Results
4. RESULTS

4.1. Isolation of fungi from soil samples

Soil samples were collected and more than 200 fungal isolates were recovered by different techniques and 167 isolates were characterized based on colony morphology and microscopic characteristics. The isolates belonged to the genera *Aspergillus* (*A. niger, A. flavus, A. terreus, A. fumigatus, A. nidulans, A. versicolor*), *Penicillium*, *Trichoderma*, *Fusarium*, *Cladosporium*, *Paecillomyces*, *Gliocladium*, *Scopulariopsis*, *Verticillium*, *Curvularia*, *Alternaria*, *Rhizopus* and *Mucor*. Some *Aspergillus* and *Penicillium* isolates were characterized only up to genus level.

Different techniques such as serial dilution followed by spread plate technique, soil direct plating, Warcup method and stress techniques were employed for the isolation of fungi from soil. The serial dilution technique was helpful in reducing the fungal load of the sample. Very common species of the genera namely *Aspergillus, Penicillium, Fusarium, Trichoderma, Rhizopus, Mucor, Cladosporium* and *Alternaria* were obtained by spread plate technique. Species of *Aspergillus, Penicillium, Fusarium, Trichoderma, Rhizopus, Mucor, Cladosporium, Curvularia, Paecillomyces* and *Verticillium* were isolated by soil direct plating technique. Warcup method was successful in isolating species of *Aspergillus, Penicillium, Fusarium, Trichoderma, Rhizopus, Mucor, Curvularia* and *Paecillomyces*. For the isolation of less common fungi from soil, stress techniques (high temperature and 70% alcohol) were applied and along with some isolates of *Aspergillus* and *Penicillium*, species of *Paecillomyces, Scopulariopsis* and *Gliocladium* were obtained. Some isolates were not identified as they were devoid of sporulation and
they were starch hydrolysis negative. The table 7 and plate 2 reveals the isolation techniques used and the corresponding isolates obtained.

**Table 7: Inoculation methods and the fungi isolated**

<table>
<thead>
<tr>
<th>Isolation technique</th>
<th>Fungal isolates obtained</th>
</tr>
</thead>
</table>
| Serial dilution and Spread plate technique | Aspergillus spp.  
Penicillium spp.  
Fusarium spp.  
Trichoderma spp.  
Rhizopus spp.  
Mucor spp.  
Cladosporium spp.  
Alternaria spp. |
| Soil direct plating technique           | Aspergillus spp.  
Penicillium spp.  
Fusarium spp.  
Trichoderma spp.  
Rhizopus spp.  
Mucor spp.  
Cladosporium spp.  
Curvularia spp.  
Paecillomyces spp.  
Verticillium spp. |
| Warcup method                           | Aspergillus spp.  
Penicillium spp.  
Fusarium spp.  
Trichoderma spp.  
Rhizopus spp.  
Mucor spp.  
Curvularia spp.  
Paecillomyces spp. |
| Heat pasteurization                     | Aspergillus spp.  
Penicillium spp.  
Paecillomyces spp.  
Scopulariopsis spp.  
Unidentified |
| Alcohol pasteurization                  | Paecillomyces spp.  
Scopulariopsis spp.  
Unidentified |

Fungi are very much diverse in their nutritional requirement. Hence, different media were used for the isolation of fungi from soil. The soil samples were inoculated on media *viz.* PDA, SDA, PCA and CZA amended with
Plate 2: Isolate obtained from different method

Soil direct plating

Spread plate technique

Warcup method
Chloramphenicol to prevent the growth of bacteria. The SDA and PCA media were also prepared by incorporating Rose Bengal in order to inhibit the fast growers such as *Rhizopus, Mucor* and *Trichoderma*. Different types of media used and the isolates obtained are shown in the table 8.

Table 8: Isolates obtained on different media

<table>
<thead>
<tr>
<th>Media used</th>
<th>Name of the isolates obtained</th>
</tr>
</thead>
</table>
4.2. Screening of fungal isolates for Amylase production

The fungal isolates were screened for starch hydrolysis by inoculating the isolates on starch agar plates and flooding Gram’s iodine solution on the media after incubation. The plates were observed for the clear zone (hydrolysis of starch) surrounding the colony.

4.2.1. Primary screening: More than 85% of the isolates showed the amylase production. Table 9 depicts the results of primary screening for the production of amylase. Figure 4 and 5 shows the starch hydrolysis pattern on starch agar plate by two isolates *Aspergillus* sp. MO 43 and *Aspergillus* sp. MO 199 respectively.

Table 9: Primary screening of the fungal isolates for amylase production

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Isolate</th>
<th>Names of isolates</th>
<th>Total No. of isolates</th>
<th>Starch hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Aspergillus niger</em></td>
<td>ANMO 1 to ANMO 19</td>
<td>19</td>
<td>+++</td>
</tr>
<tr>
<td>2.</td>
<td><em>A. flavus</em></td>
<td>AFLMO 1 to AFLMO 13</td>
<td>13</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td><em>A. terreus</em></td>
<td>ATMO 1 to ATMO 14</td>
<td>14</td>
<td>++</td>
</tr>
<tr>
<td>4.</td>
<td><em>A. fumigatus</em></td>
<td>AFuMO 1 to AFuMO 4</td>
<td>04</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td><em>A. nidulans</em></td>
<td>ANiMO 1 to ANiMO 3</td>
<td>03</td>
<td>++</td>
</tr>
<tr>
<td>6.</td>
<td><em>Aspergillus</em> isolate 16</td>
<td>AMO 16</td>
<td>01</td>
<td>+++</td>
</tr>
<tr>
<td>7.</td>
<td><em>Aspergillus</em> isolate 25</td>
<td>AMO 25</td>
<td>01</td>
<td>+++</td>
</tr>
<tr>
<td>8.</td>
<td><em>Aspergillus</em> isolate 43</td>
<td>AMO 43</td>
<td>01</td>
<td>+++</td>
</tr>
<tr>
<td>9.</td>
<td><em>Aspergillus</em> isolate 122</td>
<td>AMO 122</td>
<td>01</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Fungal Isolate</td>
<td>AMO/PMO Number</td>
<td>Activity</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-------------------------</td>
<td>----------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td><em>Aspergillus</em> isolate 160</td>
<td>AMO 160</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td><em>Aspergillus</em> isolate 199</td>
<td>AMO 199</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td><em>Penicillium</em> notatum</td>
<td>PNMO 1 to PNMO 6</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td><em>Penicillium</em> isolate</td>
<td>PMO 73</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td><em>Penicillium</em> isolate</td>
<td>PMO 33</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td><em>Trichoderma</em> spp.</td>
<td>TMO 1 to TMO 21</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td><em>Fusarium</em> spp.</td>
<td>FMO 1 to FMO 12</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td><em>Cladosporium</em> spp.</td>
<td>CiMO 1 to CiMO 13</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td><em>Pacillomyces</em> spp.</td>
<td>PAEMO 6</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td><em>Gliocladium</em> spp.</td>
<td>G1M0 3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td><em>Scopulariopsis</em> spp.</td>
<td>ScMO 1 and ScMO 2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td><em>Verticillium</em> spp.</td>
<td>VMO 1</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td><em>Curvularia</em> spp.</td>
<td>CuMO 1 to CuMO 6</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td><em>Alternaria</em> spp.</td>
<td>A1M0 1 to A1M0 5</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td><em>Rhizopus</em> spp.</td>
<td>RMO 1 to RMO 17</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>25.</td>
<td><em>Mucor</em> spp.</td>
<td>MuMO 1 to MuMO 8</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

(‘-’ No activity; ‘+’ activity; ‘++’ moderate activity; ‘+++’ marked activity; ‘++++’ high activity)
4.2.2. Secondary screening: Based on results of primary screening, 14 best performers that formed wider zone of hydrolysis were selected for secondary screening by surface fermentation. The amount of extracellular protein produced and the specific amylase activity of the isolates were estimated and are shown in the table 10. In the secondary screening, the production of extracellular proteins was found to be more in Aspergillus isolates compared to the isolates of other genera. In Aspergillus isolates, isolates MO 199 and MO 43 produced high extracellular protein (169±07 μg/ml and 160±04 μg/ml respectively). The Aspergillus isolates
MO 199 and MO 43 showed more specific amylase activity (4.98 and 4.93 respectively).

Table 10: Secondary screening for amylase production

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the isolate</th>
<th>Extracellular protein (µg/ml)</th>
<th>Specific activity (µmol/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td><em>Aspergillus niger</em></td>
<td>139 ± 10</td>
<td>4.08 ± 0.09</td>
</tr>
<tr>
<td>02.</td>
<td><em>A. terreus</em></td>
<td>112 ± 07</td>
<td>2.91 ± 0.10</td>
</tr>
<tr>
<td>03.</td>
<td><em>A. flavus</em></td>
<td>119 ± 09</td>
<td>3.02 ± 0.12</td>
</tr>
<tr>
<td>04.</td>
<td><em>A. oryzae</em></td>
<td>102 ± 08</td>
<td>3.27 ± 0.07</td>
</tr>
<tr>
<td>05.</td>
<td><em>A. versicolor</em></td>
<td>141 ± 09</td>
<td>3.28 ± 0.13</td>
</tr>
<tr>
<td>06.</td>
<td><em>A. fumigatus</em></td>
<td>123 ± 05</td>
<td>3.19 ± 0.16</td>
</tr>
<tr>
<td>07.</td>
<td><em>Aspergillus</em> isolate 16</td>
<td>144 ± 12</td>
<td>3.52 ± 0.05</td>
</tr>
<tr>
<td>08.</td>
<td><em>Aspergillus</em> isolate 25</td>
<td>132 ± 07</td>
<td>3.30 ± 0.11</td>
</tr>
<tr>
<td>09.</td>
<td><em>Aspergillus</em> isolate MO 43</td>
<td>160 ± 04</td>
<td>4.93 ± 0.07</td>
</tr>
<tr>
<td>10.</td>
<td><em>Aspergillus</em> isolate MO 199</td>
<td>169 ± 07</td>
<td>4.98 ± 0.06</td>
</tr>
<tr>
<td>11.</td>
<td><em>Penicillium notatum</em></td>
<td>78 ± 06</td>
<td>2.01 ± 0.03</td>
</tr>
<tr>
<td>12.</td>
<td><em>Penicillium</em> isolate 73</td>
<td>86 ± 11</td>
<td>2.95 ± 0.06</td>
</tr>
<tr>
<td>13.</td>
<td><em>Fusarium</em> sp.</td>
<td>75 ± 09</td>
<td>2.92 ± 0.10</td>
</tr>
<tr>
<td>14.</td>
<td><em>Rhizopus</em> sp.</td>
<td>58 ± 04</td>
<td>2.12 ± 0.08</td>
</tr>
</tbody>
</table>

Based on the extent of extracellular protein produced and specific amylase activity, two fungal isolates namely, *Aspergillus* isolate MO 43 and *Aspergillus* isolate MO 199 were selected for the further studies regarding the optimization of amylase production, partial purification, catalytic properties and characterization of amylases.

4.3. Characterization of selected isolates

The fungal isolates were characterized and identified based on the morphology of the colony, shape and arrangements of spore bearing structures, shape and arrangements of spores and molecular characterization.
4.3.1. Morphological and colony characteristics:

4.3.1.1. *Aspergillus* sp. MO 43

*Figure 6: Aspergillus* sp. MO 43 colonies on SDA plate

**Colony characteristics:** Colonies on SDA and PDA plates were green, cottony and rough with green colored aerial mycelia. The colony was surrounded peripherally by whitish zone. Upon prolonged incubation red colored pigmentation surrounding the colony and back pigmentation was observed. The colonies were furrowed with reddish metabolites on the colony (figure 6).

**Microscopic characteristics:** Conidiophores are smooth, columnar, colorless or slightly greenish in terminal areas. Vesicles were typically globose to subglobose and fertile over the entire surface. Sterigmata typically in two series, with primary series often much enlarged (figure 7). Conidia were echinulate.
Figure 7: Microscopic view of *Aspergillus* sp. MO 43

4.3.1.2. *Aspergillus* sp. MO 199

Figure 8: *Aspergillus* sp. MO 199 colony on SDA Plate

Figure 9: Microscopic view *Aspergillus* sp. MO 199
Colony characteristics: Colonies on SDA and PDA plates were white, cottony, with white colored aerial mycelia. Pigmentation surrounding the colony and back pigmentation were absent. White conidial heads were present (figure 8).

Microscopic characteristics: Conidiophores were smooth, colorless or slightly yellow in terminal areas. Vesicles were typically globose to subglobose and fertile over the entire surface. Sterigmata typically in two series, with primary series often much enlarged. Conidia were globose or subglobose and smooth (figure 9).

4.3.2. Molecular Characterization based on ITS (Internal Transcribed Spacers) sequencing: Aspergillus sp. MO 43 and Aspergillus sp. MO 199 were subjected for molecular identification by ITS sequencing. Figure 10 shows the PCR products of MO 43 and MO 199 on 1% agarose gel. The partial ITS nucleotide sequences of the isolates, BLAST algorithm to identify the ITS sequences with high degree of similarity and the phylogenetic tree are given below.

Figure 10: Analysis of PCR products on 1% agarose
4.3.2.1. Molecular identification of *Aspergillus* sp. MO 43

**Table 11: Partial ITS nucleotide sequences of *Aspergillus* sp. MO 43**

Assembly of ITS1 and ITS4

`cactctcggtgctaatctctgcttcgccggtctccgccccccccccaggggtttttcccggaagcacataagtttac`

`acggtcgagttggccgctgagcagccccccactctctgtaatgctcttgggtggtgggcctgtgcctgccgaaggaagtggtgctttgttctgcaggtcttgccttgcgctctctctctcccgggacggcccgacggcctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctc
ITS4_43
ACATTAGTATTTCAGGTGGGAGGGTTGGGCGCCTGGAGGCAGCCCGCACT
CAGTAATGA 116
Consensus
TCCGTAAGGGGTTCACCTGCAGAAGGCATCATTACTGAGTGCGGGCTGCCT
CCGGGCGCC 174
ITS1_43 -------------------------------GGGCGCC 7
ITS4_43
TCCGTAAGGGGTTCACCTGCAGAAGGCATCATTACTGAGTGCGGGCTGCCT
CCGGGCGCC 174
Consensus
CAACCTCCCCACCCGTAATACCTAACACTGGTTGCTTCGGCCGGGAACCCC
CTCGGGGGG 232
ITS1_43
CAACCTCCCCACCCGTGAATACCTAACACTGGTTGCTTCGGCCGGGAACCCC
CTCGGGGGG 65
ITS4_43
CAACCTCCCCACCCGTGAATACCTAACACTGGTTGCTTCGGCCGGGAACCCC
CTCGGGGGG 232
Consensus
CGAGCCGCCGGGACTACTGAACCTTCATGCCTGAGAGTGATGCAGTCTG
AGTCTGAAT 290
ITS1_43
CGAGCCGCCGGGACTACTGAACCTTCATGCCTGAGAGTGATGCAGTCTG

97
AGTCTGAAT 123
ITS4_43
CGAGCCGCGGGGACTACTGAACCTCATGCCTGAGAGTGATGCAGTCTG
AGTCTGAAT 290
Consensus
ATAAAAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGA
TGAAGAAC 348
ITS1_43
ATAAAAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGA
TGAAGAAC 181
ITS4_43
ATAAAAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGA
TGAAGAAC 348
Consensus
GCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAGTCTTTG 406
ITS1_43
GCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAGTCTTTG 239
ITS4_43
GCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAGTCTTTG 406
Consensus
AACGCACATTCGGCCCTGGCATCCGGGGGCGATGCCTGTCCGGTGCGAGCG
TCATTGCTG 464
ITS1_43
AACGCACATTGCGCCCTGGCATTTCCGGGGGCAATGCCGTTCGCAGCG
TCATTGCTG 297
ITS4_43
AACGCACATTGCGCCCTGGCATTTCCGGGGGCAATGCCGTTCGCAGCG
Consensus
CCCATCAAGCCCGGCTTGTGTTGCTTCGTCGTCACCCCCCCCGGGGGACGG
GCCCGAAA 522
ITS1_43
CCCATCAAGCCCGGCTTGTGTTGCTTCGTCGTCACCCCCCCCGGGGGACGG
GCCCGAAA 355
ITS4_43
CCCATCAAGCCCGGCTTGTGTTGCTTCGTCGTCACCCCCCCCGGGGGACGG
GCCCGAAA 522
Consensus
GGCAGCGGCGGCACCGTGTCCGGTCCTCGAGCTATGGGGCTTTGTCAC
CCGCTCGAC 580
ITS1_43
GGCAGCGGCGGCACCGTGTCCGGTCCTCGAGCTATGGGGCTTTGTCAC
CCGCTCGAC 413
ITS4_43
GGCAGCGGCGGCACCGTGTCCGGTCCTCGAGCTATGGGGCTTTGTCAC
CCGCTCGAC 580
Consensus
TAGGGCCGGCCGGCAGCCGACGTCCTCCAACCATTTTTCTTCAGGTT
GACCTCGG 638
ITS1_43
TAGGGCCGGCCGGCAGCCGACGTCCTCCAACCATTTTTCTTCAGGTT
GACCTCGG 471
ITS4_43
TAGGGCCGGCCGGCAGCCGACGTCCTCCAACCATTTTTCTTCAGGTT
GACC---- 634
Consensus
ATCAGGTAGGGATACCCGCTGAACCTTAAGCATA 671
ITS1_43
ATCAGGTAGGGATACCCGCTGAACCTTAAGCATA 504
ITS4_43
------------------------------------------
634

Sequence: ITS1_43
Sequence: ITS4_43

The table 12 represents top ten hits in NCBI BLAST for MO 43
Table 12: NCBI BLAST for *Aspergillus* MO 43 isolate

Sequences producing significant alignments:

<table>
<thead>
<tr>
<th>Accession</th>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query coverage</th>
<th>E value</th>
<th>Max ident</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM833159.1</td>
<td>Aspergillus sp. G-7 18S ribosomal RNA gene, partial sequence</td>
<td>584</td>
<td>1123</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>GQ178932.1</td>
<td>Uncultured fungus clone F19 internal transcribed spacer 1</td>
<td>584</td>
<td>1120</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>GQ198462.1</td>
<td>Aspergillus sp. N22 18S ribosomal RNA gene, partial sequence</td>
<td>584</td>
<td>1123</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>GU931440.1</td>
<td>Uncultured eukaryote clone N307T_271 18S ribosomal RNA</td>
<td>582</td>
<td>1117</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>HQ932360.1</td>
<td>Aspergillus sp. BMP3039 18S ribosomal RNA gene, internal transcribed spacer</td>
<td>582</td>
<td>1117</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>HM891179.1</td>
<td>Aspergillus sp. G-91 18S ribosomal RNA gene, partial sequence</td>
<td>582</td>
<td>1116</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>GQ122787.1</td>
<td>Aspergillus versicolor strain RF5 18S ribosomal RNA gene, partial sequence</td>
<td>582</td>
<td>1117</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>G028980.1</td>
<td>Aspergillus versicolor strain LTBF 011-1 18S ribosomal RNA</td>
<td>582</td>
<td>1117</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>G0120961.1</td>
<td>Fungal endophyte isolate 254 18S ribosomal RNA gene, partial sequence</td>
<td>582</td>
<td>1117</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FJ543807.1</td>
<td>Uncultured endophytic fungus clone R3-6 18S ribosomal RNA</td>
<td>582</td>
<td>1117</td>
<td>100%</td>
<td>0.0</td>
<td>99%</td>
</tr>
</tbody>
</table>

There is a sequence match score based on the same as seen in the table.

Figure 11: Phylogenetic tree arrangement for *Aspergillus* sp. MO 43

- Aspergillus sydowii 18S rRNA gene (partial), 1...
- Aspergillus sp. E6814a 18S ribosomal RNA gene
- Aspergillus sp. A-18 18S ribosomal RNA gene, partial sequence
- Aspergillus sydowii 18S rRNA gene (partial), 1...
- Penicillium sp. HZ-4 18S ribosomal RNA gene, partial sequence
- Aspergillus sydowii 18S rRNA gene (partial), IT5
- Aspergillus versicolor strain 4-9 18S ribosomal RNA gene
- Uncultured fungus clone F5 internal transcribed spacer
- Aspergillus sydowii 18S rRNA gene, 5.8S rRNA
- Aspergillus sydowii isolate NRRL 4768 18S ribosomal RNA gene
- Aspergillus sydowii 18S rRNA gene (partial), IT5
- Aspergillus sydowii 18S ribosomal RNA gene, partial sequence
- lcl116279
- Aspergillus sydowii strain 25 18S ribosomal RNA
According to the phylogenetic analysis, the isolate *Aspergillus* sp. MO 43 was characterized as *Aspergillus sydowii* and here onwards it is designated as *Aspergillus sydowii* MO 43. The systematic position is as follows.

**Domain:** Eukarya  
**Kingdom:** Fungi  
**Phylum:** Ascomycota  
**Class:** Eurotiomycetes  
**Order:** Euratiales  
**Family:** Trichocomaceae  
**Genus:** *Aspergillus* Michaili, 1729  
**Species:** sydowii

The nucleotide sequence of 671 bp was submitted to NCBI GenBank and Accession number was obtained (*KC442245*). The sequences were released on 26.06.2013.

### 4.3.2.2. Molecular identification of *Aspergillus* sp. MO 199

**Table 13: Partial ITS nucleotide sequences of *Aspergillus* sp. MO 199**

Assembly of ITS4 and ITS1

```
aggtcaaacctgtagaaatgttgggtgtgtgctggctggccggcgccggctggacagacgggtgacaaa
gcccccatagctcagagacggacgccggtgcgcggtgcctttgccgcccggccggtaccggggg
cgggcccagcatactgctgacagggccagcatgacgctggacaggcatgcctacagtgacatcgcctttgcggttccggcgggcgncgccgggagggg
dcgccgacagctgctgagggcaggtctggacaggcatgcccccggggaggggtaccgggg
cgggcccagcatactgctgacagggccagcatgacgctggacaggcatgcccccggggagggg
dcgccgacagctgctgagggcaggtctggacaggcatgcccccggggaggggtaccgggg
cgggcccagcatactgctgacagggccagcatgacgctggacaggcatgcccccggggagggg
dcgccgacagctgctgagggcaggtctggacaggcatgcccccggggaggggtaccgggg
cgggcccagcatactgctgacagggccagcatgacgctggacaggcatgcccccggggagggg
dcgccgacagctgctgagggcaggtctggacaggcatgcccccggggaggggtaccgggg
cgggcccagcatactgctgacagggccagcatgacgctggacaggcatgcccccggggagggg
dcgccgacagctgctgagggcaggtctggacaggcatgcccccggggaggggtaccgggg
cgggcccagcatactgctgacagggccagcatgacgctggacaggcatgcccccggggagggg
dcgccgacagctgctgagggcaggtctggacaggcatgcccccggggaggggtaccgggg
cgggcccagcatactgctgacagggccagcatgacgctggacaggcatgcccccggggagggg
```
Isolation, Purification and Characterization of Amylases from Potent Fungal Isolates by Solid State Fermentation

tgaagcccaaccccccgtgtatagctacctctgtgttctgcgcggcgcctcaaggctgcagggggtggcggcgtgcctcgcgccccggggccccggccgctgaatacaccacccagaacaggatctggaagggagaaggcggag

Consensus

GAGGTCAACCTGTAGAAAAATGGTTGGTTGCTGGGTGGCGCCGGCCGGGCCCTGCAGA 58
ITS4_199
GAGGTCAACCTGTAGAAAAATGGTTGGTTGCTGGGTGGCGCCGGCCGGGCCCTGCAGA 58
ITS1_199 ------------------------------------- 0

Consensus

GCGGGTGACAAAGCCTACGCTGTCAGGACCCGACGGCCTGGCCGCGCCGC
TGCCTTTTCG 116
ITS4_199
GCGGGTGACAAAGCCTACGCTGTCAGGACCCGACGGCCTGGCCGCGCCGC
TGCCTTTTCG 116
ITS1_199 ------------------------------------- 0

Consensus

GGCCCGTCCCCGCGGGTACCACGGGACGGGGGCCCCAACACAAGCCGTGC
TTGAGGGCA 174
ITS4_199
GGCCCGTCCCCGCGGGTACCACGGGACGGGGGCCCCAACACAAGCCGTGC
TTGAGGGCA 174
ITS1_199 -------------------------------------
GACGGGGCCCAACACACAAAGCCGCTTGAGGGCA 35
Consensus
GCAATGACGCTCGGACAGGCATGCCCCCCCAGGAATACCAGGGGCGCAAT
GTGCGTTCA 232
ITS4_199
GCAATGACGCTCGGACAGGCATGCCCCCCCAGGAATACCAGGGGCGCAAT
GTGCGTTCA 232
ITS1_199
GCAATGACGCTCGGACAGGCATGCCCCCCCAGGAATACCAGGGGCGCAAT
GTGCGTTCA 93
Consensus
AAGACTCGATGATTCACCTGAATTCTGCAATTCCACATTAGTTATCGCATT
CGCTGCCT 290
ITS4_199
AAGACTCGATGATTCACCTGAATTCTGCAATTCCACATTAGTTATCGCATT
CGCTGCCT 290
ITS1_199
AAGACTCGATGATTCACCTGAATTCTGCAATTCCACATTAGTTATCGCATT
CGCTGCCT 151
Consensus
TCTTCATCGATGCCGGAACCAGAGATCCATTGTTGAAAGTTTTGACTGA
TTGGTAAC 348
ITS4_199
TCTTCATCGATGCCGGAACCAGAGATCCATTGTTGAAAGTTTTGACTGA
TTGGTAAC 348
ITS1_199
TCTTCATCGATGCCGGAACCAAGAGATCCATTGTGTTGAAAGTTTTTGACTGA
TTGGTAAC 209
Consensus
AATCGACTCAGACTGCACTTTTCAGACAGTGTTCGTGTTGGGGTCTTCGG
CGGGCGCG 406
ITS4_199
AATCGACTCAGACTGCACTTTTCAGACAGTGTTCGTGTTGGGGTCTTCGG
CGGGCGCG 406
ITS1_199
AATCGACTCAGACTGCACTTTTCAGACAGTGTTCGTGTTGGGGTCTTCGG
CGGGCGCG 267
Consensus
GGCCCGGGGGCGCGAGGCCCCCCGGCGGCCGTGAGGCGGGCCCGCCGA
AGCAACAGGG 464
ITS4_199
GGCCCGGGGGCGCGAGGCCCCCCGGCGGCCGTGAGGCGGGCCCGCCGA
AGCAACAGGG 464
ITS1_199
GGCCCGGGGGCGCGAGGCCCCCCGGCGGCCGTGAGGCGGGCCCGCCGA
AGCAACAGGG 325
Consensus
TACGGTATACACGGGTGGGAGGTTGGGCTTCAGAGAAACCCTCACTCGG

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Isolation, Purification and Characterization of Amylases from Potent Fungal Isolates by Solid State Fermentation

TAATGATCC 522
ITS4_199
TACGGTATACACGGGTGGGAGGTTGGGTTCAGAGAAACCCTCACTCGG
TAATGATCC 522
ITS1_199 TACGGTATACACGGGTGGGAGGTTGGGCTTCAGAGAAACCCTCACTCGG

Consensus
TTCCGCAAGTTTCAGCCAACTGACGAGTGAGGGTTTCTCTGAAGCCCAAC
CTCCCACC 580
ITS4_199
TTCCGCAAGTTTCAGCCAACTGACGAGTGAGGGTTTCTCTGAAGCCCAAC
CTCCCACC 580
ITS1_199

Consensus
GTGTATACGTACCCTGTTGCTTCGGCGGGCCCGCCTCACGTCGCAGGGGG
GCTCGCGC 638
ITS4_199
GTGTATACGTACCCTGTTGCTTCGGCGGGCCCGCCTCACGTCGCAGGGGG
GCTCGCGC 638
ITS1_199

Consensus
CCCCGGGCGGCGCTGGAATACACCACCCAGAACAGGATCTGGAAAGGA
GAAGGCGGA 696
ITS4_199
Isolation, Purification and Characterization of Amylases from Potent Fungal Isolates by Solid State Fermentation

CCCGGGGCCCCGGCGCTGAATACACCACCCAGAAGGATCTGGAAAGGA
GAAGGC CGGA 696
ITSI_199 ----------------------------------- 353

Table 14 represents top ten hits in NCBI BLAST for Aspergillus sp. MO 199. There is a sequence match score based on the same as seen in the table.
Table 14: NCBI BLAST for *Aspergillus* sp. MO 199

Sequences producing significant alignments:

<table>
<thead>
<tr>
<th>Accession</th>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query coverage</th>
<th>E value</th>
<th>Max ident</th>
</tr>
</thead>
<tbody>
<tr>
<td>FJ441660.1</td>
<td>Aspergillus candidus strain MKY78 18S ribosomal RNA gene, 18S rRNA gene</td>
<td>951</td>
<td>1109</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FJ441667.1</td>
<td>Aspergillus candidus strain TJ16 18S ribosomal RNA gene, 18S rRNA gene</td>
<td>951</td>
<td>1109</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>AY739441.1</td>
<td>Aspergillus candidus strain ATCC 1002 18S ribosomal RNA gene</td>
<td>951</td>
<td>1109</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>AY779441.1</td>
<td>Aspergillus candidus strain SACC 310 18S ribosomal RNA gene</td>
<td>951</td>
<td>1109</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FJ775136.1</td>
<td>Aspergillus candidus strain SRRC 185 ribosomal RNA gene, 18S rRNA gene</td>
<td>924</td>
<td>1102</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FJ775138.1</td>
<td>Aspergillus candidus strain SRRC 185 ribosomal RNA gene, 18S rRNA gene</td>
<td>924</td>
<td>1102</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FJ775137.1</td>
<td>Aspergillus candidus strain SRRC 185 ribosomal RNA gene, 18S rRNA gene</td>
<td>924</td>
<td>1102</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FR723131.1</td>
<td>Aspergillus candidus strain ATCC 5.8S ribosomal RNA gene, 5.8S rRNA gene</td>
<td>924</td>
<td>1102</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FR723132.1</td>
<td>Aspergillus candidus strain ATCC 5.8S ribosomal RNA gene, 5.8S rRNA gene</td>
<td>924</td>
<td>1102</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FR723133.1</td>
<td>Aspergillus candidus strain ATCC 5.8S ribosomal RNA gene, 5.8S rRNA gene</td>
<td>924</td>
<td>1102</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>FR723134.1</td>
<td>Aspergillus candidus strain ATCC 5.8S ribosomal RNA gene, 5.8S rRNA gene</td>
<td>924</td>
<td>1102</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
</tbody>
</table>

Figure 12: Phylogenetic tree arrangement for *Aspergillus* sp. MO 199
As per the top 10 hits of the sequences that produced significant alignments and phylogenetic analysis, the isolate *Aspergillus* sp. MO 199 was characterized as *Aspergillus candidus* and here onwards it is designated as *Aspergillus candidus* MO 199. The systematic position is as follows.

**Systematic position:**
- **Domain:** Eukarya
- **Kingdom:** Fungi
- **Phylum:** Ascomycota
- **Class:** Eurotiomycetes
- **Order:** Euratiales
- **Family:** Trichocomaceae
- **Genus:** *Aspergillus* Michaeli, 1729
- **Species:** candidus

The nucleotide sequence of 696 bp was submitted to NCBI GenBank and Accession number was obtained ( KC430922 ). The sequences were released on 03.04.2013.

### 4.4. Optimization of amylase production parameters

Optimization of production of amylase by *Aspergillus sydowii* MO 43 and *Aspergillus candidus* MO 199 was done in triplicates varying one parameter at a time on a completely randomized design. The following parameters were optimized. Primarily the solid substrate material was optimized. The effect of ten different solid substrates (wheat bran, rice bran, ragi bran, jower flour, coconut cake, ground nut cake, cotton seed cake, sweet potato powder, cassava powder and amorphophalus powder); carbon sources (starch, glucose, fructose, Maltose, Lactose and sucrose); nitrogen sources (peptone, yeast extract, urea, ammonium chloride and ammonium
nitrate); pH of the production media (4, 5, 6, 7, 8, 9 and 10); incubation temperature (25, 32, 37, 45 and 52°C); initial starch concentration in the production media (0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 w/v); incubation period (24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours); salt concentration (0, 1, 2, 4, 6, 8 and 10% NaCl w/v); amino acids (L-glutamic acid, L-histidine, L-arginine, L-lysine, L-cysteine, L-tryptophan, L-tyrosine and DL-methionine); inoculum load (5, 10, 20, 30, 40 and 50% w/v); light (complete light, complete dark and usual incubation condition) and metal salts namely ZnSO₄ (0.001, 0.01, 0.1, 0.5, 1, 2, 3, 4 and 5%) and HgCl₂ (0.001, 0.01, 0.1, 0.5, 1, 2, 3, 4 and 5%) were optimized for amylase production.

The result is provided as Mean ± SD. Significance has been presented in the form of probability (p<0.05) values.

4.4.1. Optimization of amylase production by *Aspergillus sydowii* MO 43

4.4.1.1. Effect of solid substrate materials: Ten different solid substrates were tested for their ideal use as solid substrate material. Wheat bran showed maximum amylase activity (2.33±0.04) and was found to be the most ideal solid substrate material followed by cassava (2.28±0.05), rice bran (2.11±0.05), sweet potato (2.00±0.04) and powdered groundnut cake (2.00±0.05). Cotton seed cake showed least amylase activity (0.72±0.04). Hence, all the further optimization studies were done using wheat bran as the substrate material (Table 15; Figure 13).
Table 15: Effect of solid substrates on amylase production by A. sydowii MO 43

<table>
<thead>
<tr>
<th>Solid substrate Materials</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gds/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice Bran</td>
<td>3800</td>
<td>2.11±0.05</td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>4300</td>
<td>2.33±0.04</td>
</tr>
<tr>
<td>Ragi Bran</td>
<td>3876</td>
<td>1.78±0.05</td>
</tr>
<tr>
<td>Jower Flour</td>
<td>2200</td>
<td>1.22±0.04</td>
</tr>
<tr>
<td>Groundnut Cake</td>
<td>3600</td>
<td>2.00±0.02</td>
</tr>
<tr>
<td>Coconut Cake</td>
<td>2400</td>
<td>1.33±0.04</td>
</tr>
<tr>
<td>Cotton Seed Cake</td>
<td>1300</td>
<td>0.72±0.03</td>
</tr>
<tr>
<td>Sweet Potato</td>
<td>3600</td>
<td>2.00±0.02</td>
</tr>
<tr>
<td>Cassava</td>
<td>4100</td>
<td>2.28±0.02</td>
</tr>
<tr>
<td>Amorphoplallus</td>
<td>3300</td>
<td>1.83±0.03</td>
</tr>
</tbody>
</table>

Figure 13: Effect of solid substrate materials on amylase production by A. sydowii MO 43

4.4.1.2. Effect of carbon sources on amylase production: Carbon sources are most important for fungal growth. Wheat bran was amended with different carbon sources and their effect on amylase production was studied. Control set was kept without any added carbon sources. Table 16 and figure 14 indicate that maltose + starch followed by maltose were the ideal carbon sources. 73.8% increase in amylase
production was observed as compared to control. Glucose reduced the production of amylase probably by catabolite repression.

**Table 16: Effect of carbon sources on amylase production by A. sydowii MO 43**

<table>
<thead>
<tr>
<th>Carbon Sources</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (μmol/gds/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat Bran</td>
<td>4200</td>
<td>2.33± 0.03</td>
</tr>
<tr>
<td>Glucose</td>
<td>3400</td>
<td>1.89± 0.03</td>
</tr>
<tr>
<td>Glucose + Starch</td>
<td>5300</td>
<td>2.94±0.03</td>
</tr>
<tr>
<td>Starch</td>
<td>6980</td>
<td>3.87±0.04</td>
</tr>
<tr>
<td>Maltose</td>
<td>7240</td>
<td>4.05±0.04</td>
</tr>
<tr>
<td>Lactose</td>
<td>3750</td>
<td>2.06±0.03</td>
</tr>
<tr>
<td>Maltose + Starch</td>
<td>7600</td>
<td>4.22±0.04</td>
</tr>
</tbody>
</table>

**Figure 14: Effect of carbon sources on amylase production by A. sydowii MO 43**

4.4.1.3. **Effect of Nitrogen sources:** The effect of nitrogen sources on amylase production was studied by amending different nitrogen sources in the production medium. Among the nitrogen sources tested, *A. sydowii* MO 43 preferred inorganic nitrogen sources, ammonium chloride and ammonium nitrate with an amylase activity of 4.05 and 3.88 respectively (Table 17 and Figure 15).
Table 17: Effect of nitrogen sources on amylase production by *A. sydowii* MO

<table>
<thead>
<tr>
<th>Nitrogen Sources</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (μmol/gds/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>5800</td>
<td>3.22±0.03</td>
</tr>
<tr>
<td>Ammonium Chloride</td>
<td>7300</td>
<td>4.05±0.03</td>
</tr>
<tr>
<td>Ammonium Nitrate</td>
<td>7000</td>
<td>3.88±0.04</td>
</tr>
<tr>
<td>Peptone</td>
<td>3620</td>
<td>2.01±0.05</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>4160</td>
<td>2.31±0.03</td>
</tr>
</tbody>
</table>

Figure 15: Effect of nitrogen sources on amylase production by *A. sydowii* MO

4.4.1.4 Effect of pH on amylase production: The effect of initial pH of the production medium on amylase production was assessed by preparing the wheat bran medium with varying pH (4 to 10). Results provided in table 18 and figure 16 suggest that pH 6 was ideal (amylase activity of 4.22) for maximum amylase production. Amylase production was affected badly at alkaline pH.
Table 18: Effect of pH on amylase production by *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>pH</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gds/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2950</td>
<td>1.64±0.02</td>
</tr>
<tr>
<td>5</td>
<td>5740</td>
<td>3.18±0.02</td>
</tr>
<tr>
<td>6</td>
<td>7600</td>
<td>4.22±0.03</td>
</tr>
<tr>
<td>7</td>
<td>7200</td>
<td>3.99±0.05</td>
</tr>
<tr>
<td>8</td>
<td>6100</td>
<td>3.38±0.01</td>
</tr>
<tr>
<td>9</td>
<td>3020</td>
<td>1.67±0.01</td>
</tr>
<tr>
<td>10</td>
<td>1800</td>
<td>1.02±0.03</td>
</tr>
</tbody>
</table>

Figure 16: Effect of pH on amylase production by *A. sydowii* MO 43

4.4.1.5. Effect of incubation temperature on amylase production: The effect of incubation temperature on amylase production was found out by incubating the inoculated wheat bran medium at varied temperatures (25, 32, 37, 45 and 52°C). The results shown in table 19 and figure 17 shows that amylase production by *A. sydowii* was observed to occur at 25, 32 and 37°C and maximum production was observed at 37°C (amylase activity 3.58). The further increase in incubation temperature (45 and 52°C) drastically affected amylase production with activity 1.64 and 0.50 respectively.
Table 19: Effect of incubation temperature on amylase production by *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>Incubation of Temperature</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (μmol/gds/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>5840</td>
<td>3.24±0.04</td>
</tr>
<tr>
<td>32</td>
<td>6130</td>
<td>3.40±0.03</td>
</tr>
<tr>
<td>37</td>
<td>6460</td>
<td>3.58±0.03</td>
</tr>
<tr>
<td>45</td>
<td>2970</td>
<td>1.64±0.04</td>
</tr>
<tr>
<td>52</td>
<td>915</td>
<td>0.50±0.05</td>
</tr>
</tbody>
</table>

Figure 17: Effect of incubation temperature on amylase production by *A. sydowii* MO 43

4.4.1.6. Effect of inoculum load on amylase production: The table 20 and figure 18 shows the effect on inoculum load on amylase production. The effect of inoculum load was examined by inoculating different concentrations of inoculum (5, 10, 20, 30, 40 and 50% w/v). *A. sydowii* was able to produce amylase from 5% inoculum concentration. The further increase in inoculum load enhanced amylase production only up to 20%, where maximum amylase production was encountered (amylase activity 3.75). The further increase in inoculum load lowered amylase production where it became nearly stagnant.
Table 20: Effect of inoculum load on amylase production by *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>Inoculum load (%)</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2020</td>
<td>1.12±0.02</td>
</tr>
<tr>
<td>10</td>
<td>4800</td>
<td>2.66±0.05</td>
</tr>
<tr>
<td>20</td>
<td>6760</td>
<td>3.75±0.05</td>
</tr>
<tr>
<td>30</td>
<td>6200</td>
<td>3.44±0.05</td>
</tr>
<tr>
<td>40</td>
<td>5900</td>
<td>3.27±0.03</td>
</tr>
<tr>
<td>50</td>
<td>5830</td>
<td>3.24±0.05</td>
</tr>
</tbody>
</table>

Figure 18: Effect of inoculum load on amylase production by *A. sydowii* MO 43

4.4.1.7. Effect of incubation period on amylase production: For evaluating the effect of incubation period on amylase production, the inoculated media were incubated for varied periods from 24 hrs to 240 hrs. Amylase production gradually started from the first day and reached maximum (amylase activity 3.85) by 120 hours. With the further incubation up to 192 hrs, the amylase production got lowered and seemed to reach stagnancy. The incubation beyond 192 hrs reduced amylase production drastically (Table 21; Figure 19).
Table 21: Effect of incubation period on amylase production by *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>Incubation period in hours</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.00±0.01</td>
</tr>
<tr>
<td>24</td>
<td>990</td>
<td>0.55±0.03</td>
</tr>
<tr>
<td>48</td>
<td>2100</td>
<td>1.17±0.02</td>
</tr>
<tr>
<td>72</td>
<td>4560</td>
<td>2.53±0.05</td>
</tr>
<tr>
<td>96</td>
<td>5700</td>
<td>3.16±0.02</td>
</tr>
<tr>
<td>120</td>
<td>6940</td>
<td>3.85±0.02</td>
</tr>
<tr>
<td>144</td>
<td>6900</td>
<td>3.83±0.03</td>
</tr>
<tr>
<td>168</td>
<td>6720</td>
<td>3.73±0.04</td>
</tr>
<tr>
<td>192</td>
<td>6000</td>
<td>3.33±0.05</td>
</tr>
<tr>
<td>216</td>
<td>4220</td>
<td>2.34±0.04</td>
</tr>
<tr>
<td>240</td>
<td>3160</td>
<td>1.75±0.04</td>
</tr>
</tbody>
</table>

Figure 19: Effect of incubation period on amylase production by *A. sydowii* MO 43

### 4.4.1.8. Effect of concentration of starch on amylase production:

The effect of initial concentrations of starch in the medium on amylase production was studied by varying the concentration of starch from 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5% w/w in the media and the results are shown in the table 22 and figure 20. Enzyme production by *A. sydowii* was observed at all concentrations but maximum enzyme production (enzyme activity 4.29) was observed at starch concentration...
1.5%. The starch concentration beyond 1.5% did not increase the amylase production.

Table 22: Effect of initial starch concentration on amylase production by *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>% Starch Concentration</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>6620</td>
<td>3.67±0.04</td>
</tr>
<tr>
<td>0.50</td>
<td>6900</td>
<td>3.83±0.03</td>
</tr>
<tr>
<td>1.00</td>
<td>7650</td>
<td>4.25±0.05</td>
</tr>
<tr>
<td>1.50</td>
<td>7720</td>
<td>4.29±0.04</td>
</tr>
<tr>
<td>2.00</td>
<td>7300</td>
<td>4.05±0.02</td>
</tr>
<tr>
<td>2.50</td>
<td>7040</td>
<td>3.91±0.02</td>
</tr>
<tr>
<td>3.00</td>
<td>7000</td>
<td>3.89±0.02</td>
</tr>
<tr>
<td>3.50</td>
<td>6500</td>
<td>3.61±0.03</td>
</tr>
<tr>
<td>4.00</td>
<td>6600</td>
<td>3.66±0.04</td>
</tr>
<tr>
<td>4.50</td>
<td>6020</td>
<td>3.34±0.03</td>
</tr>
<tr>
<td>5.00</td>
<td>6000</td>
<td>3.33±0.01</td>
</tr>
</tbody>
</table>

Figure 20: Effect of initial starch concentration on amylase production by *A. sydowii* MO 43

4.4.1.9. Effect of salt concentration on amylase production: The effect of salt (NaCl) concentration on amylase production was examined by preparing the wheat bran media with varied concentrations of NaCl (0 to 10%) and the results are shown in table 23 and figure 21. *A. sydowii* showed maximum amylase activity (enzyme
activity 4.39) at 4% NaCl concentration. At 8 and 10% NaCl concentrations, the
growth as well as the amylase production reduced drastically.

Table 23: Effect of salinity concentration on amylase production by *A. sydowii*

<table>
<thead>
<tr>
<th>MO 43 NaCl Concentration ( %)</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (μmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6760</td>
<td>3.75±0.03</td>
</tr>
<tr>
<td>1</td>
<td>6980</td>
<td>3.87±0.04</td>
</tr>
<tr>
<td>2</td>
<td>7620</td>
<td>4.23±0.04</td>
</tr>
<tr>
<td>4</td>
<td>7910</td>
<td>4.39±0.05</td>
</tr>
<tr>
<td>6</td>
<td>7300</td>
<td>4.05±0.05</td>
</tr>
<tr>
<td>8</td>
<td>3970</td>
<td>2.20±0.05</td>
</tr>
<tr>
<td>10</td>
<td>230</td>
<td>0.13±0.03</td>
</tr>
</tbody>
</table>

Figure 21: Effect of salinity concentration on amylase production by *A. sydowii*

4.4.1.10. Effect of amino acids on amylase production: The effect of various
amino acids on amylase production was evaluated by incorporating 0.1% w/w
different amino acids to the wheat bran medium. As the effect is not apparent and
convincing, the experiment was also conducted by submerged fermentation.
Compared to the control where no amino acid was added, the tyrosine and
tryptophan showed maximum activity (amylase activity 0.80 and 0.59) respectively
(Table 24 and Figure 22).
Table 24: Effect of different amino acids on amylase production by A. sydowii MO 43

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSF</td>
<td>SmF</td>
</tr>
<tr>
<td>Control</td>
<td>7100</td>
<td>350</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>7100</td>
<td>960</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>6990</td>
<td>460</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>7040</td>
<td>570</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>7080</td>
<td>600</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>6050</td>
<td>640</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>7000</td>
<td>1060</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>7130</td>
<td>1440</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>6840</td>
<td>500</td>
</tr>
</tbody>
</table>

Figure 22: Effect of different amino acids on amylase production by A. sydowii MO 43

4.4.1.11: Effect of metal salts on amylase production: The effect of metal salts on the production of amylase was assessed by incorporating the different concentrations (0.001, 0.01, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0% w/v) of HgCl₂ and ZnSO₄ to the production media. The studies were done by SmF also, as the influence was not recognizable through SSF. The table 25 and figure 23 shows the influence of HgCl₂ on amylase production. In SSF, the growth and amylase production ceased only at
higher concentrations. In SmF, at a concentration of 1% HgCl$_2$ the growth was completely inhibited.

Table 25: Effect of HgCl$_2$ on amylase production by *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>Concentration of HgCl$_2$ (%)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSF</td>
</tr>
<tr>
<td>0.001%</td>
<td>4.20±0.05</td>
</tr>
<tr>
<td>0.01%</td>
<td>4.30±0.04</td>
</tr>
<tr>
<td>0.1%</td>
<td>4.10±0.03</td>
</tr>
<tr>
<td>0.5%</td>
<td>4.20±0.04</td>
</tr>
<tr>
<td>1%</td>
<td>3.90±0.01</td>
</tr>
<tr>
<td>2%</td>
<td>2.70±0.03</td>
</tr>
<tr>
<td>3%</td>
<td>1.80±0.02</td>
</tr>
<tr>
<td>4%</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td>5%</td>
<td>0.00±0.04</td>
</tr>
</tbody>
</table>

Figure 23: Effect of HgCl$_2$ on amylase production by *A. sydowii* MO 43

Similarly the result of effect of ZnSO$_4$ on amylase production is depicted in table 26 and figure 24. A concentration dependent increase in amylase production was observed with highest production (5.35) at 2% in SSF and (1.80) at 0.5% in SmF. It was also observed that the amylase productivity was negatively affected with the further increase in concentration of ZnSO$_4$. 

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Table 26: Effect of ZnSO₄ on amylase production by A. sydowii MO 43

<table>
<thead>
<tr>
<th>Concentration of ZnSO₄</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSF</td>
</tr>
<tr>
<td>0.001%</td>
<td>4.10±0.02</td>
</tr>
<tr>
<td>0.01%</td>
<td>4.55±0.02</td>
</tr>
<tr>
<td>0.1%</td>
<td>4.60±0.03</td>
</tr>
<tr>
<td>0.5%</td>
<td>5.00±0.05</td>
</tr>
<tr>
<td>1%</td>
<td>5.20±0.05</td>
</tr>
<tr>
<td>2%</td>
<td>5.30±0.02</td>
</tr>
<tr>
<td>3%</td>
<td>5.25±0.03</td>
</tr>
<tr>
<td>4%</td>
<td>2.34±0.05</td>
</tr>
<tr>
<td>5%</td>
<td>1.84±0.05</td>
</tr>
</tbody>
</table>

Figure 24: Effect of ZnSO₄ on amylase production by A. sydowii MO 43

4.4.1.12. Effect of light on amylase production: The effect of light on the production of amylase was conducted by incubating the inoculated media at three different incubation conditions namely, incubation in the presence of light, in dark and usual incubation condition. From the table 27 and figure 25, it is evident that A. sydowii produced amylase to the greater extent (amylase activity 4.42) in the presence of light, followed by incubation in the complete absence of light (amylase activity 2.34).
activity 4.20) and 12 hours light and dark incubation conditions (amylase activity 3.82).

Table 27: Effect of light on amylase production by *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Light</td>
<td>7960</td>
<td>4.42±0.04</td>
</tr>
<tr>
<td>Complete Dark</td>
<td>7560</td>
<td>4.20±0.04</td>
</tr>
<tr>
<td>Normal</td>
<td>6880</td>
<td>3.82±0.03</td>
</tr>
</tbody>
</table>

Figure 25: Effect of light on amylase production by *A. sydowii* MO 43

4.4.2. Optimization of amylase production by *Aspergillus candidus* MO 199

4.4.2.1. Effect of solid substrate materials: Ten different solid substrates were tested for their ideal use as solid substrate material. The table 28 and figure 26 shows that wheat bran showed maximum amylase activity (2.86± 0.04) and was found to be the most ideal solid substrate material followed by cassava (2. 55± 0.05) and rice bran (2.47± 0.05). Jower flour and Amorphophallus produced least activity.
Table 28: Effect of solid substrates on amylase production by *A. candidus* MO 199

<table>
<thead>
<tr>
<th>Solid substrates</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (μmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice bran</td>
<td>4476</td>
<td>2.47±0.03</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>5163</td>
<td>2.86±0.05</td>
</tr>
<tr>
<td>Ragi bran</td>
<td>3876</td>
<td>2.11±0.04</td>
</tr>
<tr>
<td>Jower flour</td>
<td>2600</td>
<td>1.44±0.04</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>4200</td>
<td>2.33±0.03</td>
</tr>
<tr>
<td>Coconut cake</td>
<td>3200</td>
<td>1.77±0.01</td>
</tr>
<tr>
<td>Cotton Seed cake</td>
<td>2900</td>
<td>1.60±0.03</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>3100</td>
<td>1.72±0.02</td>
</tr>
<tr>
<td>Cassava</td>
<td>4600</td>
<td>2.55±0.01</td>
</tr>
<tr>
<td>Amarphoplallus</td>
<td>3800</td>
<td>1.05±0.04</td>
</tr>
</tbody>
</table>

Figure 26: Effect of solid substrates on amylase production by *A. candidus* MO 199

4.4.2.2. Effect of carbon sources on amylase production: For evaluating the effect of different carbon sources on amylase production, wheat bran was amended with different carbon sources. Control set was kept without any added carbon sources. Table 29 and figure 27 indicate that glucose was ideal carbon source (with amylase activity of 4.12) for amylase production and a 43.05% increase in amylase production was observed compared to control.
Table 29: Effect of carbon sources on amylase production by *A. candidus* MO 199

<table>
<thead>
<tr>
<th>Carbon Sources</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (μmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran</td>
<td>5200</td>
<td>2.88±0.04</td>
</tr>
<tr>
<td>Glucose</td>
<td>7440</td>
<td>4.12±0.05</td>
</tr>
<tr>
<td>Glucose + Starch</td>
<td>7050</td>
<td>3.90±0.03</td>
</tr>
<tr>
<td>Starch</td>
<td>6946</td>
<td>3.85±0.02</td>
</tr>
<tr>
<td>Maltose</td>
<td>7306</td>
<td>4.05±0.02</td>
</tr>
<tr>
<td>Lactose</td>
<td>3880</td>
<td>2.15±0.05</td>
</tr>
<tr>
<td>Maltose + Starch</td>
<td>7020</td>
<td>3.89±0.03</td>
</tr>
</tbody>
</table>

Figure 27: Effect of carbon sources on amylase production by *A. candidus* MO 199

4.4.2.3. Effect of nitrogen source on amylase production: The effect of nitrogen sources on amylase production was studied by amending different nitrogen sources in the production medium. *A. candidus* preferred organic sources, yeast extract followed by peptone in particular with an amylase activity of 4.14 and 3.77 respectively. Urea found to inhibit amylase production with amylase activity of 0.62 (Table 30 and Figure 28).
Table 30: Effect of nitrogen sources on amylase production by *A. candidus* MO 199

<table>
<thead>
<tr>
<th>Nitrogen sources</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>1125</td>
<td>0.62±0.02</td>
</tr>
<tr>
<td>Ammonium Chloride</td>
<td>2350</td>
<td>1.31±0.03</td>
</tr>
<tr>
<td>Ammonium Nitrate</td>
<td>2500</td>
<td>1.38±0.03</td>
</tr>
<tr>
<td>Peptone</td>
<td>6800</td>
<td>3.77±0.04</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>7460</td>
<td>4.14±0.05</td>
</tr>
</tbody>
</table>

Figure 28: Effect of nitrogen sources on amylase production by *A. candidus* MO 199

4.4.2.4. Effect of pH on amylase production: The effect of pH of the production medium on amylase production was assessed by preparing the wheat bran medium with varying pH (4 to 10). Results provided in table 31 and figure 29 suggest *A. candidus* produced more amylase at pH 5 (amylase activity of 4.40). Amylase production was affected badly at alkaline pH.
Table 31: Effect of pH on amylase production by *A. candidus* MO 199

<table>
<thead>
<tr>
<th>pH of the medium</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7000</td>
<td>3.88±0.03</td>
</tr>
<tr>
<td>5</td>
<td>7920</td>
<td>4.40±0.03</td>
</tr>
<tr>
<td>6</td>
<td>6900</td>
<td>3.83±0.04</td>
</tr>
<tr>
<td>7</td>
<td>4030</td>
<td>2.23±0.05</td>
</tr>
<tr>
<td>8</td>
<td>3150</td>
<td>1.75±0.02</td>
</tr>
<tr>
<td>9</td>
<td>1820</td>
<td>1.01±0.05</td>
</tr>
<tr>
<td>10</td>
<td>140</td>
<td>0.07±0.01</td>
</tr>
</tbody>
</table>

Figure 29: Effect of pH on amylase production by *A. candidus* MO 199

4.4.2.5. Effect of incubation temperature on amylase production: The effect of incubation temperature on amylase production was found out by incubating the inoculated wheat bran medium at varied temperatures (25, 32, 37, 45 and 52°C). *A. candidus* was found to show maximum amylase production (amylose activity 3.69) at 25°C compared to all other incubation temperatures. Temperatures of 32 and 37°C were also found produce amylase by the organism (amylose activity 3.61 and 3.45) respectively (Table 32 and Figure 30). At incubation temperature of 52°C the amylase production was completely inhibited probably because of the evaporation of water content from the production media.
Table 32: Effect of incubation temperature on amylase production by *A. candidus* MO 199

<table>
<thead>
<tr>
<th>Incubation Temperature (°C)</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>6640</td>
<td>3.69±0.02</td>
</tr>
<tr>
<td>32</td>
<td>6500</td>
<td>3.61±0.03</td>
</tr>
<tr>
<td>37</td>
<td>6220</td>
<td>3.45±0.04</td>
</tr>
<tr>
<td>45</td>
<td>3840</td>
<td>2.13±0.02</td>
</tr>
<tr>
<td>52</td>
<td>190</td>
<td>0.11±0.05</td>
</tr>
</tbody>
</table>

Figure 30: Effect of incubation temperature on amylase production by *A. candidus* MO 199

4.4.2.6. Effect of inoculum load on amylase production: The effect of inoculum load on amylase production was examined by inoculating different concentration of inoculum (5, 10, 20, 30, 40 and 50% w/v) and the results are shown in the table 33 and figure 31. *A. candidus* was able to produce amylase from 5% inoculum concentration. The further increase in inoculum load enhanced amylase production only up to 20%, where maximum amylase production was encountered (3.72). The further increase in inoculum load lowered amylase production where it became nearly stagnant.
Table 33: Effect of inoculum load on amylase production by *A. candidus* MO 199

<table>
<thead>
<tr>
<th>Inoculum load (%)</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (μmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1600</td>
<td>0.89±0.03</td>
</tr>
<tr>
<td>10</td>
<td>2800</td>
<td>1.55±0.05</td>
</tr>
<tr>
<td>20</td>
<td>4620</td>
<td>2.56±0.02</td>
</tr>
<tr>
<td>30</td>
<td>5500</td>
<td>3.05±0.01</td>
</tr>
<tr>
<td>40</td>
<td>6700</td>
<td>3.72±0.01</td>
</tr>
<tr>
<td>50</td>
<td>6400</td>
<td>3.55±0.01</td>
</tr>
</tbody>
</table>

Figure 31: Effect of inoculum load on amylase production by *A. candidus* MO 199

4.4.2.7. Effect of incubation period on amylase production: For evaluating the effect of incubation period on amylase production, the inoculated media were incubated for varied periods from 24 hrs to 240 hrs. The results presented in the table 34 and figure 32 shows amylase production gradually started from first day and reached maximum by 168 hrs (4.07). With the further increase in incubation period, the amylase production got in to stagnancy.
<table>
<thead>
<tr>
<th>Incubation period in hours</th>
<th>Amount of Maltose liberated (µg/g)</th>
<th>Amylase activity (µmol/g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.00±0.02</td>
</tr>
<tr>
<td>24</td>
<td>720</td>
<td>0.40±0.03</td>
</tr>
<tr>
<td>48</td>
<td>1560</td>
<td>0.87±0.05</td>
</tr>
<tr>
<td>72</td>
<td>2360</td>
<td>1.31±0.01</td>
</tr>
<tr>
<td>96</td>
<td>3800</td>
<td>2.11±0.01</td>
</tr>
<tr>
<td>120</td>
<td>5620</td>
<td>3.12±0.03</td>
</tr>
<tr>
<td>144</td>
<td>7300</td>
<td>4.05±0.05</td>
</tr>
<tr>
<td>168</td>
<td>7340</td>
<td>4.07±0.05</td>
</tr>
<tr>
<td>192</td>
<td>6810</td>
<td>3.78±0.05</td>
</tr>
<tr>
<td>216</td>
<td>6610</td>
<td>3.67±0.04</td>
</tr>
<tr>
<td>240</td>
<td>6040</td>
<td>3.35±0.03</td>
</tr>
</tbody>
</table>

**Figure 32: Effect of incubation period on amylase production by *A. candidus* MO 199**

4.4.2.8. **Effect of initial starch concentration on amylase production:** The effect of initial concentrations of starch in the medium on amylase production was studied by varying the concentration of starch from 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0% w/w in the media. From the table 35 and figure 33, it can be inferred that *A. candidus* MO 199 produced maximum amylase production (amylase activity...
4.04) at 2% initial concentration. The starch concentrations below and above 2% showed decreased amylase activity.

**Table 35: Effect of initial starch concentration on amylase production by A. candidus MO 199**

<table>
<thead>
<tr>
<th>Starch Concentration (%)</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>6900</td>
<td>3.83±0.02</td>
</tr>
<tr>
<td>0.50</td>
<td>7000</td>
<td>3.89±0.03</td>
</tr>
<tr>
<td>1.00</td>
<td>7220</td>
<td>4.01±0.04</td>
</tr>
<tr>
<td>1.50</td>
<td>7200</td>
<td>4.00±0.05</td>
</tr>
<tr>
<td>2.00</td>
<td>7280</td>
<td>4.04±0.02</td>
</tr>
<tr>
<td>2.50</td>
<td>6980</td>
<td>3.87±0.03</td>
</tr>
<tr>
<td>3.00</td>
<td>5400</td>
<td>3.00±0.05</td>
</tr>
<tr>
<td>3.50</td>
<td>5800</td>
<td>3.22±0.03</td>
</tr>
<tr>
<td>4.00</td>
<td>4260</td>
<td>2.36±0.04</td>
</tr>
<tr>
<td>4.50</td>
<td>4400</td>
<td>2.44±0.04</td>
</tr>
<tr>
<td>5.00</td>
<td>4440</td>
<td>2.46±0.02</td>
</tr>
</tbody>
</table>

**Figure 33: Effect of initial starch concentration on amylase production by A. candidus MO 199**

4.4.2.9. **Effect of salt concentration on amylase production:** The effect of salt (NaCl) concentration on amylase production by A. candidus was examined by preparing the wheat bran media with varied concentrations of NaCl (0 to 10%). A. candidus showed maximum amylase activity (4.01) at 6% NaCl concentration. At 8% NaCl concentration, the amylase production reduced to a little extent and at 10%
NaCl concentration, the growth as well as the amylase production reduced drastically (Table 36 and Figure 34).

Table 36: Effect of salinity concentration on amylase production by *A. candidus* MO 199

<table>
<thead>
<tr>
<th>NaCl concentration (%)</th>
<th>Amount of Maltose liberated (μg/m/gds)</th>
<th>Amylase activity (μmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6900</td>
<td>3.83±0.02</td>
</tr>
<tr>
<td>1</td>
<td>6140</td>
<td>3.41±0.01</td>
</tr>
<tr>
<td>2</td>
<td>6900</td>
<td>3.83±0.03</td>
</tr>
<tr>
<td>4</td>
<td>6940</td>
<td>3.85±0.01</td>
</tr>
<tr>
<td>6</td>
<td>7220</td>
<td>4.01±0.005</td>
</tr>
<tr>
<td>8</td>
<td>5900</td>
<td>3.27±0.04</td>
</tr>
<tr>
<td>10</td>
<td>1490</td>
<td>0.83±0.01</td>
</tr>
</tbody>
</table>

Figure 34: Effect of salinity concentration on amylase production by *A. candidus* MO 199

4.4.2.10. Effect of amino acids on amylase production: The effect of amino acids on amylase production was tested by incorporating 0.1% of the respective amino acids to the media. The results of SSF did not show any significant difference and hence SmF was performed. Among the amino acids tested, Methionine (0.92) and Arginine (0.86) produced more amylase compared to control containing wheat bran
only (0.23). Glutamic acid showed least amylase activity (0.20) (Table 37 and Figure 35).

**Table 37: Effect of different amino acids on amylase production by *A. candidus* MO 199**

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Amount of Maltose liberated (µgm/gds)</th>
<th>Amylase activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSF</td>
<td>SmF</td>
</tr>
<tr>
<td>Control</td>
<td>6800</td>
<td>450</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>6880</td>
<td>960</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>6990</td>
<td>460</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>7900</td>
<td>570</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>7080</td>
<td>600</td>
</tr>
<tr>
<td>L-Cystein</td>
<td>6050</td>
<td>640</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>7200</td>
<td>1060</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>7130</td>
<td>1440</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>6840</td>
<td>500</td>
</tr>
</tbody>
</table>

**Figure 35: Effect of different amino acids on amylase production by *A. candidus* MO 199**

**4.4.2.11: Effect of metal salts on amylase production:** The effect of metal salts on the production of amylase was assessed by incorporating the different concentrations (0.001, 0.01, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0%) of HgCl₂ and ZnSO₄ to the production media. The studies were done by SmF also as the effect of metal salts were not recognizable in SSF. Table 38 and figure 36 depicts that the fungus was...
sensitive to HgCl₂ at higher concentration. The growth and amylase production was more in case of SSF. In SmF, the maximum activity (0.38) was observed at 0.01% HgCl₂.

Table 38: Effect of HgCl₂ on amylase production by _A. candidus_ MO 199

<table>
<thead>
<tr>
<th>Concentration of HgCl₂</th>
<th>Amylase Activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSF</td>
</tr>
<tr>
<td>0.001%</td>
<td>2.40±0.01</td>
</tr>
<tr>
<td>0.01%</td>
<td>3.30±0.04</td>
</tr>
<tr>
<td>0.1%</td>
<td>3.70±0.02</td>
</tr>
<tr>
<td>0.5%</td>
<td>3.60±0.04</td>
</tr>
<tr>
<td>1%</td>
<td>3.70±0.03</td>
</tr>
<tr>
<td>2%</td>
<td>2.90±0.03</td>
</tr>
<tr>
<td>3%</td>
<td>2.80±0.05</td>
</tr>
<tr>
<td>4%</td>
<td>2.00±0.01</td>
</tr>
<tr>
<td>5%</td>
<td>1.80±0.05</td>
</tr>
</tbody>
</table>

Figure 36: Effect of HgCl₂ on amylase production by _A. candidus_ MO 199

The effect of ZnSO₄ on amylase production is depicted in table 39 and figure 37. A concentration dependent increase in amylase production was observed with highest
production (4.84) at 2% in SSF and (1.20) at 0.5% in SmF. It was observed that the amylase productivity was observed even at 5% ZnSO₄ in SSF.

**Table 39: Effect of ZnSO₄ on amylase production by *A. candidus* MO 199**

<table>
<thead>
<tr>
<th>Concentration of ZnSO₄</th>
<th>Amylase Activity (µmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSF</td>
</tr>
<tr>
<td>0.001%</td>
<td>3.60±0.02</td>
</tr>
<tr>
<td>0.01%</td>
<td>3.40±0.05</td>
</tr>
<tr>
<td>0.1%</td>
<td>3.62±0.02</td>
</tr>
<tr>
<td>0.5%</td>
<td>4.34±0.03</td>
</tr>
<tr>
<td>1%</td>
<td>4.22±0.01</td>
</tr>
<tr>
<td>2%</td>
<td>4.84±0.04</td>
</tr>
<tr>
<td>3%</td>
<td>3.00±0.05</td>
</tr>
<tr>
<td>4%</td>
<td>2.42±0.01</td>
</tr>
<tr>
<td>5%</td>
<td>2.40±0.05</td>
</tr>
</tbody>
</table>

**Figure 37: Effect of ZnSO₄ on amylase production by *A. candidus* MO 199**

4.4.2.12. **Effect of light on amylase production:** The effect of light on the production of amylase was conducted by incubating the inoculated media at three different incubation conditions such as incubation in the presence of light, in dark and usual incubation condition. From the table 40 and figure 38, it can be inferred that the amylase production was found to be inhibited in the absence of light (3.34).
Amylase production was highest when incubated in the presence of light (3.89) and usual incubation condition (3.88).

Table 40: Effect of light on amylase production by *A. candidus* MO 199

<table>
<thead>
<tr>
<th>Incubation Conditions</th>
<th>Amount of Maltose liberated (μgm/gds)</th>
<th>Amylase activity (μmol/gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Light</td>
<td>7010</td>
<td>3.89±0.05</td>
</tr>
<tr>
<td>Complete Dark</td>
<td>6020</td>
<td>3.34±0.02</td>
</tr>
<tr>
<td>Normal</td>
<td>6990</td>
<td>3.88±0.03</td>
</tr>
</tbody>
</table>

Figure 38: Effect of light on amylase production by *A. candidus* MO 199

4.5. Optimization of Amylase activity:

The catalytic properties of amylases of *A. sydowii* and *A. candidus* were studied. The effect of temperature, pH, thermostability, pH stability, effect of incubation time, enzyme concentration and substrate concentration were the optimized parameters. All the studies were done in triplicates and the results are provided as Mean ± SD. Significance has been presented in the form of probability (p<0.05) values.
4.5.1. Effect of incubation temperature on amylase activity: The figure 39 depicts the effect of temperature on amylase activity of *A. sydowii* and *A. candidus*. Amylase of *A. sydowii* showed maximum activity at 40°C (amylase activity 18.3). The increase in incubation temperature to 50°C slightly decreased the amylase activity (17.2) and at 60°C, the amylase activity decreased drastically (2.6) because of the denaturation of the enzyme. At 70°C, the enzyme activity reached to almost nil (0.1). Amylase of *A. candidus* showed maximum activity (28.3) at 50°C and at 60°C, the activity drastically reduced to 4.99, reaching least activity at 70°C. This is essentially because of the denaturation of the enzyme.

![Figure 39: Effect of incubation temperatures on amylase activity](image)

4.5.2. Effect of pH on amylase activity: The effect of various pH on amylase activity is shown in the figure 40 which infers that the *A. sydowii* amylase showed maximum activity (10.7) at alkaline pH of 8. The enzyme showed activity at acidic pH (pH 5 and 6), neutral pH (pH 7) and alkaline ranges (pH 9 and 10) also. *A. candidus* amylase showed maximum activity at pH 5 and pH 6 (29.4 and 28.2)
respectively. The amylase showed activity at neutral and basic pH (enzyme activity 16.7, 17.2 and 8.44 at pH 7, 8 and 9 respectively).

Figure 40: Effect of pH on amylase activity

4.5.3. Temperature stability of amylases: The temperature stability profiles were studied by incubating the partially purified enzyme at respective temperatures for 1hr. The residual activity was measured and the data is presented in the figure 41 and 42. Amylase of \textit{A. sydowii} was more stable at 40°C and the stability went on decreasing with the increase in incubation temperature. At 70°C, the enzyme was completely denatured and the activity was zero. \textit{A. candidus} amylase was stable even at 50°C where it showed maximum activity. At 60°C and 70°C also the enzyme retained minimum activity.
4.5.4. pH stability of amylases: The pH stability profile was studied by incubating the partially purified amylases in buffers of different pH for 1 hr and estimating the residual activity. The figure 43 and 44 suggest that both the amylases were stable at wide pH ranges. Even after 1 hr, the normal activity was retained at pH ranges 5 to 10. Amylases of *A. sydowii* and *A. candidus* were found to be more stable at pH 6, 7, 8, and 9.
4.6.5. Effect of incubation period on amylase activity: The effect of incubation period on amylase activity was studied by incubating the enzyme and substrates for varied periods (10, 20, 30, 40, 50, 60, 70, 80 and 90 minutes). As the time of incubation increased, amylase activity also increased. Amylases of both *A. sydowii* and *A. candidus* showed maximum activities at 50 minutes of incubation. With the further incubation, the rate of amylase activity did not increase. Therefore, from the figure 45, it can be inferred that the optimum incubation period for amylase activity is 50 minutes.
4.5.6. **Effect of enzyme concentration on amylase activity**: The effect of enzyme concentration on amylase activity was investigated by adding different volumes of enzyme sample (0.1 to 1.0ml) to the constant volume of starch solution. Figure 46 shows that amylase activities of both *A. sydowii* and *A. candidus* increased gradually from 0.1 ml till 0.4 ml enzyme concentration. The amylase activity reached maximum at 0.5 ml enzyme with enzyme activity 5.0 and 5.2 in case of *A. sydowii* and *A. candidus* respectively. The further increase in enzyme concentration did not show any increment in amylase activity.
4.5.7. **Effect of substrate concentration on amylase activity**: The effect of substrate (starch) concentration on amylase activity was studied by varying the substrate concentration (1mg to 7mg starch/ml), keeping the enzyme concentration (0.5ml) constant. The figure 47 depicts the effect of substrate concentration on amylase activity and it is evident that the activity of amylases increased gradually with the increase in starch concentration. The activity of amylase of *A. sydowii* reached maximum at a starch concentration of 4 mg and the activity of amylase of *A. candidus* reached maximum at a starch concentration of 3 mg. The further increase in starch concentration however did not enhance the amylase activity in both the fungi.
The double reciprocal Lineweaver - Burke plot for amylase of *A. sydowii* MO 43 is provided in figure 48. The Km value was found to be 0.052mM (0.009/0.171).

Similarly, the double reciprocal Lineweaver - Burke plot for amylase of *A. candidus* MO 199 is provided in figure 49. The Km value for 199 is 0.11mM (0.019/0.172).
$Y = mx + C$ it is straight line equation. $K_m$ is calculated by the values of this equation shown figures 48 and 49. $K_m = C/m$ Lower the $K_m$ value more will be the affinity of enzyme towards substrate.

4.6. Partial purification of amylases:

The culture filtrate was directly used as crude enzyme. The crude enzyme was fractionated into three fractions of 40%, 70% and 95% followed by dialysis and ion exchange chromatography. The total yield during each stage of protein purification is provided in table 41. The crude culture filtrates of *A. sydowii* and *A. candidus* showed a specific activity of 2.06 and 1.944 respectively. After ion exchange chromatography the specific activities were increased to 4.42 and 4.15 respectively.
Table 41: Purification of amylases and their respective amylase activities

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample</th>
<th>Protein Concentration (µg/ml)</th>
<th>Enzyme Activity (U/ml)</th>
<th>Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A. sydowii MO 43</td>
<td>A. candidus MO 199</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Crude enzyme</td>
<td>21.50</td>
<td>44.44</td>
<td>2.066</td>
</tr>
<tr>
<td></td>
<td></td>
<td>92.62</td>
<td>114.72</td>
<td>1.238</td>
</tr>
<tr>
<td>2</td>
<td>Ammonium sulphate ppt. Enzyme</td>
<td>48.30</td>
<td>213.54</td>
<td>4.42</td>
</tr>
<tr>
<td>3</td>
<td>Ion exchange purified enzyme</td>
<td>61.22</td>
<td>254.22</td>
<td>4.15</td>
</tr>
</tbody>
</table>

Ion exchange chromatography elution profiles of *A. sydowii* MO 43 are depicted in the figure 50 which shows two peaks in protein concentration and a single peak of amylase activity.

**Figure 50: Elution Pattern of amylase of A. sydowii**

Ion exchange chromatography elution profiles of *A. candidus* MO 199 is depicted in figure 51 which shows a single peak for both protein concentration and amylase activity.
4.7. Characterization of amylases

4.7.1. Determination of Molecular Weight by SDS-PAGE: The active fraction from the ion exchange chromatography was eluted and amylases were run in electrophoresis by SDS-PAGE. After staining and destaining the gel, the approximate molecular weights of the amylases were found out by comparing the relative distance moved by the standard protein markers. According to the gel run the active fractions form the organisms *A. sydowii* MO 43 and *A. candidus* MO 199 were found to form single band of 40 kDa and 50 kDa respectively (figure. 52).
Figure 52: SDS PAGE showing separated protein fractions

Lane 1, 2 and 3- Ellutant from ion exchange chromatography – *A. sydowii MO 43*

Lane 5, 6 and 7- Ellutant from ion exchange chromatography – *A. candidus MO 199*

Lane 4- Standard protein markers

Band 1- 20 kDa
Band 2-30 kDa
Band 3- 40 kDa
Band 4- 50 kDa
Band 5- 60 kDa
Band 6-75 kDa
4.7.2. Amino acid sequencing of amylases by LC-MS/MS:

4.7.2.1. Amino acid sequencing of amylase of *Aspergillus sydowii* MO 43:

![Figure 53: SDS PAGE of the amylase of *A. sydowii* MO 43 (used for Ingel digestion)](image)

Table 42: Deduced amino acid sequence of amylase of *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>GDVIYQIIID</th>
<th>RFYDGDTTN</th>
<th>NPAKSYGLYD</th>
<th>PTKMYW</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGDLEGVRQIEEHFGNW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTFDTLVND</td>
<td>HQNGIKVIVD</td>
<td>FVPNHPFKGY</td>
<td>FHHNGDISNW</td>
</tr>
<tr>
<td>DDRYEAKWKFNSGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKSLADKLYQ</td>
<td>KKYANNS</td>
<td>GVNLDFDLN</td>
<td>TVIRNVGTTF</td>
</tr>
<tr>
<td>VNQTGNEYKY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KENLITFDN</td>
<td>HDMSRFLSVN</td>
<td>SNKANLHQAL</td>
<td>AFILTSRGTP</td>
</tr>
<tr>
<td>AGGNCPYNGR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMPAFDTTTT</td>
<td>AFKEVSTLAG</td>
<td>LRRNNAIQY</td>
<td>GTTTQRWINN</td>
</tr>
<tr>
<td>NDVVLVAINRGF</td>
<td>GGTQIQG</td>
<td>TVTFGGVTAT</td>
<td>VKSWTSNRHE</td>
</tr>
<tr>
<td>TDVKVTAGGV</td>
<td>148</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SSNLYSYNIL SGTQTSVVFT VK

**Molecular weight:**

**Results for 356 residue sequence starting "GDVIYQIIID".**

The protein weighs **40.19** kilodaltons

**User-provided sequence:**

10 20 30 40 50 60

GDVIYQIIID RFYDGDTTNN NPAKSYGLYD PTKMYWGGDL EGVRQIEEHF

GNWTTFDTLV

70 80 90 100 110 120

NDAHQNGIKV IVDFVPNHST PFKGYFHHNG DISNWDDRYE

AQWKHFNSGF SKSLADKLYQ

130 140 150 160 170 180

KKYANNSGVN VLDFDLNTVI RNVFGTFTQT MYDLNNMVNQ

TGNEYKYKEN LITFIDNHDM

190 200 210 220 230 240

SRFLSVNSNK ANLHQALAFI LTSRGTPSIY YGTEQYMAGG

NDPYNRGMMP AFDTTTTAFK

250 260 270 280 290 300

EVSTLAGLRR NNAAIQYGTT TQRWINNDVY IYERKFFNDV VLVAIRNGFG

TTQGTVTFGG

310 320 330 340 350 6

VTATVKSSTS NRIEVYVPNM AAGLTDVKVT AGGVSSNLYS

YNILSGTQTS VVFTVK

**Number of amino acids:** 356
Molecular weight: 40179.6

Table 43: Amino acid composition of the deduced sequence of *A. sydowii* MO 43

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Total No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>19</td>
<td>5.30%</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>13</td>
<td>3.70%</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>36</td>
<td>10.10%</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>23</td>
<td>6.50%</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>13</td>
<td>3.70%</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>10</td>
<td>2.80%</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>30</td>
<td>8.40%</td>
</tr>
<tr>
<td>His (H)</td>
<td>8</td>
<td>2.20%</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>19</td>
<td>5.30%</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>19</td>
<td>5.30%</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>17</td>
<td>4.80%</td>
</tr>
<tr>
<td>Met (M)</td>
<td>8</td>
<td>2.20%</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>21</td>
<td>5.90%</td>
</tr>
<tr>
<td>Pro (P)</td>
<td>8</td>
<td>2.20%</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>20</td>
<td>5.60%</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>36</td>
<td>10.10%</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>6</td>
<td>1.70%</td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>22</td>
<td>6.20%</td>
</tr>
<tr>
<td>Val (V)</td>
<td>28</td>
<td>7.90%</td>
</tr>
<tr>
<td>Pyl (O)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>Sec (U)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>(B)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>(Z)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>(X)</td>
<td>0</td>
<td>0.00%</td>
</tr>
</tbody>
</table>
Total number of negatively charged residues (Asp + Glu): 33
Total number of positively charged residues (Arg + Lys): 30
The atomic composition of the deduced sequence is as follows:

Carbon C 1801
Hydrogen H 2705
Nitrogen N 483
Oxygen O 550
Sulfur S 8

**Formula:** C1801H2705N483O550S8

**Total number of atoms:** 5547

**Extinction coefficients:**

Extinction coefficients are in units of M-1 cm-1, at 280 nm measured in water.

Ext. coefficient 65780

Abs 0.1% (=1 g/l) 1.637

**Estimated half-life:**

The N-terminal of the sequence considered is G (Gly).

**Instability index:**

The instability index (II) is computed to be 24.30

This classifies the protein as stable.

**Aliphatic index:** 69.78

**Grand average of hydropathicity (GRAVY):** -0.432

The user provided amino acid sequence was submitted to Protein Homology/analogy Recognition Engine V 2.0 (PHYRE²) bioinformatics tool so as
to get the ss report, that provide an idea of the possible secondary structure and probable tertiary structure for the sequence submitted.

**Secondary structure: ss report from Phyre2 (Plate 3 and 4):** The ss report provides the possible secondary structure of the enzyme. The prediction is 3-state: either α-helix, β-strand or coil. Green helices represent α-helices, blue arrows indicate β-strands and faint lines indicate coil. The 'SS confidence' line indicates the confidence in the prediction, with red being high confidence and blue low confidence. The 'Disorder' line contains the prediction of disordered regions in your protein and such regions are indicated by question marks (?). The weakest region of helix prediction coincides with a relatively strong prediction of disorder. Disordered regions can often by functionally very important.
Secondary structure and disorder prediction


Secondary structure: 

Disorder: ??

Confidence: 


Secondary structure: 

Disorder: ??

Confidence: 


Secondary structure: 

Disorder: ??

Confidence: 


Secondary structure: 

Disorder: ??

Confidence: 


Secondary structure: 

Disorder: ??

Confidence: 


Secondary structure: 

Disorder: ??

Confidence: 

Confidence Key:

- High (9)
- Low (0)

- Alpha helix
- Disordered

Confidence Key:
- High (9)
- Medium (6)
- Low (0)

Disorder:
- Alpha helix
- Beta strand
After Ingel digestion, the peptides subjected to LC MS/MS and peptides were identified. The peptides obtained from Ingel digestion of amylase of *A. sydowii MO 43* resulted in to 356 residue sequence starting "GDVIYQIIID. The calculated molecular weight of the sequence was 40.19kDa. Glycine was the N-terminal amino acid and Lysine was the C-terminal amino acid. The atomic composition of the deduced sequence was Carbon (C)- 1801, Hydrogen (H) 2705, Nitrogen (N) 483, Oxygen (O) 550 and Sulfur (S) 8 with total number of 5547 atoms. The deduced molecular formula was C_{1801}H_{2705}N_{483}O_{550}S_{8}. 

---

**Tertiary structure:**

*Figure 54: The possible 3D structure of amylase of *A. sydowii* MO 43*
4.7.2.2. Amino acid sequencing of amylase of *Aspergillus candidus* MO 199:

Figure 55: SDS PAGE of the amylase of *A. candidus* MO 199 (used for Ingel Digestion)
Isolation, Purification and Characterization of Amylases from Potent Fungal Isolates by Solid State Fermentation

Table 44: Deduced amino acid sequence of amylase of *A. candidus* MO 199

<table>
<thead>
<tr>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIAGMDNKSQNVMGGEKYDGLVEKMSGERNIYEAGIEKYNRLGWKRLT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VVLIVEAIALGSLISPGSFAATLMGVAGVLTVGLGFVAIYTSYVVQVKLKF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DVAHYEAGRLMFGRVGYEIVYVMLGLQLLFLTGSHC1TG1AFINITEDGV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSVFAVVSAILLFAIPPSFAEMAILGYYDFVSI1AAIL1TIM1ATGITASDSTA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLSGVNWASWPKEG1FTDAF1AVTNIVFAYSFAMCFSSFMDEMHTPKDYV</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>KSIWALG1EIVYTLTGALIYAFVGQDVQSPALLSAGSMVVRVAFGIALPV1F</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISGSINTVYLG1M1HG1FKKNSTIFN1KT1GMG1LTWLALITVFWVA1EVI</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFFS1LS1SSSLFSIFGFTFYFPA1PMWFL1VREGKW1TPK1N1MLG1ALNL1C1II</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GLV1LTG1AG1TYASVD1I1N1Y1RN1S1V1RG1V1FTC</td>
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<td></td>
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**User-provided sequence:**

<table>
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<tr>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSLSIPGSFA1TLMVAGVL1TVGLGFVAIY1TSYVVQVKL1KFPDAHYE</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGR1LMFGR1VG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>130</th>
<th>140</th>
<th>150</th>
<th>160</th>
<th>170</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEIVYVMLGLQ1LLFLTGSHC1LTG1AFIN1TEDGVCSVVF1AVVSAILL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAIPPSFAEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>190</th>
<th>200</th>
<th>210</th>
<th>220</th>
<th>230</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>AILGYVFVS1IAAILITMI1ATGITASDSTAGLS1GVNW3ASWPKEG1FTDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF1AVTNIVF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>250</th>
<th>260</th>
<th>270</th>
<th>280</th>
<th>290</th>
<th>300</th>
</tr>
</thead>
</table>
Isolation, Purification and Characterization of Amylases from Potent Fungal Isolates by Solid State Fermentation

AYSFAMCFFS FMDEMHTPKD YVKSIWALGL IEIVIYTLTG ALIYAFVGD
VQSPALLSAG
310 320 330 340 350 360
SMVKRVAFGI ALPVIFISGS INTVVLGRMI HGRIFKNSTI RFIINTEKMGWL
TWLALITVIT
370 380 390 400 410 420
WVAFVIAEVI PFFSDLSSSIS SSLFISGFIF YFPALMWFL YVREGKWNTPK
NLMLGALNLC
430 440 450 5
CLIIGLVTLG AGTYASVDDI ILNYRNGSVR GVFTC

Number of amino acids: 455

Molecular weight: 49423.3

Theoretical pI: 5.66

Amino acid composition:
Table 45: Amino acid composition of the deduced sequence of *A. candidus* MO 199

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Total No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>39</td>
<td>8.60%</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>12</td>
<td>2.60%</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>15</td>
<td>3.30%</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>14</td>
<td>3.10%</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>6</td>
<td>1.30%</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>5</td>
<td>1.10%</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>17</td>
<td>3.70%</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>43</td>
<td>9.50%</td>
</tr>
<tr>
<td>His (H)</td>
<td>4</td>
<td>0.90%</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>48</td>
<td>10.50%</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>48</td>
<td>10.50%</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>15</td>
<td>3.30%</td>
</tr>
<tr>
<td>Met (M)</td>
<td>16</td>
<td>3.50%</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>31</td>
<td>6.80%</td>
</tr>
<tr>
<td>Pro (P)</td>
<td>12</td>
<td>2.60%</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>32</td>
<td>7.00%</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>29</td>
<td>6.40%</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>9</td>
<td>2.00%</td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>16</td>
<td>3.50%</td>
</tr>
<tr>
<td>Val (V)</td>
<td>44</td>
<td>9.70%</td>
</tr>
<tr>
<td>Pyl (O)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>Sec (U)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>(B)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>(Z)</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>(X)</td>
<td>0</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

Total number of negatively charged residues (Asp + Glu): 31

Total number of positively charged residues (Arg + Lys): 27

Atomic composition:

Carbon C 2303
Hydrogen H 3583
Nitrogen N 543
Oxygen O 615
Sulfur S 22

**Formula:** $\text{C}_{2303}\text{H}_{3583}\text{N}_{543}\text{O}_{615}\text{S}_{22}$

**Total number of atoms:** 7066

**Extinction coefficients:**

Extinction coefficients are in units of M$^{-1}$ cm$^{-1}$, at 280 nm measured in water.

Ext. coefficient 73715
Abs 0.1% (=1 g/1) 1.492, assuming all pairs of Cys residues form cystines

Ext. coefficient 73340
Abs 0.1% (=1 g/1) 1.484, assuming all Cys residues are reduced

The N-terminal of the sequence considered is E (Glu).

**Instability index:**

The instability index (II) is computed to be 29.59

This classifies the protein as stable.

**Aliphatic index:** 118.90

**Grand average of hydropathicity (GRAVY):** 0.815.

**Secondary structure: ss report from Phyre2:**

**Tertiary structure**
The peptides obtained from Inge digestion of amylase of \textit{A. candidus} MO 199 resulted in to 455 residue sequence starting “EIAGMDNKSQ”. The calculated molecular weight of the sequence was 49423.3kDa. Glutamic acid was the N-terminal amino acid and Cysteine was the C-terminal amino acid. The atomic composition of the deduced sequence was Carbon (C) 2303, Hydrogen (H) 3583, Nitrogen (N) 543, Oxygen (O) 615 and Sulfur (S) 22 with total number of 5547 atoms. The deduced molecular formula was \( \text{C}_{2303}\text{H}_{3583}\text{N}_{543}\text{O}_{615}\text{S}_{22} \).