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## Research Article *In silico* Structural Modelling and Comparative Analysis of β-mannanases from Psychrophilic and Mesophilic *Arthrobacter* sp.

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### Abstract

**Background and Objective:** Endo-1,4-β-mannanase (β-mannanase, EC 3.2.1.78) is an industrially important enzyme which catalyses the hydrolysis of mannan-based polysaccharides. This enzyme is produced by psychrophilic and mesophilic groups of *Arthrobacters*p., yet none of them were structurally characterized. This study aims to decipher the structural features of *Arthrobacter*β-mannanases that might responsible for their cold adaptation. **Material and Methods:** Thirty amino acid sequences encoding β-mannanases from *Arthrobacter* sp. were retrieved from GenBank and subjected to series of analysis of amino acid profiling and structural homology modelling using Phyre2 and SWISS-MODEL. **Results:** Structural alignment showed the catalytic residues (2 glutamic acids) were conserved among β-mannanase suggesting that they might shares catalytic mechanism. Psychrophilic β-mannanases showed remarkable differences from the mesophilic ones in the content of hydrophilic, particularly negatively charged, residues and proline, which were thought to be important for its cold adaptation. Three-dimensional model of all *Arthrobacter* β-mannanases forms a classic α/β barrel motif consisting of 8 helices and 9 β-sheets structures, except for psychrophilic ones, which having 8 helices and 8 β-sheets. **Conclusion:** Adaptation of *Arthrobacter* β-mannanases towards cold temperature involves structural adjustment particularly on structural flexibility and amino acid distribution.

Key words: β-mannanase, mannan, Arthrobacter sp., structural modelling, cold-adaptation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

β-mannanase or specifically endo-1,4-β-mannanase (EC 3.2.1.78) is an enzyme belonging to the glycosyl hydrolase (GH) families that catalyses the hydrolysis of β-D-1,4mannosidic linkages in mannan based polysaccharides, leaving shorter chains of oligosaccharides<sup>1-3</sup>. These enzymes offer many applications, has many demands in various industries and is produced by many microorganisms in extracellular forms<sup>3,4</sup>. Arthrobacter sp. is one of the bacterial species known to produce β-mannanases and is widely found in soil and extreme contaminated environments including the Antarctic region<sup>5-8</sup>. This group has been found to have a growing temperature of 0-45°C which makes this bacteria to be either categorised as psychrophilic or mesophilic group<sup>9,10</sup>. GenBank houses at least 30 amino acid sequences of β-mannanases from Arthrobacter sp. which have been isolated from psychrophilic and moderate mesophilic Arthobacter sp. Nevertheless, to our knowledge, none of β-mannanases of Arthrobacter sp. has been structurally studied extensively so far. While a number β-mannanases structures were deposited in protein data bank (PDB), none of them originated from Arthobactersp. In addition, there are no inclusive reports on psychrophilic β-mannanases from any organism.

Structural studies of psychrophilic enzymes gain wide interest as the mechanisms by which the enzymes adapt to low temperature are diverse. Particularly, the cold adaptation mechanism of psychrophilic β-mannanases remains poorly understood. X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy are undoubtedly powerful tools to decipher the protein structures at atomic level. However, the technologies are costly and tedious<sup>11,12</sup>. On the other hand, it is important to note that amino acid sequence of proteins dictate the structural properties of the protein<sup>13</sup>. Furthermore, the amino acid sequence stores the information required for the determination and characterization of a protein molecule's functions, physical and chemical properties<sup>14</sup>. Accordingly, harnessing the amino acid sequence through a series of various platforms of computational biology (in silico) analysis is considerably a viable tool as an alternative over other costly technologies. In silico structural analysis, structural homology modelling is known to be getting more popular within this decade. This is due to the sigmoidal growth of deposited experimental data in the protein data bank (PDB) and a rapid progress of computation technology. The availability of experimental data at PDB provides various structural templates for the structural modelling with better accuracy<sup>15</sup>.

In this study, structural features of *Arthobacter*  $\beta$ -mannanases isolated from various environments were analysed using various *in silico* platforms. This involves amino acid composition, chemical properties and three-dimensional structural models of these  $\beta$ -mannanases. The comparative analysis between mesophilic and psychrophilic *Arthobacter*  $\beta$ -mannanases was then used as a basis to discuss the cold adaptation mechanism of this enzyme.

#### **MATERIALS AND METHODS**

Sequence and structural retrieval: The amino acid sequences of 30 mannan endo-1,4- $\beta$ -mannosidase ( $\beta$ -mannanase) protein was retrieved from the GenBank (https://www. ncbi.nlm.nih.gov/genbank/). The study was conducted at Biotechnology Research Institute, Universiti Malaysia Sabah, Malaysia, for the period of October, 2018-March, 2019. For comparison, previously reported  $\beta$ -mannanases were included in this study including CfMan26A (*Cellulomonas fimi*), MeMan5A (*Mytilus edulis*) and TrMan5A (*Trichoderma reesei*). The complete list of organisms used in this study is shown in Table 1.

The POWER web interface (http://power.nhri.org.tw/) was connected for phylogenetic tree development and investigation in light of the Dayhoff-PAM method using the amino acid sequence of the proteins<sup>16</sup>. The Kitsch (http:// caps.ncbs.res.in/iws/phylip\_files/kitsch.html) program (Fitch-Margoliash slightest squares strategy) was utilized for investigation of phylogenetic tree<sup>17</sup>. The unwavering quality of the assessed trees was assessed by using the Bootstrap strategy with 1000 replications.

**Primary structural analysis and secondary structural analysis:** ExPasy (Expert Protein Analysis System) ProtParam server<sup>18</sup> has been 100 utilized for physiochemical characterization of the β-mannanases. These parameters include theoretical isoelectric points (pl), molecular weights, the total number of positive and negative residues, instability index, aliphatic index and the grand average hydropathy (GRAVY)<sup>19-21</sup>. PSIPRED was used for secondary structure analysis of the proteins<sup>22</sup>. It is a server that uses the principle of 2 feeds which are the forward and neural networks.

**Analysis of conserved catalytic residues:** Analysis of the sequences was conducted using Clustal Omega-MSA for obtaining pairwise distance<sup>23,24</sup>. The catalytic residues of the  $\beta$ -mannanases were determined using sequence alignment

| Table 1: List of Arthobacter strains producing | β-mannanases used in this study |
|--|---------------------------------|
|--|---------------------------------|

| Strains                              | Codes | Accession numbers (GenBank) | Growing temperature (°C)/type | References               |
|--------------------------------------|-------|-----------------------------|-------------------------------|--------------------------|
| 135MFCol5.1                          | A1    | WP_018762220.1              | N/A                           |                          |
| 162MFSha1.1                          | A2    | WP_026265823.1              | N/A                           |                          |
| 31Cvi3.1E                            | A3    | SKB67575.1                  | N/A                           |                          |
| 49Tsu3.1M3                           | A4    | SKB72436.1                  | N/A                           |                          |
| Agilis                               | A5    | WP_087028622.1              | 20-30/Mesophile               | JGI                      |
| Br18                                 | A6    | WP_052274099.1              | 15/Psychrophile               | JGI                      |
| Enclensis                            | A7    | KSU78669.1                  | 30/Mesophile                  | Dastager <i>et al.</i> , |
| EPSL27                               | A8    | KUM33307.1                  | N/A                           |                          |
| FB24                                 | A9    | WP_043430353.1              | Mesophile                     | JGI                      |
| Globiformis                          | A10   | WP_003803021.1              | Psychrophilic                 | Berger <i>et al.</i> ,   |
| L77                                  | A11   | WP_052274099.1              | Psychrophile                  | Singh <i>et al.,</i>     |
| Leaf137                              | A12   | WP_056075972.1              | Mesophile                     | JGI                      |
| Leaf141                              | A13   | WP_056596147.1              | N/A                           |                          |
| Leaf234                              | A14   | WP_055769075.1              | Mesophile                     | JGI                      |
| Leaf337                              | A15   | WP_055797900.1              | Mesophile                     | JGI                      |
| Luteus                               | A16   | AQQ16388.1                  | N/A                           |                          |
| Nitrophenolicus                      | A17   | ELT45426.1                  | 30/Mesophile                  | Arora and Jain           |
| OV608                                | A18   | WP_091416456.1              | N/A                           |                          |
| OY3WO11                              | A19   | WP_066280927.1              | 22/Mesophile                  | Town <i>et al.</i> ,     |
| P2b                                  | A20   | WP_079596712.1              | N/A                           |                          |
| Pseudarthrobacter chlorophenolicus   | A21   | WP_015938113.1              | Mesophile                     | JGI                      |
| Pseudarthrobacter phenanthrenivorans | A22   | WP_052259887.1              | 30/Mesophile                  | JGI                      |
| Pseudarthrobacter sulfonivorans      | A23   | WP_058932997.1              | Psychrophilic                 | Zhang <i>et al.</i> ,    |
| RIT-PI-e                             | A24   | WP_049830849.1              | Mesophile                     | Tran <i>et al</i> .,     |
| Soil761                              | A25   | KRE76147.1                  | Mesophile                     | JGI                      |
| Soil764                              | A26   | WP_056329268.1              | Mesophile                     | JGI                      |
| SPG23                                | A27   | WP_043479121.1              | Mesophile                     | Gkorezis <i>et al.</i> , |
| U41                                  | A28   | WP_069949883.1              | N/A                           |                          |
| UNC362MFTsu5.1                       | A29   | WP_043439180.1              | Mesophile                     | JGI                      |
| ZBGIO                                | A30   | WP_050683930.1              | Mesophile                     | JGI                      |

from 3 reported and well-studied  $\beta$ -mannanases from *Cellulomonas fimi, Mytilus edulis* and *Trichoderma reesei.* The active sites of those  $\beta$ -mannanases (E175/E282, E177/E308 and E169/E276, for *Cellulomonas fimi, Mytilus edulis* and *Trichoderma reesei*, respectively) were used as references<sup>25-27</sup>.

**Homology protein modelling:** The modelling of the 3D structure of the β-mannanases was performed using SWISS-MODEL and Phyre2 servers<sup>28</sup> and displayed using PyMoL software<sup>29</sup>. The models were then evaluated by Verify 3D (http://toolkit.tuebingen.mpg.de/modeller/verity3d/), the Ramachandran plot using RAMPAGE (mordred.bioc.cam.ac. uk/~rapper/rampage.php), GMQE (Global Model Quality Estimation) and QMEAN (the Qualitative Model Energy Analysis) scores<sup>30</sup>. The B-factor of each protein was calculated from the ResQ server (http://zhanglab.ccmb.med.umich. edu/)<sup>31</sup>.

#### RESULTS

**Arthrobacter** producing  $\beta$ -mannanases: Table 1 showed the 30 *Arthrobacter* strains producing 1,4- $\beta$ -D mannanase from various environments including soil, water and plants. Based

on their growth temperatures, 4 strains were identified as psychrophilic bacteria while 16 strains were classified as mesophilic bacteria. In addition, the optimum growth temperatures for the rest of 10 strains are unknown. Phylogenetic analysis of the thirty  $\beta$ -mannanases and 3 of the control was shown in Fig. 1. The distance between *Arthrobacter*  $\beta$ -mannanases were relatively close to each other, exceptionally with the three controls and A16 which is a slightly different species as mentioned previously.

**Primary structure profile:** Primary structure analysis showed that all  $\beta$ -mannanases from these bacteria were multi-domain proteins with a catalytic domain as a structural region responsible for the catalytic activity (Fig. 2). Amino acid sequences of these  $\beta$ -mannanases were found to have low similarities to the well-studied  $\beta$ -mannanases: TriMan5A, CFMan26, MeMAN5A (Table 2). Interestingly, the similarities among *Arthrobacter*  $\beta$ -mannanases also varied from 17.67-87.95%. Further analysis of the amino acid sequences shown in Fig. 3 revealed that the theoretical sizes and isoelectric point (pl) of the full-length were higher than the catalytic domain. Further, the instability index values, the aliphatic index and the grand average of hydrophobicity

(GRAVY) of the full-length of *Arthrobacter*  $\beta$ -mannanases were also remarkably higher than that of the catalytic domain.

**Amino acid profile:** Figure 4 revealed that the full-length of *Arthrobacter* β-mannanases were dominated by hydrophobic

residues followed by neutral, hydrophilic, charged and pro residues. Similar patterns were also found in the catalytic domain. Nevertheless, the hydrophilic residues were found to be higher than the neutral residues. Overall, the full-length had remarkable higher hydrophobic, neutral and proline



Fig. 1: Phylogenetic tree analysis among Arthrobacter β-mannanases and 3 controls (highlighted in red boxes)



Fig. 2: Continue



Fig. 2: Continue

| The second that are been been been been been been been be  | 1 2 2   | THE MENT OF THE THE PARTY OF TH   | 2 4 4   |
|--|---|--|---|
| CFMan26  | 166   | IS TRANSPORTATION A Y-GSEE NOLL BETWY Y ROUNDY NEL ANGE  | 219   |
| MeMANSA  | 119   | IL FFTLWN AVNOSTHERLN LNE DTRKLOST DHAL FEANA KNEW LGGWDIN NEF   | 178   |
| AL   | 223   | RPVELRFAILMING NWY PWAEGVIGNOFGDYVRA WRHVHDVVA ATGRANVCWVWSP   | 277   |
| AZ   | 231   | NPV LRIAL MNG - NWY PWAEGVNGNOEGDYVAA WRHVHDVVA ATGAANV WVWSP  | 285   |
| A3<br>A4   | 164   | THE REAL SHE   | 212   |
| 85   | 225   | CPVMLBTAN MNG  | 279   |
| AG   | 228   | IPVNLRIGHIMNGNWY-PWEEGENGNEEGDYVER-WENVHDVVR-STGRENESWVWDP   | 282   |
| A7   | 231   | YPVCLRFANIMIG-DWY PWAISVNGNCEGDYVCA WRHVHDVVE ACGAINVWWVWSP  | 285   |
| AB   | 225   | HPVMLRTENTMNGNWY-PWAEGINGNEFGCLARA-WRNVHDVVA-ATGAIN TWVWNP   | 279   |
| 210  | 253   | CPVCLRPATIANG NW PWARONGNE GDPVCA WRIVIDVVA ATGA NV WVW P  | 313   |
| A11  | 201   | HPY HE FALL NIG - LWY - PWARGVIGHT AGDY AR - CHVHDVVA - STGALNE WVMNP  | 255   |
| A12  | 230   | YPVCLRFANIMNG-EWY-PWAIEVNGNC-GDYACA-WRNVHDVVE-ACGAINVEWVWSP  | 284   |
| A13  | 230   | KPVELRIAIIMNGNWY-PWAEGVNENCAGDYVEA-WRNVNDVVA-ACGAANVCWVWEP   | 284   |
| A14  | 201   | RPVYLRIENIMNGDWY-PWSEGVNGNGAGDYVAA-NRNVHDVVR-STGATNVSWVWNP   | 255   |
| A15  | 219   | OPVOLATIAL ING - NW - PWATEVNENS FEDEVICE - WRITINGVVA - ANGALINAWWEF  | 273   |
| A17  | 225   | NPVELETAL MNG-NWY-PWSCGVNGNCHGDY EA-WONVHELVA-BAGACNVCWVWSP  | 279   |
| A18  | 224   | YPVELRIAN MNG-NWY-PWAEGVNGNGAGDYVCA-WRNVHDVVA-ATGAENVEWVWSP  | 278   |
| A19  | 238   | HPUELRIEIIIMNG-NWY-PWEECVNGNGEGDYVAA-WCHVHEVVE-ARGAENEEWVWEP   | 292   |
| A20  | 229   | YPECLRFANIMNGDWY PWAEGVNGNEAGDYVCA WRNVNDVER ATGAENVEWVWSP   | 283   |
| A21  | 232   | TPUCLEFAILMING - EWY - PWARE VNGNO EGDY VCA - WENVNDVVE - AGGAEN VEWVWS P  | 286   |
| A23  | 256   | NPVELREAL MNG NWY PWARSANGNGAGDYVAA WRIVHDVVA ATGAENVWEWSP   | 310   |
| A24  | 236   | CPVTLRIENMNG   | 290   |
| A25  | 231   | NPVELRIANIMNGNWY-PWAEGVNGNOEGDYVAA-WRHVHDVVA ATGAANVOWVMSP   | 285   |
| A20  | 211   | 2 PUCKETAL MING - INV - PWAR VNGNO SGDYAAR WRIVIND VA AGGAGIN V WVWB P   | 265   |
| A28  | 238   | MPV LETER MNG NY PWARGYNGNGEGDYARA WENVEDVIR - TGAENVEWYNNP  | 292   |
| A29  | 219   | CPVELRFANIMNG NWY PWAEGVNGNOEGDYVCA WRNVHDVVA ATGAENVEWVWSP  | 273   |
| DEA  | 230   | xpvolrfaningnwy-pwaegvnoncagdyvse-wrnvhdvva-acgaanvcwvwsp  | 284   |
|  |   |  |   |
| TriMansa   | 167   | GNEPRCVKSLD  | 1.95  |
| CFMan26  | 220   | GGGFG  | 225   |
| MeMANSA  | 179   | EGEIKPGESSSEPCFDTRNLSGSGAGWAGNLYSAQEIGRF <u>VNNQ</u> AAAIKEVD  | 230   |
| AL   | 278   | NV   | 283   |
| A2   | 286   |  | 291   |
| 84   | 281   | NW STATE GOFAARPGTPDFAALD  | 286   |
| AS   | 280   |  | 285   |
| AS   | 283   | NV   | 288   |
| A7   | 286   |  | 291   |
| AB   | 280   |  | 285   |
| A10  | 306   |  | 311   |
| A11  | 256   | SV   | 261   |
| A12  | 285   | NV   | 290   |
| ALS  | 285   | NM   | 290   |
| D15  | 274   |  | 279   |
| A16  | 282   | NGSGVEYLDMV  | 310   |
| A17  | 280   | <u>₩</u>   | 285   |
| A18  | 279   | NM   | 284   |
| A19  | 284   |  | 289   |
| A21  | 287   | NV   | 292   |
| A22  | 283   | NV   | 288   |
| A23  | 311   |  | 316   |
| A24  | 291   | NMPM2C   | 296   |
| 826  | 282   |  | 231   |
|  |   |  | × D /   |
|  |   |  | 207   |
| TriManha   | 196   |  | 207   |
| TriMan5A<br>CFMan26  | 196   |  | 229   |
| TriMan5A<br>CFMan26<br>MeMAN5A   | 196<br>226<br>231   | SN   | 229<br>244<br>268   |
| TriMan5A<br>CFMan26<br>MeMAN5A<br>Al   | 196<br>226<br>231<br>284  | SN   | 229<br>244<br>268<br>301  |
| TriMan5A<br>CFMan26<br>MaMAN5A<br>A1<br>A2<br>22   | 196<br>226<br>231<br>284<br>292   | SN   | 229<br>244<br>268<br>301<br>309   |
| TriMan5A<br>CFMan26<br>MeMAN5A<br>A1<br>A2<br>A3<br>A4   | 196<br>226<br>231<br>284<br>292<br>239<br>287   | DN   | 229<br>244<br>268<br>301<br>309<br>266<br>304   |
| TriMan5A<br>CFMan26<br>MeMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5   | 196<br>226<br>231<br>284<br>292<br>287<br>287<br>286  | IL TI GDEGLGLSTGDGAY PYTYGEGTDFAKN<br>NRDVYLRTYFC DI VIJ<br>PGANVTVGSWNMKADTDANGFMINISDHCLVKAGSKOS<br>II TACIN FGAVYO<br>DGTLTM SDDYA FYN FGAVYO<br>TDN DGTLTM SDDYA FYN FGAVYO<br>II FF COVYC SI VY   | 229<br>244<br>268<br>301<br>309<br>246<br>309<br>206<br>304   |
| TriMan5A<br>CFMan26<br>MeMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A6   | 196<br>2231<br>2234<br>2239<br>2239<br>2239<br>228<br>228<br>228<br>228<br>228<br>228<br>228<br>228<br>228<br>22  | SN   | 2244<br>2244<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>20   |
| TriMan5A<br>CFMan26<br>MeMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A6<br>A5<br>A6<br>A7   | 196<br>2234<br>2234<br>2239<br>2282<br>2289<br>2289<br>2289<br>2289<br>2289<br>2289   | SN   | 2244<br>2244<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>20   |
| TriMan5A<br>CFMan26<br>MeMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A6<br>A7<br>A0<br>A9   | 196<br>2234<br>2234<br>2239<br>228<br>239<br>228<br>239<br>228<br>239<br>228<br>239<br>200<br>230<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>200   | SN   | 2244<br>2244<br>2244<br>2244<br>2200<br>2244<br>200<br>2064<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>20  |
| TriMan5A<br>CIFMan26<br>MeMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A6<br>A7<br>A8<br>A8<br>A9<br>A10   | 12238937692602<br>222222222222222222222222222222222   | SN   | 229<br>244<br>268<br>309<br>266<br>303<br>306<br>303<br>306<br>303<br>303<br>303<br>303<br>303<br>3   |
| TriMan5A<br>CFMan26<br>MeMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A6<br>A7<br>A8<br>A9<br>A10<br>A11   | 122314<br>22314<br>22314<br>22318<br>2238<br>2288<br>2388<br>2388<br>2388<br>2388<br>238  | SN   | 229<br>2244<br>2301<br>2266<br>3005<br>3005<br>3005<br>3005<br>3005<br>3005<br>3005<br>30   |
| TriMan5A<br>CEMman26<br>MemMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A6<br>A7<br>A8<br>A0<br>A10<br>A11<br>A12<br>A12   | 19261<br>2334<br>2337<br>2842<br>2337<br>2869<br>2889<br>2889<br>2880<br>2380<br>2380<br>2380<br>2380<br>2380<br>2380<br>2380   | DN   | 229<br>2244<br>2301<br>3056<br>3003<br>3005<br>3003<br>3005<br>3003<br>3037<br>3025<br>3037<br>3025<br>3037<br>3025<br>3025<br>3025<br>3025<br>3025<br>3025<br>3025<br>3025   |
| TriMan5A<br>CFMan26<br>MaMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A6<br>A7<br>A7<br>A7<br>A7<br>A7<br>A7<br>A7<br>A1<br>A1<br>A12<br>A13<br>A14  | 1223142397699260222<br>22334222238889260222<br>22338288892222<br>233822223<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>234002222<br>2340022222<br>23400222222<br>234002222222<br>234002222222222   | SN   | 229<br>2244<br>2268<br>3056<br>3066<br>3056<br>3057<br>3057<br>3057<br>3057<br>3057<br>3057<br>3058<br>3059<br>3059<br>3059<br>3059<br>3059<br>3059<br>3059<br>3059   |
| TriMan5A<br>CFMan26<br>MekAN5A<br>Al<br>A2<br>A3<br>A4<br>A5<br>A6<br>A5<br>A6<br>A7<br>A8<br>A9<br>A10<br>A11<br>A12<br>A13<br>A14<br>A15   | 1223142937692260222<br>2233429338892260222<br>22338893882222<br>223382222<br>233382222<br>233382222<br>233382222<br>2333822222<br>2333822222<br>233382222222<br>2333822222222   | SN   | 229<br>2244<br>301<br>3066<br>305<br>305<br>303<br>305<br>303<br>303<br>303<br>303<br>303<br>303  |
| TriMan3A<br>CFMan26<br>MawAN5A<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3   | 122389387692602281<br>22389387692602281<br>2238938889980228<br>2238938889980228<br>223893888<br>2389388<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238938<br>238958<br>238938<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238958<br>238956<br>23895756<br>23895756<br>23895756<br>23895756<br>23895756<br>23895756<br>23895756<br>23895756<br>23895756<br>23895756<br>23895756<br>23895756<br>238957575757575757575757575757575757575757   | SN   | 2244<br>2268<br>3005<br>2266<br>3005<br>3005<br>3005<br>3005<br>3005<br>3005<br>3005<br>30  |
| TriMan5A<br>CFMan26<br>MewAN5A<br>Al<br>A2<br>A3<br>A4<br>A5<br>A6<br>A3<br>A1<br>A12<br>A13<br>A13<br>A13<br>A15<br>A15<br>A17<br>0   | 1221429769260221122012<br>223893976926022216912<br>223888998221691120016<br>23888998221691120016<br>23888998221691120016<br>23888998221691120016<br>23888998221691120016<br>23888998221691120016<br>23888998221691100016<br>23888998221691100016<br>23888998221691100016<br>23888998221691100016<br>23888998221691100016<br>238889998221691100016<br>238889998221691100016<br>238889998221691100016<br>238889998221691100016<br>238889998221691100016<br>2388899982216911000000000000000000000000000000000  | SN   | 2244<br>2246<br>2306<br>4300<br>2300<br>2300<br>2300<br>2300<br>2300<br>2300<br>2300  |
| TriMan36<br>CFMAn26<br>ManAN58<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A10<br>A112<br>A116<br>A116<br>A116<br>A17<br>A18<br>A19<br>A19<br>A19<br>A19<br>A19<br>A19<br>A19<br>A19<br>A19<br>A19  | 122318938769260222116016<br>223289387692222216936818982222222222222222222222222222222222  | SN   | 2244<br>2246<br>2246<br>2306<br>430<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>200<br>2   |
| TriMan5A<br>CTMan26<br>MaRAN5A<br>Al<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3   | 122789769260221120166990<br>9278978889982169968118899<br>2278928889982169968118899<br>2288222222223   | SN     ILL VT     GDEGLGISTGDGAY PYTYGEGT DFAKN       PGANVTVGSWNMKA DT DANG FINIS SDICL MAGGRQS     INROVYLRTYFG PA       TDN     DGTLTM     IST LAGLY PGAGYVD N       TDN     DGTLTM     IST LAGLY PGAGYVD N       TDN     DGTLTM     IST LAGLY PGAGYVD N       IST LAGLY PGAGYVD N     IST LAGLY PGAGYVD N       IST LAGLY PGAGYVD N     IST LAGLY PGAGYVD N       IST LAGLY PGAGYVD N     IST LAGLY PGAGYVD N       IST LGCY PGAGYVD N     IST LGCY PGAGYVD N  | 2244811964369379988976<br>22468196436937998897663267<br>333643383333333333333333333333333333333   |
| TriMan26<br>CFWan26<br>NaMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A2<br>A3<br>A4<br>A5<br>A10<br>A12<br>A14<br>A12<br>A15<br>A12<br>A14<br>A15<br>A19<br>A12<br>A19<br>A22<br>A2   | 1 2 2 3 1 4 2 3 7 6 9 2 6 0 2 2 1 1 2 2 3 1 9 3 3 7 6 9 2 6 0 2 2 1 1 2 2 3 0 9 3 8 8 8 9 9 1 2 1 6 9 9 6 8 1 1 8 8 9 9 0 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2   | SN   | 2244811964369379988976632670<br>224332604369379988976632670   |
| TriMan5A<br>CFMan26<br>MARAN5A<br>A12<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3  | 1 2 2 1 4 2 9 7 6 9 2 6 0 2 2 1 2 1 2 0 1 6 8 9 0 7 9 7 9 1 2 2 1 6 9 9 6 0 1 1 2 1 9 9 9 0 7 9 7 9 1 2 1 2 9 9 6 7 9 7 9 1 2 1 2 9 9 9 6 7 9 9 6 7 9 7 9 7 9 7 9 7 9 7 9   | SN   | 2 2 4 4 8 1 9 6 6 4 3 6 9 3 7 9 9 8 8 9 7 6 3 2 6 7 0 6 4 2 8 0 0 8 6 4 3 8 9 3 7 9 9 8 8 9 7 6 3 2 6 7 0 6 4 3 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1   |
| TriMan26<br>MamAan26<br>MamAan5A<br>Al<br>A2<br>A3<br>A4<br>A5<br>A10<br>A12<br>A14<br>A10<br>A12<br>A13<br>A14<br>A15<br>A16<br>A17<br>A12<br>A14<br>A15<br>A17<br>A12<br>A14<br>A15<br>A17<br>A22<br>A23<br>A22<br>A23<br>A24  | 1223199789899169246924112216996919977<br>22319978899982169996811819998119<br>223222222222222222222222222222222222   | SN     ILL VT     GDEGLGISTGDGAY PYTYGEGT DFAKN       PGANVTVOSWNMKA DT DANG FININS SDHOL VKAGSKOS     IT       DTDI     DGTLTM     IT       DGTLTM     IT     IT       DGTLTM     IT  | 22468196433693799889763267064<br>2242332233233333333333333333333333333  |
| TriMan3A<br>CFMAn26<br>ManAN5A<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3   | 1 2 2 3 4 2 9 7 6 9 2 6 0 2 8 1 1 2 0 1 6 5 9 0 7 9 7 7 2<br>2 2 3 4 9 3 5 5 5 6 9 2 6 0 2 8 1 1 2 6 9 9 6 1 9 9 9 9 1 9 9 9 1 1 9 9 9 1 1 9 9 9 1 1 9 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 9 1 1 9 1 9 1 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 1 9 1 1 9 1 9 1 9 1 9 1 9 1 9 1 1 9 1 9 1 1 9 1 9 1 1 9  | 9N   | 2 24481964369379988976326706449<br>22468096000032799889765226706449   |
| TriMan5A<br>CFMan26<br>MaRAN5A<br>Al<br>Al<br>A3<br>A5<br>A3<br>A5<br>A3<br>A5<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3   | 661420976926022112011659077728<br>122719781819191221699681181999961998<br>1227297222222201299221699681181999961998  | BN   | 2 2448129643693799889763267064499<br>224680960000032799889763267064499<br>2222332333333333333333332223333333333   |
| TriMan26<br>CFMMAN26<br>NA126<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3  | 1 2 2 3 4 2 9 7 6 9 2 6 0 2 2 1 1 2 0 1 6 3 9 0 8 9 7 7 2 8 2 2 3 4 9 3 8 5 8 9 8 2 1 6 9 9 6 6 1 1 8 8 9 9 8 1 9 9 1 9 1   | 9N   | 2 24481964369379988976326706449592<br>224680060003270079500107010310081<br>200003337333333333332223333333333333333  |
| TriMan5A<br>CFMan26<br>MawAN5A<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3   | 661472976926022112016996077728200<br>122319938899821699681188999819981998<br>1223232938221699681188999819981998<br>1293222000000000000000000000000000000000   | SN     ILL VT     GDE GLGLS TGDGAY PYTYGEGT DFAKN       PGANVTVGSWNMKA DT DNG FINION SDNCT MAGGRQS     IT       TDN     DGT LTAGLY PGAYVON       TDN     DGT LTM       ST FLGO Y PGA     YCA       ST FLGO Y PGA        ST FLGO  | 224681<br>224681<br>330643<br>330643<br>330643<br>330643<br>330643<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307988<br>3307998<br>3307988<br>3307988<br>3307998<br>3307978<br>3307978<br>3307978<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3307674<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>30050577<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>3005977<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>30059777<br>300597777<br>3005977777777777777777777777777777777777  |
| TriMan26<br>CFMan26<br>Al<br>Al<br>Al<br>Al<br>Al<br>Al<br>Al<br>Al<br>Al<br>Al<br>Al<br>Al<br>Al  | 66147.07687.087801147.01404.00199777.882.8001<br>1223125312531253247.265952111252552125217718170100<br>12232247.00000000000000000000000000000000000   | DN   | 2244<br>2246<br>300<br>226<br>300<br>300<br>300<br>300<br>300<br>300<br>300<br>300<br>300<br>30   |
| TriMan26<br>CFMAn26<br>Man26<br>Man26<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3  | $\begin{array}{c} 1966\\ 2261\\ 2261\\ 22219222222222222222$  | 9N   | 2254<br>2261<br>30166<br>3009<br>3009<br>3009<br>3009<br>3009<br>3009<br>3009<br>30   |
| TriMan26<br>MaMAN5A<br>A1<br>A2<br>A3<br>A4<br>A5<br>A4<br>A5<br>A10<br>A12<br>A13<br>A10<br>A12<br>A13<br>A14<br>A15<br>A16<br>A17<br>A13<br>A14<br>A15<br>A16<br>A17<br>A13<br>A14<br>A15<br>A22<br>A23<br>A22<br>A22<br>A22<br>A22<br>A22<br>A23<br>A22<br>A23<br>A22<br>A23<br>A22<br>A23<br>A22<br>A23<br>A22<br>A23<br>A23   | 1966<br>2262<br>2314<br>2922<br>2824<br>2922<br>2922<br>2920<br>2920<br>2920<br>2920<br>2911<br>2622<br>2912<br>2920<br>2910<br>2920<br>2920<br>2920<br>2920<br>29  |  | 2249<br>2248<br>3001<br>2268<br>3001<br>2268<br>3002<br>2268<br>3002<br>3003<br>3003<br>3005<br>3008<br>3008<br>3008<br>3008<br>3008  |
| TriMan36<br>CFMan26<br>MaMAN58<br>A2<br>A3<br>A3<br>A4<br>A5<br>A6<br>A7<br>A10<br>A112<br>A116<br>A112<br>A116<br>A116<br>A117<br>A18<br>A22<br>A22<br>A22<br>A22<br>A22<br>A22<br>A22<br>A22<br>A22<br>A2  | 19261238422923284922923238422992323842299232384229923223849229923238492299232384929923492923232323232323232323232323232   |  | 229 9<br>2248<br>32061<br>30056434<br>300593<br>300933<br>300933<br>300933<br>300933<br>300933<br>300933<br>300933<br>300933<br>300933<br>300933<br>300956668<br>300933<br>30096668<br>300956668<br>300956668<br>300956668<br>300956668<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>300956688<br>3009566688<br>300956688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>3009566688<br>30095666688<br>3009566688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>3009566688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>30095666688<br>300000000000000000000000000000000  |
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                           |   | DN       ILL ST_G GDEGLGLSTGDGAY PYTYGEGT DFAKN         POAMVTVGSWNMAA DTIANG FILIDISEDICINAA GRQS         TDN       DTTVAGTVGSWNMAA DTIANG FILIDISEDICINAA GRQS         TDN       DDTTVAGTVGSWNMAA DTIANG FILIDISEDICINAA GRQS         TDN       DDTTVAGTWGSWNMAA DTIANG FILIDISEDICINAA GRQS         TDN       DTTVAGTWGSWNMAA DTIANG FILIDISEDICINAA GRQSGGGTGTA FINGY PYCASAAS         TDSTGTFALY POACTURE       TSTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT  | 222948<br>222948<br>2261<br>3005666<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30053<br>30054<br>30053<br>30054<br>30053<br>30054<br>30053<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>30054<br>300    |
| TriMan35A<br>CFMMAN57<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3  |   |  | W         224601996446397988           2242601996446497988         23101001797888           233266446497988         2310002379988           233266446497988         2310002379988           233266446497988         2310002379988           233266446497988         2310002379988           233266446497988         2310002379988           233266446497988         2310002379988           240002379988         2310002379988           233264464979988         2310002379988           240002379988         2310002379988           2333333333333333333333333333333333333   |
| TriMan26<br>CMMMAN5A<br>A1<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3   | 1223107171692020200000000000000000000000000000000   | PN       ILL TI GDEGLGLSTGDGAY PYTYGEGT PFAKN         PGAMVTVGSWINNA DT DANG FINIONS DILLT MAAGKQS       ILL TI STORE STADDAY VO NOT THE STADDAY VO  | 2244<br>2244<br>22461<br>2300<br>2300<br>2300<br>2300<br>2300<br>2300<br>2300<br>230  |
| TriMan36A<br>CFMAR26<br>Man26<br>Man26<br>Man26<br>A2<br>A3<br>A3<br>A4<br>A5<br>A2<br>A3<br>A4<br>A5<br>A10<br>A123<br>A14<br>A16<br>A123<br>A14<br>A16<br>A123<br>A14<br>A16<br>A22<br>A220<br>A223<br>A220<br>A223<br>A220<br>A223<br>A220<br>A223<br>A220<br>A223<br>A220<br>A223<br>A220<br>A223<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A223<br>A225<br>A227<br>A220<br>A227<br>A220<br>A223<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A225<br>A227<br>A227  |   | DN       ILL ST       GDEGLGJ.STGDGAY PYTYGEGT DFAKN         POAMVTVGSWNMA DT JANG FINISME DINLIMKA GRQS       ILL ST       GDEGLGJ.STGDGAY PYTYGEGT DFAKN         TDN       DGTLATY PYDGYTY       ILL ST       GDEGLGJ.STGDGAY PYTYGEGT DFAKN         TDN       DGTLATY       FID TASTY PYDGYTY       ILL ST       GDEGLGJ.STGDGAY PYTYGEGT DFAKN         TDN       DGTLATY       FID TASTY PYDGYTY       ILL ST       GDEGLGJ.STGDGAY PYTYGEGT DFAKN         TDN       DGTLATY       FID TASTY PYDGYTY       ILL ST       GDEGLGJ.STGDGAY PYTYGEGT DFAKN         TDN       DGTLATY       FID TASTY POASTYN       ILL ST       GDEGLGJ.STGDGAY PYTYGEGT DFAKN         GT TGN PGGT PYDGAY PYDGASAN       GT TGN PGGT PANNON       ILL ST       GDEGLGJ.STGDGAY PYTYGEGT DFAKN         GT TGN PGGT PANNON       GT TGN PGGT PANNON       GT TGN PGGT PANNON       ILL ST       GT TGN PGGT PANNON         GT TGN PGGT PANNON       GT TGN PGGT PANNON       GT TGN PGGT PANNON       ILL ST       GT TGN PGGT PANNON       ILL ST         GT TGN PGGT PANNON       GT TGN PGGT PANNON       GT TGN PGGT PANNON       ILL ST       GT TGN PGGT PANNON       I  | 224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           2300         94         8           3200         94         94           3200         94         94           3200         94         94           3200         77         98           3200         77         98           3200         74         94           3213         3210         76           3214         94         94           3213         3214         94           3213         3214         94           3214         94         94           3213         3213         3213           3233         3233         3333           3233         3333         3333           3233         3333         3333   |
| TriMan15A<br>CFMan26<br>MaMAN5A<br>A1<br>A2<br>A3<br>A3<br>A4<br>A56<br>A7<br>A10<br>A12<br>A13<br>A10<br>A12<br>A13<br>A14<br>A16<br>A10<br>A12<br>A13<br>A14<br>A17<br>A12<br>A14<br>A15<br>A17<br>A12<br>A22<br>A22<br>A22<br>A22<br>A22<br>A22<br>A22<br>A22<br>A22  | 12222232420769222116550029772828290191<br>966142877692296601165500297772822900191<br>1266010704809982028601<br>14660107048000019477280090891<br>14660107048000010480008957428282800<br>24660107048800089584   | PN       ILL ST GDEGLGLSTGDGAY PYTYGEST PAKN         PGANVTYGSWNMADT DANG FUNGY SDUCL MAAGKQS         TDN       DST FASDY SDAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N       TST FASDY SGAY YO N         TST FASDY SGAY YO N <th>v         22220019266431603370981809776432604495066678           x         22220019266431603370981766326079663266786           x         222200192664316033709786326776632677664898693798           x         22220019266326677663267766326776632677864893798333333333333333333333333333333333</th>  | v         22220019266431603370981809776432604495066678           x         22220019266431603370981766326079663266786           x         222200192664316033709786326776632677664898693798           x         22220019266326677663267766326776632677864893798333333333333333333333333333333333  |
| TriMan35A<br>CFMMAN55A<br>A12<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3  | 122220071692221121165900299772219900109704800009000794  |  | 224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           224         94         8           224         94         94           224         94         94           224         94         94           333         903         94           333         903         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94         94           333         94  |
| TriMan26<br>Man26<br>Man26<br>Man26<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3  | 1222224276922221221169900299772829010109777287290100000000000000000000000000000000000   | DN       ILL ST       GDEGLGLSTGDGAY PYTYGEST DFAKN         POANVTYGSWNNA DT DANG FILIØN SDILL MKAGKQS       ILL ST         TDN       DGT HAGDY PGADYNG         TDN DGT HAGDY PGADYNG NU       TON TOTOS NU         TD TAGUY PGADYNG NU       TON TOTOS NU         TD TAGUY PGADYNG NU       TON TOTOS NU         TT TAGUY PGADYNG NU       TOTOS NU         TT TAGUY PGADYNG NU       TOTOS NU <th>N         224201950433           224201950433         2010177988           23300433         2010177988           23300433         2010177988           23300797988         2010177988           23300797988         20101777988           23300797988         20101777988           23300797988         20101777788           23300797788         20101777788           23300797788         20101777788           23300797788         20101777788           23300797788         20101777788           23300777788         201017777788           23300777788         201017777777777777777777777777777777777</th>   | N         224201950433           224201950433         2010177988           23300433         2010177988           23300433         2010177988           23300797988         2010177988           23300797988         20101777988           23300797988         20101777988           23300797988         20101777788           23300797788         20101777788           23300797788         20101777788           23300797788         20101777788           23300797788         20101777788           23300777788         201017777788           23300777788         201017777777777777777777777777777777777  |
| TriMan15A<br>CMMANNSA<br>A12<br>A34<br>A56<br>A73<br>A34<br>A56<br>A73<br>A34<br>A56<br>A73<br>A123<br>A14<br>A123<br>A14<br>A123<br>A14<br>A123<br>A14<br>A123<br>A14<br>A123<br>A14<br>A123<br>A14<br>A123<br>A2223<br>A2256<br>A229<br>A2256<br>A229<br>A2256<br>A229<br>A2256<br>A229<br>A2256<br>A229<br>A2256<br>A229<br>A2256<br>A229<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A2256<br>A2278<br>A237<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A2578<br>A | 122320478692231414859028979892 986022324728829000 9865104800089504879890 98622232484829000 986224829000 986546478880099804879890 98650100700480009904874873141110009900104800099047448000100480009904748731411100099001048000901048000000000000000  | DN   | N         224401200000000000000000000000000000000   |
| TriMan26<br>Man26<br>Man26<br>Man26<br>Man26<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3   | 1222220769207999601221104590239772882801 0159772840010101010101010111111111111111111111   |  | W         2242019004709880778           2242019004709880779888077978880779788807797888077978880779797880797978807979788079797880779788077978807797880779788077978807777978807777880777777   |
| TriMan26<br>Man15A<br>A126<br>A126<br>A127<br>A34<br>A36<br>A34<br>A36<br>A3123<br>A34<br>A36<br>A3123<br>A314<br>A3123<br>A314<br>A3123<br>A314<br>A3123<br>A314<br>A3123<br>A314<br>A3123<br>A314<br>A3123<br>A314<br>A3123<br>A32223<br>A3225<br>A3225<br>A3225<br>A3225<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A3226<br>A326<br>A3  | 1222202107106922221212016590239772822001<br>926114797769222220222022016590239772822001<br>926147987692222222222202200000000000000000000000  |  | 224<br>22401<br>22401<br>22401<br>22401<br>22401<br>22401<br>22401<br>2300<br>2300<br>2300<br>2300<br>2300<br>2300<br>2300<br>23  |
| TriMan35A<br>CFMAR26<br>Man26<br>Man26<br>Man26<br>Man26<br>A2<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3<br>A3   | 122222076920769202211176590029772882901 0159772802010101010101010101010101010101010101  |  | V         94           224         94 <t< td=""></t<>   |

Fig. 2: Continue



Fig. 2: Multiple sequence alignment among *Arthrobacter* β-mannanases

| Table 2: Amin  | o acid perce<br>TriM   | ent identity<br>CfM  | ot <i>Arthoba</i> .<br>MeM   | <i>cter</i> β-man<br>A1   | nanases<br>A2   | A3   | A4   | A5   | A6  | Α7   | A8  | A9  | A10   | A11  | A12   | A13   | A14   |
|--|--|--|--|---|---|--|--|--|---|--|---|---|---|--|---|---|---|
| TriMan5A<br>CfMan5A<br>MeMan5A<br>A1<br>A2<br>A3<br>A5<br>A5<br>A8<br>A8<br>A8   | 100.00<br>21.55<br>20.16<br>16.75<br>17.22<br>13.78<br>16.75<br>16.75<br>16.77<br>16.77<br>16.27                           | 100.00<br>14.04<br>22.65<br>23.00<br>23.10<br>23.16<br>23.16<br>23.16<br>23.16<br>22.65  | 100.00<br>13.42<br>14.59<br>16.39<br>13.78<br>13.78<br>11.2.99<br>11.2.99<br>12.45<br>12.45                      | 100.00<br>87.16<br>61.36<br>61.36<br>58.29<br>60.88<br>60.88<br>61.17   | 100.00<br>26.51<br>62.09<br>60.15<br>61.48<br>75.43<br>61.81                  | 100.00<br>25.45<br>24.46<br>26.67<br>27.71<br>24.39  | 100.00<br>61.65<br>68.34<br>84.94  | 100.00<br>64.87<br>60.20<br>61.21  | 100.00<br>63.52<br>61.14  | 100.00   | 100.00  |   |   |  |   |   |   |
| A9<br>A11<br>A12<br>A15<br>A17<br>A17<br>A17<br>A17<br>A17<br>A17<br>A17<br>A17<br>A17<br>A17  | 14.83<br>15.79<br>15.79<br>15.31<br>17.22<br>17.22<br>15.31<br>15.79<br>15.79<br>15.79<br>15.79                            | 23.69<br>22.65<br>22.81<br>22.81<br>22.81<br>23.00<br>24.74<br>23.51<br>23.51<br>23.51<br>23.51  | 11.16<br>12.93<br>12.27<br>12.28<br>12.28<br>12.28<br>11.28<br>11.28<br>11.28<br>11.26<br>11.59<br>11.59         | 74.81<br>58.75<br>57.98<br>71.32<br>71.32<br>71.32<br>71.32<br>73.86<br>73.86<br>73.86<br>75.87<br>76.63<br>74.50 | 75.25<br>57.84<br>52.09<br>52.09<br>72.91<br>72.91<br>72.91<br>72.95<br>74.94 | 25.08<br>25.508<br>25.53<br>25.59<br>25.59<br>25.98<br>25.98<br>25.98<br>25.98<br>25.98                | 61.62<br>69.12<br>60.21<br>61.10<br>62.83<br>61.32<br>59.50<br>68.73<br>60.65<br>60.65 | 62.00<br>63.84<br>63.84<br>63.84<br>61.01<br>61.01<br>61.05<br>61.06<br>61.06<br>61.06<br>61.06<br>61.06 | 62.53<br>71.39<br>65.18<br>60.71<br>60.71<br>61.40<br>61.40<br>61.24<br>61.03<br>61.24<br>61.03 | 76.66<br>60.05<br>58.99<br>91.28<br>68.8<br>68.8<br>63.76<br>53.75<br>75.75<br>71.71<br>71.71<br>80.69<br>80.69<br>71.44<br>80.69<br>71.44 | 60.41<br>70.86<br>61.84<br>60.155<br>60.16<br>60.36<br>60.36<br>60.36<br>60.36<br>59.90<br>59.58<br>59.90<br>59.53<br>59.53 | 100.00<br>59.80<br>59.79<br>72.63<br>72.63<br>72.63<br>88.81<br>74.44<br>83.13<br>83.13<br>83.13<br>83.13<br>83.13<br>83.13 | 100.00<br>58.90<br>59.46<br>59.21<br>62.83<br>59.28<br>50.00<br>57.46<br>57.88<br>57.88 | 100.00<br>58.73<br>59.47<br>75.65<br>59.15<br>58.99<br>53.61<br>63.61<br>59.26         | 100.00<br>67.57<br>61.90<br>73.18<br>21.36<br>70.72<br>78.47<br>76.24<br>76.96        | 100.00<br>61.32<br>70.28<br>74.69<br>60.96<br>69.95                           | 100.00<br>60.74<br>19.41<br>61.11<br>62.50<br>64.02                           |
| A22<br>A23<br>A26<br>A28<br>A28<br>A28<br>A28<br>A28<br>A28<br>A28   | 16.75<br>14.29<br>16.27<br>17.22<br>15.31<br>15.31<br>15.31<br>15.79<br>16.75  | 22.30<br>20.83<br>23.78<br>23.78<br>23.00<br>21.95<br>23.26<br>23.34<br>23.34<br>23.34<br>21.60  | 12.02<br>13.03<br>11.30<br>14.59<br>11.26<br>13.27<br>13.27<br>13.79   | 83.70<br>61.85<br>55.67<br>87.65<br>71.25<br>59.19<br>76.36<br>59.19<br>75.38<br>74.07                            | 87.32<br>61.37<br>56.08<br>99.27<br>72.46<br>76.80<br>58.81<br>75.31<br>75.31 | 24.70<br>23.12<br>25.91<br>26.51<br>25.23<br>25.38<br>25.38<br>23.26<br>25.68                          | 62.06<br>61.60<br>56.50<br>62.09<br>63.85<br>68.14<br>60.81<br>62.19                   | 59.15<br>60.15<br>60.40<br>60.40<br>60.40<br>63.57<br>63.57<br>63.50<br>61.01<br>58.21                   | 60.93<br>59.55<br>61.48<br>61.48<br>61.28<br>61.28<br>61.40<br>60.46                            | 74.07<br>59.61<br>58.66<br>75.43<br>85.12<br>85.12<br>78.46<br>59.65<br>59.65<br>68.06   | 61.52<br>62.81<br>62.81<br>62.81<br>62.11<br>66.67<br>66.67<br>60.61<br>61.90   | 74.50<br>63.46<br>56.05<br>75.25<br>74.26<br>92.84<br>63.16<br>63.16<br>88.31<br>70.30                                      | 59.51<br>59.86<br>59.01<br>58.17<br>58.17<br>61.58<br>68.10<br>68.10<br>68.10<br>68.21  | 58.89<br>57.48<br>65.53<br>58.36<br>59.32<br>62.30<br>62.30<br>58.62<br>58.62<br>58.62 | 7255<br>59.75<br>58.31<br>72.66<br>87.32<br>76.86<br>61.54<br>61.54<br>61.54<br>67.08 | 72.48<br>62.41<br>56.33<br>75.79<br>67.90<br>67.90<br>61.54<br>70.28<br>99.27 | 60.37<br>60.37<br>70.53<br>62.33<br>60.32<br>61.68<br>60.48<br>60.48<br>60.79 |
| A 15<br>A 16<br>A 16<br>A 17<br>A 21<br>A 20<br>A 26<br>A 26<br>A 26<br>A 26<br>A 27<br>A 29<br>A 29<br>A 20<br>A 29<br>A 20<br>A 20<br>A 20<br>A 20<br>A 20<br>A 20<br>A 20<br>A 20 | A15<br>100.00<br>18.73<br>75.44<br>81.36<br>60.56<br>60.56<br>7.4.75<br>74.75<br>74.06<br>89.23<br>89.23<br>69.77<br>69.77 | A16<br>100.00<br>20.56<br>19.24<br>19.94<br>19.14<br>19.14<br>19.15<br>21.57<br>21.57<br>21.25<br>21.25<br>21.25<br>21.25<br>21.25<br>21.35<br>21.35 | A17<br>100.00<br>73.87<br>60.51<br>73.07<br>75.50<br>61.29<br>56.28<br>79.80<br>70.57<br>75.97<br>73.42<br>73.42 | A18<br>100.00<br>60.15<br>79.26<br>76.25<br>76.62<br>76.68<br>76.68<br>84.42<br>61.65<br>82.87<br>69.98           | A15<br>59.9<br>60.6<br>60.2<br>60.2<br>60.2<br>60.2<br>60.2<br>60.2<br>60.2   | A20<br>5 77.6<br>5 77.6<br>5 77.6<br>6 6 73.1<br>75.1<br>1 75.6<br>6 69.4<br>8 80.1<br>6 1.6<br>6 69.4 | 6 2 2 2 2 8 2 2 2 2 2 2 2 2 2 2 2 2 2 2  | 21<br>2000<br>23397<br>253<br>255<br>255<br>255<br>333<br>333<br>26<br>25<br>525<br>333                  | A22<br>00.00<br>661.33<br>56.00<br>71.32<br>76.1.32<br>76.1.32<br>74.81<br>71.74                | A23<br>00.00<br>54.32<br>54.32<br>61.37<br>64.18<br>64.18<br>64.18<br>64.18<br>61.92   | A24<br>00.00<br>55.97<br>57.77<br>55.73<br>55.83  | A25<br>100.00<br>72.46<br>76.80<br>58.81<br>75.31   | A26<br>100.00<br>76.23<br>59.95<br>67.41  | A27<br>100.00<br>63.52<br>87.95<br>71.98   | A28<br>100.00<br>61.54  | A29<br>100.00<br>69.77  | A30<br>100.00   |



Fig. 3(a-e): Continue



#### Fig. 3 (a-e): Theoretical chemical properties of Arthrobacter β-mannanases

Table 3: Overall comparison between amino acid profile and theoretical chemical properties of psychrophilic and mesophilic β-mannanases

|                                 | Psychrophilic | (n = 4)          | Mesophilic ( $n = 16$ ) |             |                  |         |
|---------------------------------|---------------|------------------|-------------------------|-------------|------------------|---------|
| Properties                      | Full length   | Catalytic domain | Average                 | Full length | Catalytic domain | Average |
| Hydrophobic (%)                 | 59.30         | 49.48            | 54.390                  | 60.16       | 53.42            | 56.790  |
| Neutral (%)                     | 22.18         | 21.85            | 22.015                  | 23.41       | 20.59            | 22.000  |
| Hydrophilic (%)                 | 22.28         | 37.30            | 29.790                  | 18.79       | 34.28            | 26.535  |
| Charged (%)                     | 13.15         | 13.35            | 13.250                  | 11.99       | 12.29            | 12.140  |
| Negatively charged residues (%) | 7.88          | 8.78             | 8.330                   | 6.88        | 8.66             | 7.770   |
| Positively charged residues (%) | 5.23          | 4.58             | 4.905                   | 5.09        | 3.57             | 4.330   |
| Proline (%)                     | 7.43          | 5.35             | 6.390                   | 6.79        | 6.58             | 6.685   |
| Instability index               | 29.05         | 17.81            | 23.430                  | 30.12       | 25.90            | 28.010  |
| Aliphatic index                 | 78.95         | 69.49            | 74.220                  | 81.43       | 74.90            | 78.165  |
| GRAVY                           | 0.147         | -0.272           | -0.0625                 | 0.11        | -0.17            | -0.030  |

Table 4: Predicted secondary structure comparison between psychrophilic and mesophilic Arthrobacter β-mannanases

|                 | Psychrophilic | : (n = 4)        |         | Mesophilic (n = 16) |                  |         |
|-----------------|---------------|------------------|---------|---------------------|------------------|---------|
| Properties (%)  | Full length   | Catalytic domain | Average | Full length         | Catalytic domain | Average |
| α-helix         | 27.52         | 33.79            | 30.655  | 29.77               | 32.65            | 31.21   |
| β-turn          | 6.36          | 8.38             | 7.370   | 6.13                | 7.69             | 6.91    |
| Extended strand | 20.22         | 15.15            | 17.685  | 20.06               | 14.94            | 17.50   |
| Random coil     | 45.90         | 42.68            | 44.290  | 44.04               | 44.72            | 44.38   |

residues than the catalytic domain. Further, Table 3 revealed that  $\beta$ -mannanases from psychrophilic bacteria were found to have higher hydrophilic and charged residues than the mesophilic group. Meanwhile, neutral residues and proline of both groups were similar. Further, Table 3 also showed that hydrophilic residues of the full-length psychrophilic *Arthrobacter*  $\beta$ -mannanases were higher than that of the mesophilic group. By contrast, remarkable differences between the catalytic domain of  $\beta$ -mannanases from psychrophilic and mesophilic groups were observed in hydrophobic and hydrophilic groups (Table 3). In addition, Table 3 also showed that the instability index of mesophilic *Arthrobacter*  $\beta$ -mannanases was found to be higher than that of psychrophilic *Arthrobacter*  $\beta$ -mannanases. This was particularly observed in the catalytic domains of both  $\beta$ -mannanases. In addition, the aliphatic index and GRAVY of full-length and catalytic domains of mesophilic *Arthrobacter*  $\beta$ -mannanases were also higher than that of psychrophilic *Arthrobacter*  $\beta$ -mannanases.

**Secondary structure profile:** Figure 5 showed the full-length and catalytic domain of *Arthrobacter*  $\beta$ -mannanases were dominated by coil structures, followed by  $\alpha$ -helix,  $\beta$ -sheet and  $\beta$ -turn secondary structures. Furthermore, Table 4 revealed that the full-length and catalytic domain of psychrophilic and mesophilic *Arthrobacter*  $\beta$ -mannanases were dominated by random coil structures, followed by  $\alpha$ -helix,  $\beta$ -sheet and  $\beta$ -turn contents (Table 4). Among these secondary structures, only



Fig. 4 (a-b): (a) Amino acid compositions of the full-length and (b) Catalytic domain of *Arthrobacter* β-mannanases



Fig. 5 (a-b): (a) Predicted secondary structure profiles of the full-length and (b) Catalytic domain of Arthrobacter β-mannanases

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 $\alpha$ -helix contents of the full-length psychrophilic and mesophilic *Arthrobacter*  $\beta$ -mannanases were found to be considerably different. Meanwhile, the catalytic domain of the psychrophilic group has lower content of random coil secondary structures than that of the mesophilic group, while

the other secondary structures were considerably comparable (about 1% difference only).

**Three-dimensional model structures:** The 30 selected models as shown in Fig. 6 were mostly built based on the template of



Fig. 6: Continue



Fig. 6: Selected 3D models generated from SWISS-MODEL for *Arthrobacter* β-mannanases (designated as A1-A30 accordingly)

Black label is those originated from the strain with unknown growth temperature, red label is mesophilic β-mannanase, blue label is psychrophilic β-mannanase, all models are viewed using Pymol software



Fig. 7 (a-b): Alignment of the glutamic acids active sites among (a) Psychrophilic and (b) Mesophilic Arthrobacterβ-mannanases

endoglucanase H of Clostridium thermocellum (PDB ID:2V3G) under the SCOP domain of D2V3GA1. All these models were selected as these models met the standards for structural quality parameters including structural geometry (Ramachandran plot), GCMQE, QMEAN and Verify-3D. The Ramachandran plot revealed that most of the residues of Arthrobacter β-mannanases were located in favoured regions (94-98%). In addition, all selected structures had GMQE scores ranging from 0.51-0.96 which were considered to be in the moderate to high score level. Meanwhile, the selected models have acceptable z-score (QMean), ranging from -4-0, which was considered a good structure model. Besides, 3D-1D score residues (Verify-3D) of all selected models were considered to be a good model as the scores were ranging from 96-100% (higher than the minimum standard of 65%). The best model selected were mostly built based on Phyre2 (19 models) and the SWISS-MODEL (11 models) platforms.

Figure 6 showed that all *Arthrobacter*  $\beta$ -mannanases folded into a classic ( $\beta/\alpha$ )<sub>8</sub>-barrel whereby the helices and strands form a solenoid that curved around to close on itself in a doughnut shape. The parallel  $\beta$ -strands formed the inner wall of the doughnut (hence, a  $\beta$ -barrel), whereas the  $\alpha$ -helices formed the outer wall of the doughnut. Each

β-strand connected to the next adjacent strand in the barrel through a long right-handed loop that included one of the helices, so that the ribbon N-to-C colouring at the top view proceeded in rainbow order around the barrel. In general, Table 5 indicated all structures showed high similarity among each other as indicated by low RMSD values of Ca-atoms (0.00-0.036 Å), with the exception of strain A16. Comparative analysis between the models of Arthrobacter β-mannanases from psychrophilic and mesophilic revealed the structures were highly similar (Table 5). Furthermore, 2 active sites of glutamic acids between the psychrophilic and mesophilic β-mannanases also indicated the residues were in highly-conserved placement (Fig. 7). In addition, B-factors for the 1st and 2nd catalytic residues of the psychrophilic group ranged from 20.00-24.10 (average: 21.93) and 25.57-32.65 (average: 29.76). Meanwhile, B-factors for the 1st and 2nd catalytic residues of the mesophilic group ranged from 20.00-24.10 (average: 21.93) and 25.01-32.51 (average: 30.71) (Table 6).

#### DISCUSSION

Primary structural analysis revealed that the size and amino acid number of the Arthrobacter  $\beta$ -mannanases are

| A15                    | 0.111<br>0.121<br>0.135<br>0.135<br>0.135<br>0.121<br>0.118<br>0.119<br>0.119<br>0.119<br>0.1118<br>0.1118<br>0.123<br>0.112 | A30 | 0.000<br>0.000<br>0.115<br>0.000<br>0.115<br>0.000<br>0.000<br>0.000<br>0.110<br>0.000<br>0.1100<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.0000<br>0.000<br>0.000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.00000<br>0.0000<br>0.0000<br>0.0000<br>0.000000   |
|------------------------|--|-----|--|
| A14                    | 0.000<br>0.000<br>0.000<br>0.115<br>0.115<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000                     | A29 | 0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.268<br>0.000<br>0.268<br>0.000<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.266<br>0.268<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.260<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.00000<br>0.0000<br>0.0000<br>0.00000<br>0.00000<br>0.00000<br>0.000000  |
| A13                    | 0.000<br>0.000<br>0.259<br>0.106<br>0.106<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000                     | A28 | 0.000<br>0.000<br>0.261<br>0.115<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.268<br>0.200<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.268<br>0.269<br>0.269<br>0.269<br>0.269<br>0.269<br>0.269<br>0.260<br>0.260<br>0.260<br>0.260<br>0.260<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.0000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.00000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.0000<br>0.00000<br>0.00000<br>0.0000<br>0.00000<br>0.000000  |
| A17                    | 0.000<br>0.000<br>0.1111<br>0.1000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000                            | A27 | 0.298<br>0.298<br>0.298<br>0.281<br>0.281<br>0.297<br>0.297<br>0.299<br>0.299<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.298<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.297<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070<br>0.2070000000000 |
| A11                    | 0,000<br>0,000<br>0,115<br>0,115<br>0,000<br>0,000<br>0,000<br>0,000<br>0,000  | A26 | 0.000<br>0.000<br>0.259<br>0.107<br>0.107<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.110<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266  |
| A10                    | 0.000<br>0.000<br>0.115<br>0.115<br>0.000<br>0.000<br>0.000<br>0.000   | A25 | 0.251<br>0.253<br>0.259<br>0.269<br>0.251<br>0.251<br>0.253<br>0.253<br>0.253<br>0.253<br>0.253<br>0.253<br>0.253<br>0.251<br>0.253<br>0.251<br>0.253<br>0.251<br>0.253<br>0.251<br>0.253<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.253<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.253<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.251<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.255<br>0.2550000000000  |
| A9                     | 0.000<br>0.000<br>0.260<br>0.106<br>0.000<br>0.000<br>0.000<br>0.000   | A24 | 0.266<br>0.271<br>0.260<br>0.089<br>0.251<br>0.271<br>0.267<br>0.267<br>0.266<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.268<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.267<br>0.269<br>0.266<br>0.267<br>0.269<br>0.266<br>0.266<br>0.267<br>0.269<br>0.266<br>0.266<br>0.267<br>0.269<br>0.267<br>0.269<br>0.266<br>0.267<br>0.269<br>0.266<br>0.266<br>0.266<br>0.266<br>0.267<br>0.266<br>0.266<br>0.266<br>0.267<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.266<br>0.267<br>0.266<br>0.267<br>0.266<br>0.267<br>0.266<br>0.267<br>0.266<br>0.267<br>0.266<br>0.267<br>0.267<br>0.267<br>0.266<br>0.267<br>0.267<br>0.266<br>0.267<br>0.267<br>0.267<br>0.266<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.267<br>0.277<br>0.277<br>0.277<br>0.277<br>0.277<br>0.2770<br>0.2770<br>0.2770<br>0.2770<br>0.27700<br>0.27700<br>0.2770000000000   |
| A8                     | 0.000<br>0.000<br>0.000<br>0.115<br>0.115<br>0.000<br>0.000<br>0   | A23 | 0.260<br>0.261<br>0.254<br>0.081<br>0.250<br>0.250<br>0.261<br>0.261<br>0.261<br>0.261<br>0.261<br>0.261<br>0.260<br>0.120<br>0.260<br>0.120<br>0.347<br>0.347<br>0.347  |
| A7                     | 0.000<br>0.000<br>0.000<br>0.115<br>0.115<br>0.000<br>0  | A22 | 0.349<br>0.358<br>0.323<br>0.335<br>0.3356<br>0.3356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356<br>0.356   |
| A6                     | 0.000<br>0.000<br>0.264<br>0.111<br>0  | A21 | 0.000<br>0.000<br>0.260<br>0.115<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.118<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000  |
| A5                     | 0.105<br>0.117<br>0.117<br>0.249<br>0  | A20 | 0.000<br>0.000<br>0.260<br>0.115<br>0.100<br>0.115<br>0.000<br>0.000<br>0.000<br>0.119<br>0.119<br>0.119<br>0.119<br>0.268<br>0.268  |
| dels<br>A4             | 0.260<br>0.263<br>0.249<br>0<br>0  | A19 | 0.268<br>0.268<br>0.256<br>0.255<br>0.267<br>0.267<br>0.267<br>0.268<br>0.268<br>0.268<br>0.268<br>0.123<br>0.123<br>0.123   |
| een all 3D mc<br>A3    | 000.0<br>000.0   | A18 | 0.302<br>0.298<br>0.113<br>0.113<br>0.298<br>0.298<br>0.298<br>0.298<br>0.298<br>0.298<br>0.298<br>0.298<br>0.298<br>0.298<br>0.276<br>0.276<br>0.276<br>0.276<br>0.000  |
| nparison betw<br>A7    | 0.000  | A17 | 0.000<br>0.000<br>0.264<br>0.109<br>0.109<br>0.000<br>0.000<br>0.000<br>0.000<br>0.000<br>0.113<br>11.943<br>0   |
| SD matrix cor<br>A1    | 0  | A16 | 12.375<br>12.735<br>7.670<br>9.124<br>7.670<br>8.046<br>7.941<br>14.061<br>8.419<br>7.541<br>7.304<br>7.304<br>0   |
| Table 5: RM<br>Strains | A1<br>A2<br>A3<br>A5<br>A5<br>A6<br>A1<br>A11<br>A11<br>A112<br>A13<br>A13<br>A15  |     | A1<br>A2<br>A5<br>A5<br>A5<br>A1<br>A1<br>A1<br>A1<br>A1<br>A1<br>A1<br>A1<br>A1<br>A1<br>A1<br>A2<br>A2<br>A2<br>A2<br>A2<br>A2<br>A2<br>A2<br>A2<br>A2<br>A2<br>A2<br>A2   |

|            | 1st catalytic residue |          | 2nd catalytic residue |          |
|------------|-----------------------|----------|-----------------------|----------|
|            |                       |          |                       |          |
| Protein ID | Position              | B-factor | Position              | B-factor |
| MeMan5A    | E177                  | -        | E307                  | -        |
| TriMan5A   | E165                  | -        | E275                  | -        |
| CfMan26    | E173                  | -        | E280                  | -        |
| A1         | E231                  | 24.15    | E342                  | 29.64    |
| A2         | E239                  | 24.11    | E350                  | 25.59    |
| A3         | E172                  | 26.59    | E325                  | 27.79    |
| A4         | E234                  | 21.89    | E345                  | 28.55    |
| A5         | E233                  | 21.67    | E344                  | 31.27    |
| A6         | E236                  | 20.76    | E347                  | 30.02    |
| A7         | E239                  | 23.85    | E350                  | 25.01    |
| A8         | E233                  | 20.75    | E344                  | 24.60    |
| A9         | E267                  | 21.20    | E378                  | 31.31    |
| A10        | E259                  | 20.00    | E370                  | 25.57    |
| A11        | E209                  | 24.10    | E320                  | 30.79    |
| A12        | E238                  | 22.92    | E349                  | 30.65    |
| A13        | E238                  | 25.00    | E349                  | 30.43    |
| A14        | E209                  | 24.42    | E320                  | 30.71    |
| A15        | E227                  | 24.46    | E338                  | 32.51    |
| A16        | D249                  | 30.17    | -                     | -        |
| A17        | E233                  | 22.45    | E344                  | 31.01    |
| A18        | E232                  | 23.97    | E343                  | 31.40    |
| A19        | E246                  | 20.88    | E357                  | 30.43    |
| A20        | E237                  | 21.61    | E348                  | 30.59    |
| A21        | E240                  | 21.16    | E351                  | 31.58    |
| A22        | E236                  | 21.86    | E347                  | 31.92    |
| A23        | E264                  | 22.84    | E375                  | 32.65    |
| A24        | E244                  | 21.02    | E355                  | 31.70    |
| A25        | E239                  | 20.32    | E350                  | 32.37    |
| A26        | E235                  | 20.35    | E346                  | 27.94    |
| A27        | E219                  | 21.70    | E330                  | 30.52    |
| A28        | E246                  | 21.86    | E357                  | 30.27    |
| A29        | E227                  | 20.70    | E338                  | 32.39    |
| A30        | E238                  | 22.84    | E349                  | 29.96    |

varied. This might be due to the presence of an additional domain (apart from its catalytic domain), which is the carbohydrate-binding domain (CBM) which functions to anchor substrates more effectively<sup>32,33</sup>. Interestingly, Fig. 1 showed that most of Arthrobacter β-mannanases (except for Strain A16 and A13) were in the distinct branches or nodes of the well-known β-mannanases (MeMan5A, TriMan5A and CfMan26A). This might suggest that the historical evolution of Arthrobacter B-mannanases are different from MeMan5A, TriMan5A and CfMan26A and possibly exhibit unique properties. Meanwhile, pl value of the Arthrobacter β-mannanases also varied which suggests different pH adaptation of these proteins. It was previously confirmed that the pl value of an enzyme is related to the pH optimum for their catalytic activity and plays an important role in the solubility of the enzyme<sup>34</sup>. The instability index values of the full-length and catalytic domain of Arthrobacter β-mannanases were found to be lower than 40 (Table 3). The index refers to the prediction of

protein stability in the test tube, whereby the greater index reflects lower stability. In particular, a protein with the index of lower than 40 is considerably stable<sup>19</sup>. This suggests that Arthrobacter β-mannanases are generally stable in the test tube.

In addition, Table 3 indicated that the aliphatic index showed that the index of mesophilic Arthrobacter β-mannanase is considerably higher than that of psychrophilic one. This is plausible since psychrophilic proteins were characterized by lower thermal stability than its mesophilic counterparts<sup>35</sup>. It is interesting that the GRAVY index value of the mesophilic Arthrobacterβ-mannanases were found to be higher than that of the psychrophilic group. This suggested that the mesophilic Arthrobacter β-mannanases is more hydrophobic than the psychrophilic group. To note, hydrophobic interaction was known to play important role in the thermal stability of protein. Therefore, thermo-stable proteins are usually characterized by higher GRAVY index and more hydrophobic residues than the thermo-labile

proteins<sup>36,37</sup>. Nevertheless, Table 3 indicated that compositions of hydrophobic residues between mesophilic and psychrophilic *Arthrobacter*  $\beta$ -mannanase were similar. Note that the composition only refers to the number of hydrophobic residues, regardless of the magnitude of their hydrophobicity. Accordingly, similar number of hydrophobic residues between these 2 groups does not necessarily imply that both are similarly hydrophobic. As the GRAVY index is obviously different, it is assumed that the psychrophilic *Arthrobacter*  $\beta$ -mannanase is dominated by less bulky hydrophobic residues which then leads to less hydrophobic than the mesophilic ones.

Overall features of amino acid composition of Arthrobacter B-mannanase indicated the domination of the hydrophobic residues (Fig. 4). Similarly, β-mannanase from alkaliphilic Bacillus sp. N16-5 was also reported to be dominated by hydrophobic residues<sup>11</sup>. Apart from the involvement of hydrophobic residues in structural stability, these residues might also be catalytically important for substrate binding and were often found in the substratebinding pocket<sup>38-40</sup>. The domination of hydrophobic residues in Arthrobacter β-mannanase was followed by polar uncharged and charged residues. The uncharged residues were reported to be heavily involved in the solvation of the protein as the residues were mostly located on the surface of the protein<sup>41</sup>. On the other hand, hydrophilic residues are believed to play a role in the formation of any ionic interaction, hydrogen bond and the Van Der Waals interaction stabilizing the proteins.

Interestingly, while generally cold-adapted enzymes are characterized by lower charged residues<sup>42,43</sup>, Table 3 indicated that charged residues of psychrophilic Arthrobacter  $\beta$ -mannanase were found to be slightly higher than the mesophilic ones (Table 3). Nevertheless, Gianese et al.44 indicated that rather than the composition (number) of charged residues, their spatial distribution is more important to cold-adaptation strategy. Thermophilic enzymes tend to have more charged residues in their flexible regions in order to increase the stability by compensating the structural entropy. In addition, the ratios of positively and negatively charged residues were also reported to be the factors for cold-adaptation<sup>45</sup>. Nevertheless, Table 3 showed that both psychrophilic and mesophilic Arthrobacter β-mannanases showed lower positively charged residues compared to the negatively charged residues. This indicated that adjustments of charged residues might not be the main strategy of thermal adaptation for *Arthrobacter* β-mannanase. Alternatively, the structural distributions of charged residues not the number,

are the main factors affecting the thermal adaptation of the enzyme. Nevertheless, further structural investigation on the distribution is needed to confirm this assumption.

Secondary structural analysis revealed that the full-length content of random coil as compared to the mesophilic one. This indicated that the full-length adopted a strategy of destabilizing some regions, particularly the coil, to adapt to the low temperature. The coil structure was known to be the flexible region in the protein structure and often determines protein stability. In some cases, the region is found to be disordered due to high flexibility<sup>46</sup>. Accordingly, cold-adapted enzymes often have a more flexible region as compared to the mesophilic or thermophilic counterparts. Further, the average helical structure content of psychrophilic *Arthrobacter*β-mannanase was considerably lower than the mesophilic one (Table 4, Fig. 5). This is believed to be associated with the structural stability as the longer helical structure is reported to be more stable than the shorter one<sup>47</sup>. It is interesting that the catalytic domain has comparable helical content (<1% differences) than the fulllength, which suggested that the adaptation to low temperature by psychrophilic *Arthrobacter* β-mannanase is dictated by its full-length structure.

Furthermore, the fact that all 3D-models of psychrophilic and mesophilic Arthrobacter β-mannanase were similar (Fig. 6, Table 5) and indicated that the tertiary structure has no or little effect on the temperature adaptation of the enzyme. Rather, secondary and local structure arrangements as discussed above, play a more important role in the adaptation. Indeed, the B-factor of the second glutamic acid active site of the psychrophilic Arthrobacter 
B-mannanase is similar yet has a wider range compared to that of the mesophilic one (Fig. 7, Table 6). The B-factor reflects the fluctuation of atoms about their average positions which suggests that the protein with higher B-factor is considerably highly dynamic (flexible)<sup>48</sup>. Accordingly, the second glutamic acid active site of the psychrophilic Arthrobacter β-mannanase exhibited higher flexibility than that of the mesophilic one. It is known that the flexibility of the area around the catalytic pocket is one of the strategies of cold-adapted enzymes. Nevertheless, the high structural similarity of psychrophilic and mesophilic Arthrobacter β-mannanase and conservation of the catalytic sites reflected that the enzymes shared similar mechanisms. This also implied that structural adjustment for thermal adaptation might be independent from the catalytic mechanism, however, it may indirectly have affected the catalysis properties.

#### CONCLUSION

β-mannanases from various *Arthrobacter* strains were shown to have high genetic relatedness and share the catalytic sites which indicated that the enzymes might employ similar catalytic mechanism. Nevertheless, comparative analysis on these β-mannanases displayed a variation of the physio-chemical and structural properties of the enzymes. The variation is believed to be associated with the adaptation of mechanisms to their respective environments. In particular, cold adaptation of psychrophilic β-mannanases was achieved by a series of adjustments on the secondary structure formation, flexibility (B-factor) around the active sites as well amino acid composition (hydrophilic, particularly negatively charged, residues and proline residues).

#### SIGNIFICANCE STATEMENT

This study discovers the structural differences of psychrophilic and mesophilic *Arthrobacter*  $\beta$ -mannanase that can be beneficial for further studies and industrial application. This study will help the researcher to uncover the critical areas of structural regulation of  $\beta$ -mannanases in thermal adaptation that many researchers were not able to explore. Thus, a new theory on the mechanism by which *Arthrobacter*  $\beta$ -mannanases adapt to low temperature may be arrived at.

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