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*Original Research Article*

# BIOPROSPECTING OF MICROBES PRODUCING COMMERCIALLY USEFUL PRODUCTS

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## ABSTRACT

Microbial bioprospecting is screening, isolating and identifying microbes from their natural sources which could be helpful in producing industrially useful products. It is estimated that more than 50 % of the molecules discovered in the world are from natural sources. Among these, microbes form an integral part in production of novel molecules. Although soil screening is an age old method in isolating commercially useful microbes, still it remains largely unexplored. In the present study, bioprospecting of soil samples around Udupi was carried out. Soil samples collected around various locations near Udupi were screened for microbes with antibiotic and amylolytic activities. Among the various isolates screened, KWF-2 showed significant antibiotic and amylolytic activity. Due to the promising result, further studies on characterization and optimization for increasing the yield of antibiotic and amylase are being carried out.

**Key words:** Screening, Bioprospecting, Amylase, Antibiotic.

## INTRODUCTION

Microbes practically can produce any molecule [1]. Discovery of antibiotics resulted in "Golden era" where there was a frenzied search for microbes producing antibiotics. Slowly, researchers started realizing the potential use of microbes in producing many other biomolecules. Biomolecules with anticancer properties, anti-inflammatory properties, enzymes with wide variety of pharmacological and industrial applications and number of novel molecules with newer pharmacological properties are continuously being discovered from microbes. So far thousands of biomolecules have been reported from microbes. The type of molecule which a microbe can produce and the kind of effect which these biomolecules can produce in a human is difficult to comprehend [2]. There are more than 50,000 microbial derived biomolecules discovered till date with various pharmacological activities [3]. These "small bugs" contribute significantly to the "billion dollar industry." The size of the market is only expected to increase in the future [4].

The hunt for original molecules begins with screening for microbes producing novel biomolecules. One can expect to come across a molecule with "uncommon structure and a novel target" to be produced by microbes [5]. Among the various sources, soil is considered to be the chief source for microbes. Environmental conditions play an important role in determining the type of organism found in a particular region. Therefore, depending on the geographical location, there is a huge difference in the type and characteristics of microbes found [6]. Microorganism which was once isolated from soil are continuously employed in production of number of industrially valuable products including amylase, protease and many other small molecules [7].

α- Amylases which were reported first in 1896 have number of applications including those in textile, food and paper industry [8]. Recently these enzymes have found increased acceptance for their possible role in medicinal and clinical application [8]. Among the various commercially available industrial enzymes, α- amylases occupies the top position in market share [9].

Microbes still remain the number one choice for isolation and identification of newer antibiotics. In the present study bioprospecting of soil sample around Udupi was carried out. Soil samples collected from 12 different places around Udupi were screened for microbes capable of producing antibiotic and α-amylase. In total, 40 isolates were checked for the above mentioned activities. An isolate, KWF-2 was found to be an amylolytic producing isolate with promising antimicrobial activity.

**Table.1.Industrially produced enzymes and their applications [10]**

| Name of the enzymes | Source | Applications |
|---|---|---|
| Takadiastase | *A.oryzae* | **Starch liquefying amylase** |
| | | Digestive aid. Supplement to bread. Syrup |
| Amylase | *B.subtilis* | Desizing textiles. Syrup. Alcohol fermentation industry. Glucose production |
| Acid resistance amylase | *A.niger* | Digestive aid |
| Amyloglucosidase | *Rhizopus niveus, A.niger Endomycopsis fibuliger* | **Starch saccharifying amylases** |
| | | Glucose production |
| Invertase | *S.cerevisiae* | Confectionaries, to prevent crystallization of sugar. Chocolate. High-test molasses |

## MATERIALS AND METHODS

### Chemicals and Reagents

All the dehydrated microbiological media were procured from Himedia, Fine chemicals from Merck Pvt. Ltd. Mumbai, India.

### Microorganism and cultural conditions :

### Sample collection, primary and secondary screening

Soil samples from sea shores were collected from twelve different places located around Udupi, India. Samples were collected from a depth of 5-10 cm using sterile spatula in a sterile screw capped container and was processed on the same day in the lab for primary screening. Enzymes were screened by pour plate technique after suitably diluting the samples. Crowded plate technique was adopted for antibiotic screening. Soil sample (1 g) was transferred to 100 ml sterile saline (0.85%) and incubated in an orbital shaking incubator for 15 min (27°C and 37°C for fungal and bacteria isolation respectively). At the end of incubation, soil samples were allowed to settle. Supernatant (1 mL) was aseptically transferred to 9 ml of sterile saline and serially diluted to sixth dilution. One ml from each dilution was transferred to molten nutrient agar medium (for bacteria) and Sabouraud dextrose medium (for fungi) and plated. The Nutrient agar plates were incubated at 37°C for 48 h and Sabouraud dextrose plates were incubated at 28°C for 96 h.

The bacterial and fungal isolates obtained were subcultured on nutrient and Sabouraud dextrose agar slants for further testing. Bacterial isolates were incubated at 37°C for 48 h while fungal slants were incubated at 28°C for seven days. All the isolates obtained were maintained at 4°C in refrigerator. These were tested for amylolytic activities. For antimicrobial screening, colonies showing a clear zone in

the crowded plate were selected and further tested.

For identifying potential isolates with amylolytic activity, the test isolates were inoculated on starch (1%) agar and at incubated at 37°C and 28°C for 48 h and 96 h for bacterial and fungal isolates respectively. At the end of incubation period the plates were flooded with dilute iodine solution and the diameter of clear zone, if any, around the colony was measured.

**Antibiotic testing**

For identifying potential isolates with antimicrobial activity, isolates obtained through primary screening were further tested for antimicrobial activity by agar diffusion method. Mueller Hinton agar and Sabouraud dextrose agar were used for antibacterial and antifungal testing respectively. For antibacterial assay, *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus* were used as standard microorganisms. For antifungal assay *Candida albicans* was used as standard microorganism. The isolates were grown in nutrient and Sabouraud dextrose broth. The supernatant (50 µl) from the production medium was added to each well cut on the agar plates swabbed with standard test organisms. The plates were kept in refrigerator for an hour at 4°C for diffusion to occur. The Mueller Hinton agar and Sabouraud dextrose agar plates were incubated for 24 h at 37°C and 28°C respectively. After incubation, diameter of Zone of Inhibition, if any, was measured.

Biochemical tests viz., gelatin liquefaction, carbohydrate, citrate and tyrosine utilization tests were carried out for partial characterization of the isolates. Fungal colonies were identified by gross morphological studies using lacto-phenol cotton blue stain.

**Characteristic of the colonies isolated**

All the isolates were preserved on agar slants (Nutrient agar for bacterial and Potato dextrose agar for fungal) and then subcultured regularly.

The isolates were then subjected to biological studies *viz.,* amylase activity and antimicrobial activity. The results of these studies are tabulated and given below.

**RESULTS FOR BIOLOGICAL ACTIVITIES**

**Primary screening**

Of all the isolates tested for amylase activity, six isolates showed positive result for amylase activity (Table 2).

**Table.2.Amylase activity of the isolates**

| Isolate | Amylase activity (zone of activity in cm) |
|---|---|
| FL2b | 1.0 |
| FL3a | 1.0 |
| FM1a | No activity |
| FM2 | 1.0 |
| KWF-2 | 2.5 |
| MC1 | 0.2 |
| MC2 | 0.5 |
| MNSS1 | No activity |

Among these isolates, KWF-2 showed the highest activity with clear zone of activity being 2.5 cm (Fig. 1), where as the isolate MC1 showed the least activity with a diameter of 0.2 cm.

KWF-2 which through gross morphological study was identified as yeast, showed good amylolytic activity. Therefore it was selected for further studies.


**Fig.1.Amylase activity of KWF2**

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KWF-2 showed clear zone during primary screening for antibiotic activity. Therefore, KWF-2 was further studied. The isolate was grown on Sabouraud dextrose medium and supernatant was tested for antimicrobial activity by agar diffusion method. Among all the isolates tested for antimicrobial activity,

KWF-2 showed maximum Zone of Inhibition. Among the test organisms studied, KWF-2 showed maximum activity against *Bacillus subtilis* shown in (Fig. 2). There was very little antimicrobial activity seen against other microorganisms. The standard drug used for comparison was Ciprofloxacin.

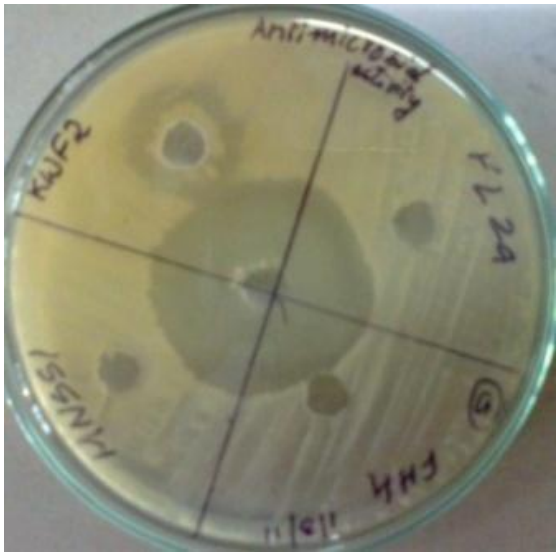


**Fig.2.Isolate KWF-2 showing antimicrobial activity against *Bacillus subtilis***

**Table.3.Diameter of Zone of Inhibition of isolate KWF-2 and Standard Ciprofloxacin (mm)**

| Isolate | Drug | Zone of Inhibition (mm) | |
|---|---|---|---|
| | | Isolate | Drug |
| KWF2 | Ciprofloxacin | 19 | 38 |

**RESULT OF BIOCHEMICAL TESTS**

Biochemical test results for KWF-2 are given below in Table 4.

**Table.4.Biochemical test for KWF-2**

| S No. | Biochemical Test | Observation |
|---|---|---|
| 1 | Gelatin Liquefaction | Positive |
| 2 | Carbohydrate utilization | Able to utilize dextrose, fructose, sucrose and maltose |
| 3 | Citrate utilization | Negative |
| 4 | Tyrosine utilization | Negative |

## DISCUSSION

Among the various isolates obtained through screening, KWF-2 was found to be a promising isolate as it showed good amylolytic activity. Yeasts are known to possess amylolytic activity. They are even capable of producing thermostable α-amylase [11]. In the present study, KWF-2 also showed antimicrobial activity against *Bacillus subtilis.* Morphological studies showed that KWF-2 could be yeast. Most of the antibiotics are obtained from bacteria including actinomycetes. Only small portion of the available antibiotics are obtained from fungi. Even among fungi, moulds including Penicillium are the main contributors. Very few reports are available on production of antibiotic by yeast. Few yeasts are reported to produce naphthaquinone antibiotics [12]. Yeast such as *Monascus purpureus* and other species are known to produce compounds such as Monascidin A and polyketide pigments. These molecules are reported to have antimicrobial properties [13,14]. The present study shows that KWF-2 is novel yeast with amylolytic and antimicrobial activities.

## CONCLUSION

Among the various isolates isolated, KWF-2 showed significant amylase activity. The isolate also showed good antibiotic activity against *Bacillus subtilis*. Currently, efforts are on to characterize the isolated organism.

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