# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbreviations</td>
<td>i-iii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>iv-vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>vii</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1-8</td>
</tr>
<tr>
<td>2. Review of Literature</td>
<td>9-43</td>
</tr>
<tr>
<td>2.1. <em>Aspergillus fumigatus</em></td>
<td>9</td>
</tr>
<tr>
<td>2.2. <em>A. fumigatus</em> as a human pathogen</td>
<td>10</td>
</tr>
<tr>
<td>2.3. Types of aspergillosis</td>
<td>11</td>
</tr>
<tr>
<td>2.3.1. Allergic bronchopulmonary aspergilosis</td>
<td>11</td>
</tr>
<tr>
<td>2.3.2. Aspergilloma</td>
<td>14</td>
</tr>
<tr>
<td>2.3.3. Chronic pulmonary aspergillosis</td>
<td>15</td>
</tr>
<tr>
<td>2.3.4. Invasive aspergillosis</td>
<td>15</td>
</tr>
<tr>
<td>2.4. Pathology of aspergillosian</td>
<td>17</td>
</tr>
<tr>
<td>2.5. Pathogenic factors of <em>A. fumigatus</em></td>
<td>18</td>
</tr>
<tr>
<td>2.5.1. Thermotolerance related molecules</td>
<td>19</td>
</tr>
<tr>
<td>2.5.2. Immunomodulatory molecules</td>
<td>19</td>
</tr>
<tr>
<td>2.5.3. Molecules protective for pathogen</td>
<td>19</td>
</tr>
<tr>
<td>2.5.4. Molecules involved in nutrient uptake, signaling and metabolic regulation</td>
<td>21</td>
</tr>
<tr>
<td>2.5.5. Allergens</td>
<td>22</td>
</tr>
<tr>
<td>2.5.6. Toxins and secondary metabolites</td>
<td>22</td>
</tr>
<tr>
<td>2.6. Immunobiology of aspergillosan</td>
<td>22</td>
</tr>
<tr>
<td>2.7. Methods for diagnosis of aspergillosis</td>
<td>23</td>
</tr>
<tr>
<td>2.7.1. Clinical diagnosis</td>
<td>24</td>
</tr>
<tr>
<td>2.7.2. Laboratory investigations</td>
<td>25</td>
</tr>
<tr>
<td>2.8. Therapy for aspergillosian</td>
<td>34</td>
</tr>
<tr>
<td>2.8.1. Chemotherapy</td>
<td>34</td>
</tr>
<tr>
<td>2.8.2. Immunotherapy</td>
<td>37</td>
</tr>
<tr>
<td>2.9. Need of novel allergens/antigens of <em>A. fumigatus</em></td>
<td>40</td>
</tr>
</tbody>
</table>
3. Materials and Methods

3.1. Pathogens

3.1.1. A. fumigatus strains
3.1.2. Culture of A. fumigatus

3.2. Antigen preparation

3.2.1. Preparation of A. fumigatus secreted proteins
3.2.2. Preparation of A. fumigatus conidial-hyphal-cytosolic proteins
3.2.3. Protein estimation

3.3. Recruitment of subjects

3.3.1. Subjects
3.3.2. Collection and analysis of blood samples

3.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

3.4.1. Gel casting
3.4.2. Electrophoresis
3.4.3. Commassie staining

3.5. Immunoblotting

3.6. Development of proteomes and immunoproteomes of A. fumigatus

3.6.1. Two dimensional gel electrophoresis
3.6.2. Silver staining
3.6.3. The 2DE Western blotting

3.7. Identification of immunoreactive proteins

3.7.1. Matching of immunoreactive spots
3.7.2. Enzymatic digestion of proteins
3.7.3. Protein identification by Q-TOF analysis

3.8. Characterization of immunoreactive proteins

3.8.1. Functional annotation and prediction of antigenic index
3.8.2. Prediction of signal peptide and cellular localization
3.9. Recombinant production of immunogenic proteins 58
3.9.1. Targets for cloning 59
3.9.2. Primer designing and synthesis 59
3.9.3. Preparation of cDNA of *A. fumigatus* 60
3.9.4. Amplification of targeted genes 61
3.9.5. Agarose gel electrophoresis 62
3.9.6. DNA elution from agarose gel 63
3.9.7. Sequencing 63
3.9.8. Transformation 63
3.9.9. Vector plasmid preparation 64
3.9.10. Restriction digestion of plasmid vectors and PCR insert 65
3.9.11. Ligation of vector plasmids and PCR inserts 66
3.9.12. Confirmation of positive clones 68
3.9.13. Induction of recombinant proteins 70
3.9.14. Purification of recombinant proteins 70
3.10. Immunological characterization of recombinants 71

4. **Results and Discussion** 72-131
4.1. Culture of pathogenic strains of *A. fumigatus* 72
4.2. Clinical observations on the subjects 74
   4.2.1. Asthma 74
   4.2.2. Skin prick test 74
   4.2.3. Radiological observations 75
   4.2.4. Serum immunoglobulins 76
4.3. Antigen profile of *A. fumigatus* on SDS gel 78
4.4. Immunoblotting 80
4.5. Proteome of *A. fumigatus* 82
   4.5.1. Secreted proteome of *A. fumigatus* 82
   4.5.2. Cytosolic proteome of *A. fumigatus* 84
4.6. Immunoproteome of *A. fumigatus* 86
   4.6.1. Secreted immunoproteome of *A. fumigatus* 86
   4.6.2. Cytosolic immunoproteome of *A. fumigatus* 89

4.7. Marking of immunoreactive spots 92

4.8. Mass spectrometric based characterization 92
   4.8.1. Identification of immunoreactive secreted proteins 92
   4.8.2. Identification of CHC immunogenic proteins 101

4.9. Selection of molecules for cloning 113
   4.9.1. The 2DE immunobloting of secreted fraction with sera of individual ABPA patients 113
   4.9.2. The 2DE immunobloting of CHC fraction with sera of individual ABPA patients 118
   4.9.3. Target molecules of *A. fumigatus* for cloning 121

4.10. Cloning and recombinant production of selected immunoreactive proteins 122
   4.10.1. PCR amplification and cloning of target proteins 122
   4.10.2. Recombinant expression of proteins 125

4.11. Immunological characterization of the recombinant proteins 129

5. Summary and Conclusions 132-138

6. References 139-159

Credentials