolite by *Streptomyces tendae.* However secondary metabolites were also produced at 8.0 pH level of culture medium but at median or low level. The lower pH level as 5.0, 5.5 & 6.0 as well as higher pH level as 9.0 and 10.0 not remained suitable for production of different types of anti-fungal secondary metabolites by *Streptomyces tendae.*

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