OPTIMIZATION FOR THE FERMENTATION CONDITIONS OF SELECTED SOIL FUNGUS *CURVULARIA* SPP

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Abstract

In the study, ten different fungi were isolated from different soil samples. It was collected from three different places in Bye Da Yaw Village in Sagaing Township, Sagaing Region for the isolation of soil fungi during May, 2022. Isolated soil fungi were tested by using five test organisms. Ten fungal strains showed antibacterial activities against the five test organisms during the preliminary screening. Among them, soil fungus SZF-02 showed the highest antibacterial activities on *Escherichia coli* (19.54mm) during the confirmatory screening. Therefore, this fungus SZF-02 was selected for further investigation such as antibacterial activities, effect of different fermentation media, effect of pH and temperature. In the present study, the maximum production of antibacterial metabolite was observed in different fermentation media with FM-1 showed distinct inhibitory zones of (22.00 mm) against *Escherichia coli* under pH 5 showed the best inhibitory zones of (21.60 mm) and incubation temperature 23°C showed the highest inhibitory zones of (21.91 mm). The microscopically characters of selected fungus SZF-02 was preliminary studied. On the basis of the morphological, microscopical characters and references key, the selected fungus SZF-02 was identified as *Curvularia* spp. Present study concludes that, soil fungus SZF-02 (*Curvularia* spp) serve as a potential source for further investigation will produce antibacterial metabolite.

Keywords: Antibacterial activities, different fermentation media, pH, Temperature

Introduction

Fermentation can be defined as a process in which biochemical changes are brought about in organic substances (carbohydrates, proteins, and fats) through the action of enzymes which are produced by microorganisms. Fermentation processes, the precursor of the specific products are not added in the medium, carbon and nitrogen sources present in the medium during their metabolism may initiate the biosynthesis of precursors that regulate the metabolism and influence the end products synthesis. Nutrients type and their concentrations in the medium play an important role in commencing the production of primary and secondary metabolites as limited supply of an essential nutrient can restrict the growth of microbial cells or product formation. Generally, carbon and nitrogen sources present in the medium can influence the metabolite production (Elibol, 2004).

Medium optimization is still one of the most critically investigated phenomenon that is carried out before any largescale metabolite production, and possess many challenges too. Before 1970s, media optimization was carried out by using classical methods, which were expensive, time consuming, involving plenty of experiments with compromised accuracy. Nevertheless, with the advent of modern mathematical/statistical techniques, media optimization has become more vibrant, effective, efficient, economical and robust in giving the results. For designing a production medium, the most suitable fermentation conditions (e.g., pH, temperature, agitation speed, etc.) and the appropriate medium components (e.g., carbon, nitrogen, etc.) must be identified and optimized accordingly. Further, by optimizing the above said parameters,

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maximum product concentration could be achieved (Gupte and Kulkarni, 2003; Franco-Lara *et al*., 2006; Wang *et al*., 2011).

Optimal fermentation conditions are very important for maximal productively of metabolites. (Phay, 1997).

The aim of the study is to the isolation and antimicrobial activities of isolated soil fungi from the collected soil samples, to study the Fermentation conditions of soil fungi for the production of antimicrobial metabolites and to identified the genus of selected soil fungus (SZF-02).

Materials and Methods

Collection of Soil Samples

Three different soil samples were collected from three different places at Bye Da Yaw Village in Sagaing Township, Sagaing Region During May, 2022. The soil texture, soil pH and soil moisture % were measured at Department of Agriculture (LAND USE). Soil interpretation of results was made in Mandalay Township.

Isolation of Soil Fungi from Chemical Treatment Dilution Method (Hayakawa and Kobayashi, 2005)

The collected soil sample was air–dried at room temperature. The soil sample was grounded and sieved. 2g of sieved soil was put into test tube and then 4 mL of sterilized water was also put into the tube containing soil samples. 14 mL of 70% of methanol solution was then added into the tube containing soil suspension and was shaken for 1 minute and diluted with sterile water. The dilution series were cultured on low carbon source medium such as glucose yeast extract agar medium (GYA).

Preliminary Study for Antibacterial Activities by Agar Well diffusion Assay(Collin, 1965)

The isolated soil fungi were cultured on potato glucose agar medium for seven days. The isolated fungi were inoculated into seed medium and incubated at 27°C for three days. Five percent of seed medium were transferred into the fermentation medium. The fermented broth was used by agar well diffusion assay method (Collin 1965) to check the antibacterial activities against five test organisms. These test organisms were supported by Department of Biotechnology of Pathein University for the cooperation research. Cork borer was used to make the wells (8mm) in diameter for antibacterial assays. The fermented broth (20µL) was impregnated and allowed to dry.

The assay medium for the antibacterial activities test was prepared and one percent of test organism was added to assay medium, then poured into plates. After solidification, agar well impregnated with samples (fermented broth) were applied on the agar plates and incubated at 27°C for 24-36 hours. Clear zones (inhibitory zones) surrounding the agar well indicated the presence of bioactive metabolites which inhibit the growth of test organisms. Five test organisms, *Agrobacterium tumefaciens*, (NITE 09678), *Escherichia coli* (AHU5436), *Pseudomonas fluorescens* (IFO94307), *Staphylococcus aureus* (AHU8465) and *Bacillus pumilus* (IFO90517) were used in agar well diffusion assay.

Basal Media Used for the Antibacterial Activities of isolated Soil Fungi (NITE, 2004)

Seed medium

Glucose	0.5 g
Yeast extract	0.3 g
KH_2PO_4	0.01 g
KNO_3	0.1 g
Soluble starch	0.3 g
$MgSO_4 7H_2O$	0.01 g
DW	100mL
pH	6.5

Fermentation Medium (Basal medium)

Glucose	2.0
Yeast extract	0.3 g
KNO_3	0.1 g
K_2HPO_4	0.01 g
$CaCO_3$	0.1 g
DW	100 mL
pH	6.5

Assay Medium

Glucose	1.0 g
Polypeptone	0.3 g
KNO_3	0.1 g
Agar	1.6 g
DW	100mL
pH	6.5

Utilization of Four Different Fermentation media for Isolated Fungus SZF-02

FM 1

Glucose	2.0 g
Sucrose	2.0 g
Polypeptone	0.3 g
KNO_3	0.1 g
K_2HPO_4	0.01 g
$CaCO_3$	0.1 g
DW	100 mL
pH	6.0

FM 2

Sucrose	2.0 g
Yeast extract	0.3 g
Polypeptone	0.3 g
KNO_3	0.1 g
K_2HPO_4	0.01 g
$CaCO_3$	0.1 g
DW	100 mL
pH	6.0

FM 3

Glycerol	2.0 g
sucrose	1.0 g
Yeast extract	0.3 g
KNO_3	0.1 g
K_2HPO_4	0.01 g
$CaCO_3$	0.1 g
DW	100 mL
pH	6.0

FM 4

Maltose	2.0 g
Yeast extract	0.3 g
Polypeptone	0.3 g
KNO_3	0.1 g
K_2HPO_4	0.01 g
$CaCO_3$	0.1 g
DW	100 mL
pH	6.0

Distinguished Characters of the Pathogenic Bacteria *E. coli*

The selected bacterium was cultured on peptone glucose agar medium. After 24 hrs of incubation, plates were observed for microscopic characteristics such as gram-negative and microscopic characters such as facultative anaerobic, rod shape and coliform bacterium of the genus *Escherichia.*

Different Fermentation Media on Selected Isolated Fungus SZF-02 (Omura 1985)

In the preliminary study, the effect carbon source (0.5g) and nitrogen source (0.3g) were used in basal medium for the study of antibacterial activities. Different carbon and nitrogen sources were replaced in basal medium for the production of antibacterial metabolites against *E. coli.*

Determination of Effects of pH on Fermentation by Isolated Fungus SZF-02

To determine the effect of pH for the production of secondary metabolite by isolate fungi SZF-02 in the fermentation media with different pH. The experiment was carried out individual at various pH 4, 5, 6, 7 and 8 incubations respectively. Adjustments of the pH were done by addition of hydrochloric acid and sodium hydroxide to achieve acidity and alkalinity respectively were incubated at room temperature.

Determination of Effects of temperature on Fermentation by Isolated Fungus SZF-02

The optimization temperature of fermentation medium (FM-1) of SZF-02 were carried out four different temperature viz. 21 °C, 23 °C, 25 °C and 27ºC and incubated for 7 days. Antibacterial activities were tested by using agar well diffusion assay method.

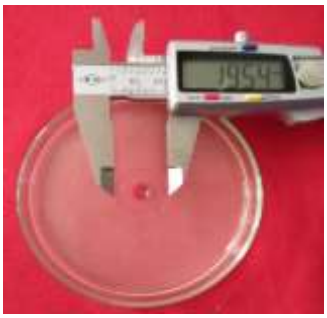
Identification of Distinguished Characters of Selected Fungus SZF-02

The morphological and microscopical characters of fungus SZF-02 was observed by the methods of Ando and Inaba, 2004, Domsch and Gams, 1993, Watanabe, 2002 and Barnett,1960 with the help of Olympus Microscope.

Results

Screening of Antibacterial Activities of Isolated Soil Fungi

In this study, total of ten fungi colony was isolated from three different soil samples. All fungal strains showed antibacterial activities against the five test organisms during the preliminary screening. Among them, soil fungus SZF-02 showed the highest antibacterial activities against *Escherichia coli* (19.54mm) during the confirmatory screening.



Fermentation periods 5 days

Agar wall = 8mm

Test organism = *E. col*

Figure 1. Antibacterial activities of selected fungus SZF-02

Effects of Different Fermentation Media of Isolated Fungi SZF-02

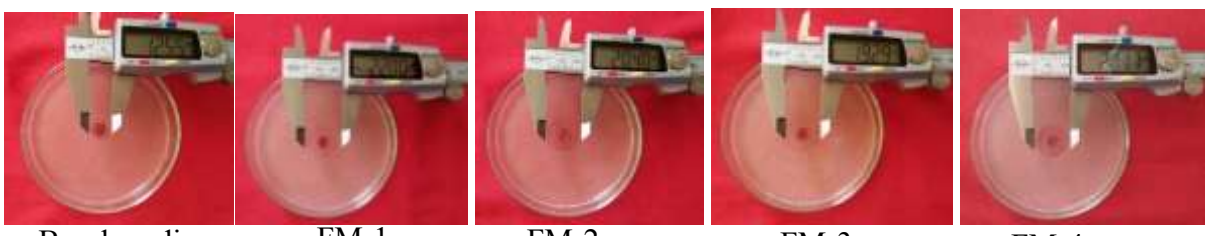
In the present study, five kinds of fermentation media were used. According to the results of antibacterial activities, fermentation medium FM-1 showed the highest inhibitory zone of (22.00 mm) in diameter.

Table 1. Basal Medium and Various Fermentation Medium of Isolated Fungus SZF-02 against *Escherichia coli*

Medium	Inhibitory zone diameter (mm)
Basal	12.55
FM-1	**22.00**
FM-2	20.40
FM-3	19.29
FM-4	20.17

Agar well= 8mm

Test organisms- *E. coli*



Basal medium FM-1 FM-2 FM-3 FM-4

Figure 2. Effect of different fermentation media of isolated fungus SZF-02

Effects of pH of Isolated Fungus SZF-02

The optimization of pH of the fermentation broth for antibacterial metabolite production was done by carrying out the fermentation at eight different pH values pH 4, 5, 6, 7 and 8, incubations. The antibacterial activities assayed by culture against test *E. coli* was found maximum at pH 5 as the inhibitory zone of 21.60 mm.

Table 2. Effect of pH on fermentation media

Isolated fungi SZF-02	
pH	**Inhibitory zone (mm) and fermentation period (5 days)**
4	17.31
5	**21. 60**
6	21.18
7	20.61
8	18.04

Agar well = 8mm

Test organism = *E. col*

pH 4 **pH 5** **pH 6** **pH 7** **pH 8**

Figure 3. Effect of pH on fermentation of SZF-02 against *E.coli*

Effect of Temperature on Fermentation of SZF-02

The optimization temperature for antibacterial metabolite production was carried out at four different incubation temperatures 21°C, 23°C, 25°C and 27°C incubated. Thus 23°C is the most suitable temperature (21.91mm) for the antibacterial metabolite production.

Table 3. Effect of incubation temperature on fermentation media

Temperature (°C)	Inhibitory zone (mm)
21	12.55
23	**21.91**
25	19.05
27	15.15

Agar well = 8mm
Test organism = *E. col*

21°C **23°C** **25°C** **27°C**

Figure 4. Effect of temperature on fermentation of SZF-02 against *E.coli*

Preliminary Study on the Morphology and Microscopical Characters of SZF-02 (Ando and Inaba, 2004)

After 5 days cultivations, it was observed that colonies on potato glucose agar medium at 25ºC. Colony is dark brown, woolly surface, reverse is dark. In microscopical characters, hyphae are septate darkly pigmented. Conidiophore are brown, erect simple or branched with bend or twisted and holding brown condia. Condia are large, porosporous, subellipsoidal, mostly 4-celled, darker brown in 2- central cells, especially curved, larger in the penultimate cells, with indistinct hilum basally.

Colony of front view Colony of reverse view Microscopical character

Figure 5. Morphological and Microscopical characters of SZF-02 on PGA medium

Discussion and Conclusion

Stanbury *et al*., 1999, also reported the quality of fermentation media is very important as it provides nutrients and energy for growth of microorganisms. In this study, ten different fungi were isolated from different soil samples. It was collected from three different places in Bye Da Yaw Village in Sagaing Township, Sagaing Region during May, 2022 by using soil dilution method (NITE, 2004).

Ten different soil fungi were isolated and their antibacterial activities were tested by using five test organisms. All fungal strains showed antibacterial activities against the five test organisms during the preliminary screening. Among them, soil fungus SZF-02 showed the highest antibacterial activities (19.54mm) on *Escherichia coli* during the confirmatory screening (Figure 1) Therefore, fermentation conditions were investigated for the production of antibacterial metabolites. This isolated fungus SZF-02 was the most promising and was selected for detailed study such as antibacterial activities, effect of different fermentation media, effect of pH and effect of various temperature. In the present study, effect of different fermentation medium with FM-1 showed distinct inhibitory zones of ingredients like each 2.0 g glucose and sucrose and polypeptone proved to be the best fermentation and showed the highest antibacterial activities (22.00mm) against *Escherichia coli* (Table 1 and Figure 2).

SZF-02 showed that, fermentation medium containing (SZF-02) pH 5 is the best for the production of antibacterial metabolite (21.60 mm) (Table 2 and Figure 3). The effect of incubation temperature 23°C showed antibacterial activity (21.91mm) (Table 3 and Figure 4) were optimized for the fermentation condition. The microscopical characters of selected fungus SZF-02 was preliminary studied. Soil fungus SZF-02 may be genus *Curvularia* sp. Based on the distinct microscopic characters of hyphae, conidiophore and conidium.

The result of antibacterial activities, different fermentation media, effect of pH and temperature on SZF-02 will be investigated for further extraction and purification of antibacterial metabolites against *E. coli.* According to Literature, the present studies on antibacterial metabolite with expectations which had the potential source to provide a therapeutic agent to the treatment and control of bacterial infections used for clinical and food industrial. Therefore, SZF-02 (*Curvularia* sp.) could be a potent source of antibacterial natural products. In this study, screening of fungus SZF-02 (*Curvularia* sp.) revealed the potential sources for further investigation will produce antibacterial metabolite.

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