Egypt. J. Vet. Sci. **Vol. 54,** No. 6, pp. 1165-1176 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Fundamental Steps to Evaluate the Possible Functionality of the Horizontally Transferred Antibiotic Resistance Genes from the Transgenic Diet into Gut Microflora in Animals

Hanaa Abdel-Sadek Oraby, Amal Ahmed Mohamed Hassan* and Nadia Aboul-Ftooh Aboul-Maaty
Cell Biology Department, Biotechnology Research Institute, National Research Centre (NRC), Cairo, Egypt.

THE horizontally transferred antibiotic resistance genes from transgenic feed to the microbiome in animals may have deleterious health impacts on consumers. The present work is a complementary step analysis conducted by coupling both experimental PCR detection assay with the available bioinformatics tools to evaluate the possible functionality of the horizontally transferred aminoglycoside 3" adenyltransferase (*aadA*) gene from the transgenic diet to the microbiome of rats after 90 days of feeding duration.

Transferred *aadA* partial segments into the bacterial genomes (284 bp-target) were investigated and confirmed by analysis of the internal nucleotide sequences of the amplified DNA segments based on nucleotide sequence determination, translation to protein, and alignment with GenBank. The results indicated that the investigated partial sequence of the *aadA* gene, and its predicted protein are completely similar to the corresponding sequences from the *aadA* gene in several cloning vectors. Results also indicated that the predicted 3D structure for the investigated aadA protein segment was modeled with 100% confidence and 48% identity by the highest-scoring template of aminoglycoside adenyltransferase in the protein database. The processed PDB X-Ray structure validation report for the model quality of the template aadA protein verified that there are no bond lengths outliers, bond angle outliers, or chirality outliers. These results suggest the possible functionality of the aadA protein only when the full-length gene is fully transferred horizontally to the bacterial genome. Results also highlight the importance of studying the whole length of the protein sequence to evaluate its general physical properties and possible functionality.

Keywords: Genetically modified crops, Gut microflora, The *aadA* gene, Bioinformatics analysis tools, Protein prediction.

Introduction

The importance of supplying the world with animal-sourced food has strived scientists to produce genetically modified (GM) crops with higher yields and better-quality traits to provide livestock with optimum nutrition. The global increase in cultivation and commercialization of GM food and feed has drawn attention to the safety of the antibiotic resistance (AR) genes inserted in the genome of the transferred crop as markers during its production to facilitate the selection of the transformed plants. The aminoglycoside 3" adenyltransferase (*aadA*) gene is usually used as a marker gene to select the plasmid transformation during the production of the transformed crops.

The antibiotic resistance genes help to protect the bacteria by producing enzymes to degrade antibiotics. Gut microbiota may gain resistance to

*Corresponding author: Amal Ahmed Mohamed Hassan, e-mail: amal.a.hassan@hotmail.com. Tel.: 01273628999
(Received 04/06/2023, accepted 06/08/2023)
DOI: 10.21608/EJVS.2023.213214.1518

antibiotics by the acquirement of resistance genes via the horizontal gene transfer (HGT) phenomenon [1, 2], which is naturally involved in the evolution, and shaping of the genome of various organisms [3- 5]. Many scientists reported the transfer of GM-DNA from the GM diet into various tissues of animals [6, 7], leucocytes of rainbow trout [8], goats [9], pigs [10, 11], and human bloodstream [12]. Transfer of antibiotic resistance genes horizontally may consequently, cause deleterious effects on animals and human health.

The ecological conditions in the animal gut are considered one of the most appropriate niches for the occurrence of horizontal gene transfer [13, 14]. HGT from a GM diet to bacterial cells inhabiting the gastrointestinal tract (GIT) is one of the issues that gained much interest from some community sectors; scientists, GMO producers, animal breeders, and GMO consumers. HGT has been dealt with from different aspects. Scientists tried to demonstrate the occurrence of HGT, especially in microbiota in GIT of animals consuming a GM diet. One study [15] revealed evidence of low-frequency transfer of a small fragment (180bp) of epsps introduced gene derived from GM soybean to microorganisms within the small intestine of a human ileostomist. Others supported the suggestion that the occurrence of HGT is uncertain [14, 16, 17]. In most cases, the possible transcription of the transferred GM-DNA was not properly addressed.

In a previous work conducted by our team, the occurrence of DNA transfer of antibiotic resistance marker genes neomycin phosphotransferase II (*nptII*) and *aadA* from GM plant material to the enteric microflora has been demonstrated in rats fed on an experimentally proved GM diet for 90 days [18]. These results drew our attention to the importance of exploring and evaluating the safety of the transferred antibiotic resistance genes utilizing the Bioinformatics prediction tools to assess the functionality of the transferred genes and their predicted protein.

Material and Methods

Male Wistar Albino rats (30 rats) were provided from the animal house of the National Research Centre shortly after birth. The protocol applied during this study was reviewed and approved by the NRC Ethical Committee (reference 12,142). This protocol fulfils all the requirements of the ARRIVE guidelines, where all animals received humane care.

The experimental animals were divided into three groups of ten animals each. The first group (G1) was immediately euthanized and considered a control group (G1). The other two groups (G2 and G3) of animals were kept in standard ambient conditions where food and water were given ad-libitum. Animals fed on a dried rodent diet for 30 or 90 days. This diet was previously proved experimentally by our team [18] genetically modified, using primers for the Cauliflower Mosaic Virus-35S promoter (CaMVP-35S) and antibiotic resistance genes *nptII* and *aadA*. At the end of the two feeding durations, animals were euthanized. Transfer of the antibiotic resistance *aadA* gene to the microflora in Male Wistar Albino rats was evaluated experimentally using conventional PCR assay and further analyzed employing bioinformatics-based protein analysis tools.

Experimental detection of horizontal gene transfer applying PCR assay

Microflorae were collected from rectums and caecal cavities of all animal groups and inoculated onto the surface of nutrient agar plates for 24-48 hours at 37°C. The DNA was extracted from cultured bacteria using GF-1 Bacterial DNA Extraction Kit (Vivantis) as described by the manufacturer. The quality and concentration of all DNA samples were determined using the NanoDrop 1000/Thermo Scientific spectrophotometer.

The conventional polymerase chain reaction (PCR) assay was applied to investigate the transfer of transgenic DNA from the GM diet into the extracted bacterial DNA samples. A set of primers for partial amplification of the cauliflower mosaic virus (CaMV) 35S promoter was used as a marker to confirm the occurrence of the horizontal transfer of GM-DNA from the GM diet into the bacterial genome. Another set of primers for partial amplification of the antibiotic resistance aminoglycoside 3" adenyltransferase (*aadA*) gene (284 bp-target) was used to detect the occurrence of horizontal transfer of the *aadA* gene from the GM-diet into the bacterial genome. Primer sequences, amplicon lengths, and annealing temperatures are presented in Table 1. PCR was conducted at least twice for each sample in TM Thermal Cycler (MJ Research PTC-100 thermocycler). PCR conditions and profiles were performed as described previously [19]. PCR products were analyzed, using the SYNGENE Bio-Imaging Gel Documentation System, for the presence of a fluorescent band of the expected level for the two investigated DNA segments (P-35S and *aadA* partial gene).

TABLE 1. List of primers used throughout the experimental duration, their sequences, amplicon lengths, and annealing temperatures.

Primers	Positions on the genomes ‡	Sequences (5'-3')	Amplicon length (bp)	Annealing Temp (°C)	Reference
P-35S	7190-7209 7364-7384	5′- GCTCCTACAAATGCCATCA -3′ 5′-GATAGTGGGATTGTGCGTCA-3′	195	57	[20]
aadA	1188-1208 1471-1451	5'- CGC TAT GTT CTC TTG CTT TTG-3' 5'- TGA TTT GCT GGT TAC GGT GAC-3'	284	63	[21]

‡: Primers positions are relevant to the cauliflower mosaic virus genome (accession no. emb|V00141.1|), and *aadA* gene (accession no. MH973510.1)

The PCR amplicons obtained from the amplification of primers for *aadA* partial gene in some bacterial DNA samples (ten samples); four samples (B5, B7, B8, B9) from the G2 group and six samples (B12, B13, B14, B17, B18, B19) from G3 group, were purified using total fragment DNA purification kit (MEGA quick-spin, iNtRON Biotechnology, Inc). Internal nucleotide sequencing of the purified DNA was performed by MWG-Biotech AG.

Bioinformatics-based protein analysis
Sequences alignment

Protein analysis in the present work is mainly based on sequence alignment using nucleotide and protein alignment software. The *aadA* DNA sequences were analyzed and aligned via Clustal Omega software [22]. Amplified aadA DNA sequences were further translated to protein using the Expasy translation tool (https://web.expasy.org/translate/).Multiple alignments of the translated protein sequences were done using Clustal Omega. The *aadA* nucleotide and translated protein sequences were compared with sequence databases using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phyre2 engine program (Protein Homology/ analogy Recognition Engine V 2.0)

We also employed a set of related software (Phyre2) available on the web to predict protein modelling, structure, function, and possible effects of mutations [23].

The possible functionality of the translated aadA protein of each bacterial sample studied (10 samples from both G2 and G3 groups), was evaluated employing the Phyre2 engine program (available at http://www.sbg. bio.ic.ac.uk/phyre2). The phyre2 system incorporates a protein sequence database and a protein structure database for predicting the 3D structure of each submitted protein sequence.

Results and Discussion

The present work is a complementary step analysis conducted by coupling both experimental PCR detection assay with the available bioinformatics tools to evaluate the possible functionality of the horizontally transferred antibiotic resistance *aadA* gene (284 bp-target) from the transgenic diet to the microbiome of rats after 90 days of feeding duration.

Experimental identification of Horizontal transfer of GM-DNA from the diet into enteric microflora of the investigated rats

To confirm the possible occurrence of gene transfer from the GM diet to gut microflora, we employed a set of primers for the CaMV35S promoter (P35S) since most of the gene constructs used in genetic transformation are under the control of the CaMV35S promoter [24].

Results as shown in Fig. 1 indicated that there was no amplification of the P35S primers occurred in the DNA of bacterial cells extracted from the control rats euthanized shortly after birth (G1). Fig. 1 also showed that primers for P35S were amplified in most bacterial DNA extracted from the microflora of the other two groups fed on the GM diet for 30 or 90 days, confirming the transfer of the genetic materials of the GM diet to the gut microflora. The transfer of the amplified P35S (195 bp-target) nucleotide sequences from the GM diet to the liver and brain tissues of experimental rats fed the GM diet for three months was previously confirmed by Oraby and colleagues [7]. Blast analysis of the nucleotide sequences of the amplified P35S in liver and brain cells [7] shared 100% homology with the corresponding segment in the cauliflower mosaic virus (emb|V0014 1.1|) as many binary vectors that are usually used in genetic transformation.

Amplification of a partial segment of the *aadA* gene in most bacterial DNA samples in the

present work (Fig. 2) proved experimentally the transfer of this segment horizontally from the transgenic diet into DNA of microflora present in the gastrointestinal tract (GIT) of rats fed on this diet for 30 or 90 days.

Bioinformatics-based protein analysis

Transfer of the partial segments (284 bp-target) from the *aadA* gene, as detected by PCR assay was further confirmed by analysis of their internal nucleotide sequences. This analysis is based on nucleotide sequence determination, translation to protein, and alignment with the GenBank database.

Sequences alignment

Analysis of the nucleotide sequences revealed that the size of the retrieved *aadA* sequences was 284 bp in all DNA investigated samples. The nucleotide sequence of the transferred DNA segment (284 bp) represents approximately 36% of the total *aadA* gene length (792 bp). Translated protein using the ExPasy program gave a partial protein stretch of 94 residues representing approximately 36% of the total aadA protein length (263 amino acids).

Multiple sequence alignments indicated that the amplified *aadA* DNA segments and their translated proteins were similar in all microbiota samples therefore one representative DNA sequence was deposited to the GenBank database (accession Number OP554293). The *aadA* nucleotide and translated protein sequences also shared 100% identity with the *aadA* gene and protein in the numbers of Cloning vectors (Table 2).

Prediction of aadA protein secondary and 3D structures

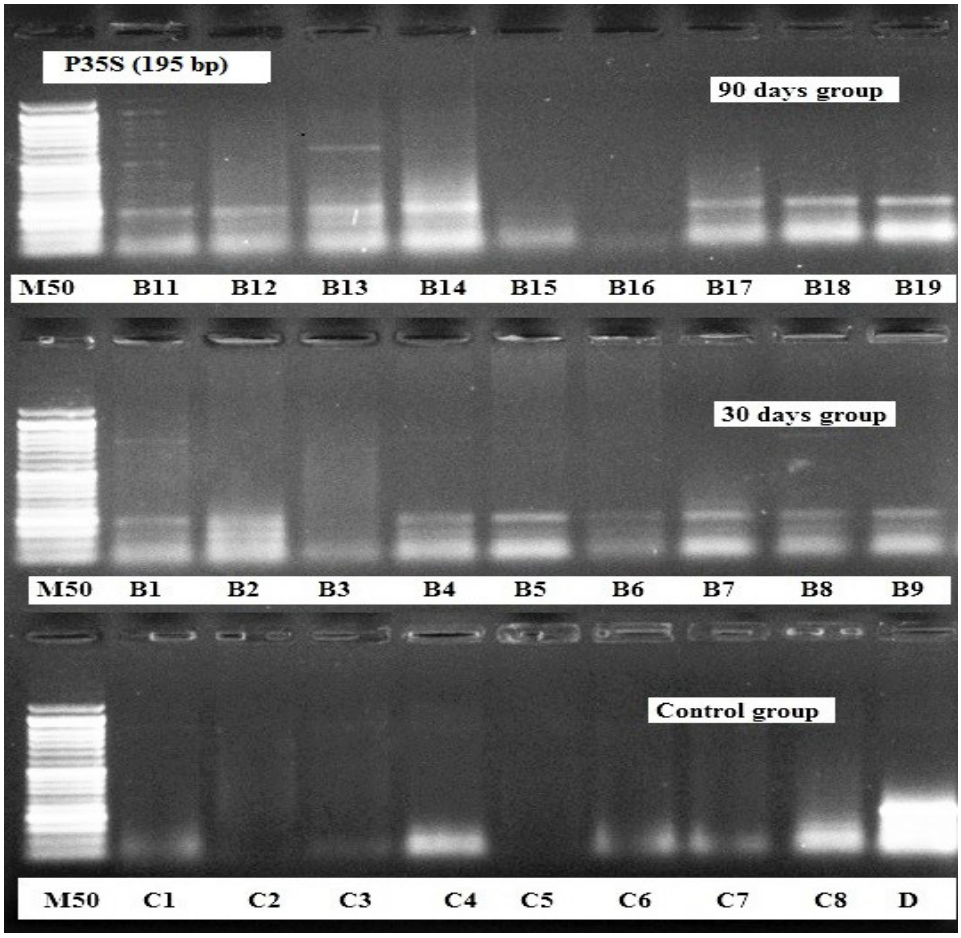

Fig. 1. PCR amplification products of P35S (195 bp) fragments in bacterial DNA samples of rats fed on GM-diet for 30, and 90 days. Control group; rats were euthanized shortly after birth. D is for the GM-diet.

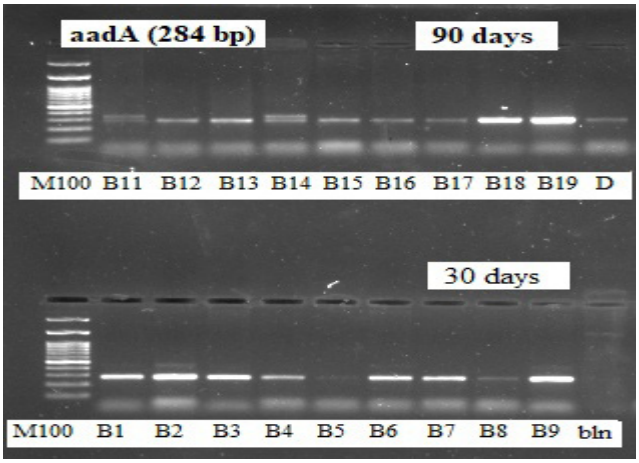

Fig. 2. PCR amplification products of *aadA* (284 bp) gene in bacterial DNA samples of rats fed on GM-diet for 30, and 90 days. D is for the GM diet, bln is for blank

TABLE 2. List of accession numbers of representative vectors that shared 100% identity with the investigated *aadA* nucleotides and predicted protein sequences.

Vector	aaA Nucleotide accession number	aadA protein accession number
Cloning vector pTJV1Sc2-tetA, complete sequence	OK423785.1	UOX75826.1
Cloning vector pTJV1Sc-rpoB, complete sequence	OK377063.1	UOX75819.1
Cloning vector pTJV1Sc3-rpoB plasmid pTJV1Sc3-rpoB, complete sequence	OK483345.1	UOX75815.1
Vector CDF-MpCYP97C-MpLCYe DNA, complete sequence	LC654938.1	BDB95794.1
Binary vector pKT-NM-erNEON, complete sequence.	MH973510.1	AYR16910.1

The predicted secondary structure of the modelled aadA protein consisted of 40% alpha helices, 14% beta strands, and 17% disordered as shown in Fig. 3. Secondary structure plays a substantial role in protein structure folding [25]. It is used to identify protein features for fold recognition [26] and can also be used to assess the quality of a model built with (a tertiary structure prediction method).

The secondary structure consists of different regions: two of these secondary structural elements are alpha helices (with the highest percentage; 40%) and beta strands. These elements are spontaneously formed as an intermediate process before the protein folds into its three-dimensional tertiary structure [27] to carry out its function. The third region consists of polypeptide segments (disordered segments). Disordered segments are not likely to form a defined three-dimensional structure, but they may be functional [28] when they reached a certain length (>30 residues). Functional disordered segments including certain disease-related proteins [29] may occur in 33.0% of eukaryotic proteins [30].

Since the translated aadA protein partial sequences in the ten investigated samples were all similar, the 3D structures of all submitted protein sequences were also similar. The predicted 3D structure (Fig. 4) has been modeled with 48% identity and 100% confidence by the single highest-scoring template of aminoglycoside adenyltransferase (c4cs6A) in the protein database (PDB DOI:http://doi.org/10.2210/pdb4CS6/pdb). The present investigated aadA protein has a reasonable sequence identity equal to 48%, given that sequences with less than 20% sequence identity can only generate completely different 3D structures [31].

The reliability of the 3-dimensional atomic

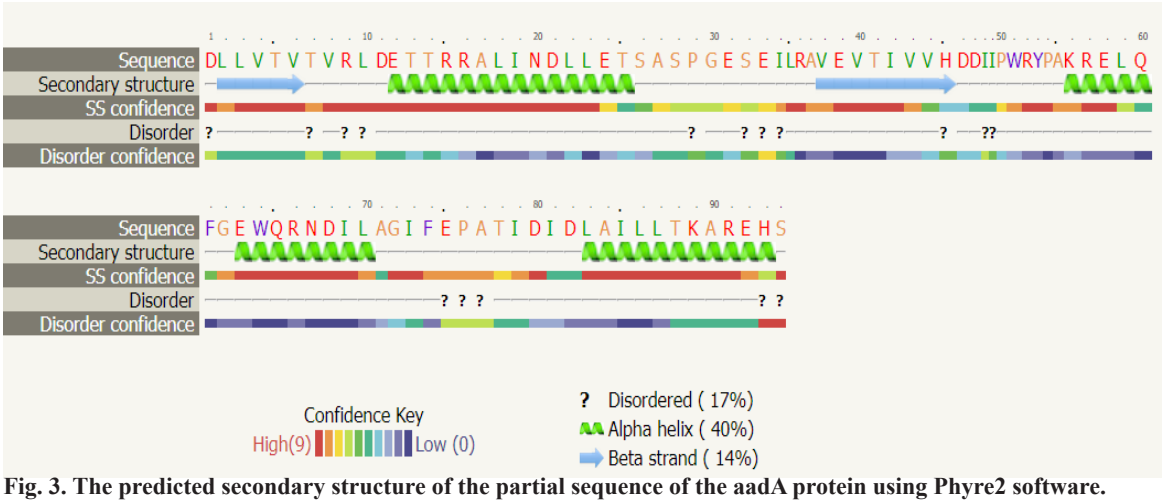

Fig. 3. The predicted secondary structure of the partial sequence of the aadA protein using Phyre2 software.

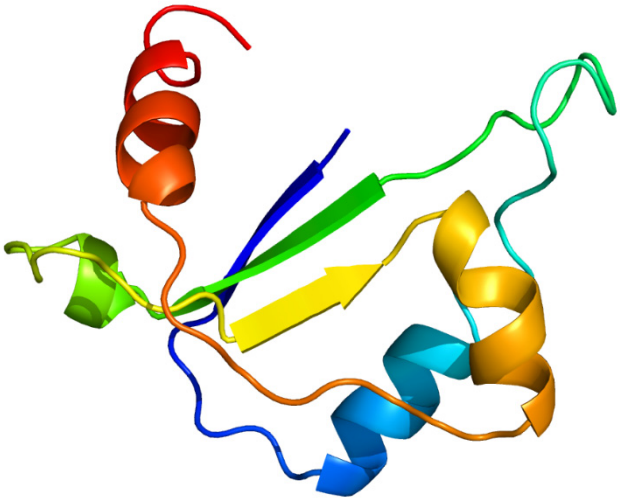

Fig. 4. The predicted 3D structure (modeled according to the template c4cs6A) for the investigated partial aadA protein sequence submitted to Phyre2 program.

models of the aadA protein and the overall quality of the predicted protein can be deduced, to some extent, from the structure quality of the template protein processed by the PDB X-Ray structure validation report. According to the structure validation report, the structure quality of the template protein covered several key quality indicators (Fig. 5) of the protein structure. The percentile ranks below (Fig. 5) revealed that the template protein model is a moderate structure. The figure shows that the model fitted to a small subset of the experimental data (Rfree): Clashes, Rotamers, and Ramachandran. The Clashscore calculated by MolProbity [32] represents the number of pairs of atoms (8 clashes) in the present model that are close to each other. The figure also demonstrates that the chosen model (C4cs6A) has no Ramachandran outliers. Ramachandran outliers are those amino acids with non-favorable dihedral angles which sometimes might play a significant role in protein function [33]. The Sidechain outliers score (5.5%) is the percentage of residues with unusual sidechain conformation concerning the total number of residues available. The last described indicator is the real-space R-value (RSRZ outliers) which measures the fitting quality between a part of the atomic model and the data in real space [34]. The RSRZ value in the present model is equal to 0 values indicating that this model has no RNA or DNA. A residue with an RSRZ value greater than two is considered an RSRZ outlier.

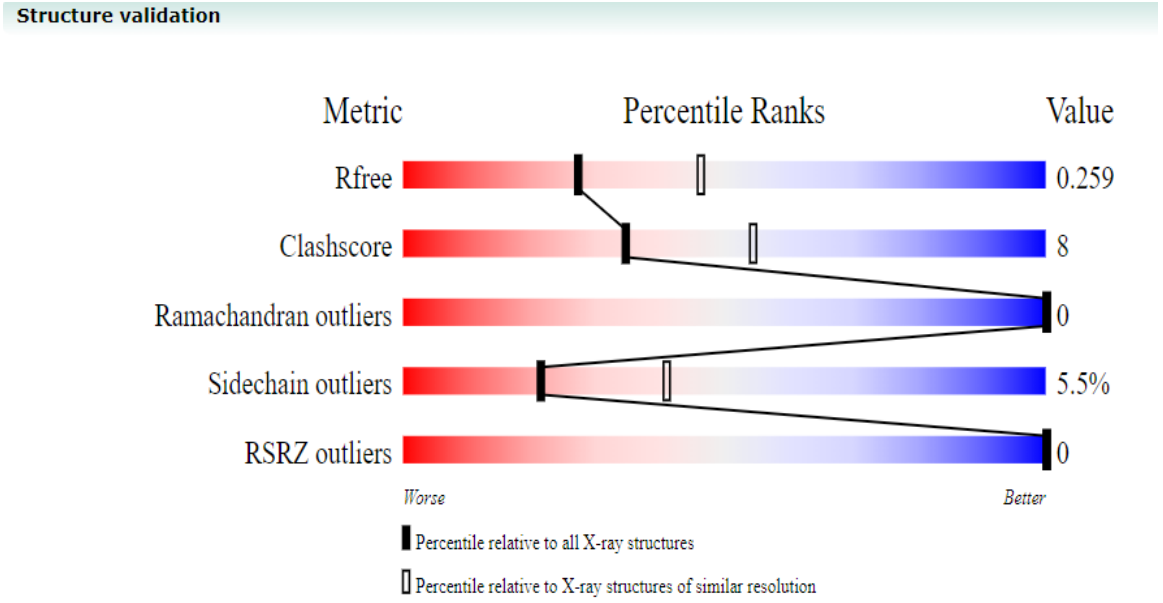

Fig. 5. Percentile ranks as quality indicators of the structure of aadA protein compared to others previously deposited to Protein Data Base.

The resulting structure validation report also indicated that the template protein model contains two unique types of molecules. Molecule 1 is the aminoglycoside adenyltransferase protein and molecule 2 is water.

The size of the investigated partial protein segment is 94 residues which covers 36% of the whole template molecule (260 residues) of the aminoglycoside adenyltransferase protein. The investigated aadA segment occupies the area between residue 46 and residue 140, as demonstrated in Fig. 6 (the amino acids stretch between the two black arrows).

Quality assessment of the aadA template protein model proceeded through standard geometry, which describes the covalent geometry parameters for protein molecules. These parameters provide information on bond lengths, angles, chirality, and planarity. The validation report for the model quality of the template aadA protein demonstrated that there are no bond lengths outliers, bond angle outliers, or chirality outliers. The only planarity outlier reported type PRO was at residue number 156. The investigated partial aadA protein, as demonstrated in Fig. 6, stretched out only between residues 46 to residue 140. All data and refinement statistics reported for the template aadA sequence revealed no significant pseudo-translation detected in the template molecule structure.

The major component of livestock feed is transgenic plant products. The possible horizontal transfer of antibiotic resistance (AR) genes to the microflora present in the gastrointestinal tract (GIT) of farm animals is one of the essential topics to address during the safety assessment of GM products. The transfer of genes from diets to microbiota in the gut has been confirmed by many authors [1, 2]. Even the transfer of a full-length gene was previously reported [12]. Nevertheless, the possible functionality of the transferred GM-DNA did not receive enough attention.

The present study confirmed experimentally the possible occurrence of gene transfer from the transgenic diet to gut microflora in GIT of rats fed on the GM diet for 90 days (Fig. 1). PCR analysis detected the presence of partial segments from the CaMV35S promoter in most DNA of the cultured bacterial samples. Oraby and colleagues [7] reported the transfer of an 80% segment of the CaMV35S promoter from the GM diet to experimental animals where part of the P35s (195bp) shared 100% homology with the corresponding segment in the cauliflower mosaic virus (emb|V0014 1.1|).

The partial DNA segment of the *aadA* gene, representing 36% of its total length of the gene, as reported in the present work (Fig. 2) also transferred to the bacterial genome of the gut microflora of experimental animals fed the GM diet for 30 or 90 days.

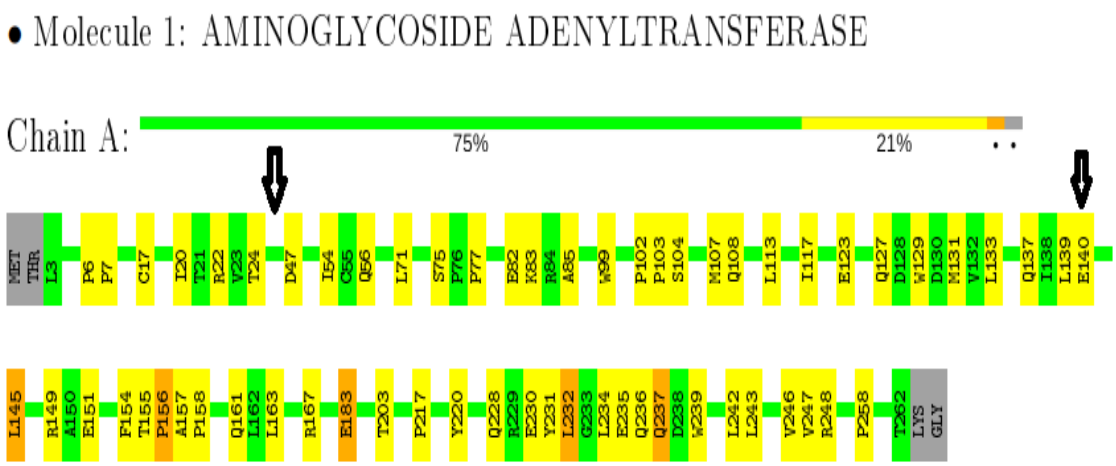

Fig. 6. Residue-property plots Outliers are colored coded; green=0 outlier, yellow= 1 outlier, orange= 2 outliers. Grey residues are not represented in the model.

Bioinformatics analysis is one of the tools used to support the occurrence of horizontal gene transfer. It is considered the most efficient way to evaluate the extent of similarity between GM event sequences and microbial genomes [35]. The present analysis of the results began by describing the internal nucleotide sequences of the PCR amplicons obtained from the amplification of the partial *aadA* gene segment in cultured bacterial samples isolated from the gut of experimental animals. The identities of the investigated sequences are determined depending on sequence databases. As presented above, multiple sequence alignments indicated that the amplified DNA segments and their translated proteins were similar in all bacterial samples and shared 100% identity with the *aadA* gene and protein in many binary vectors (Table 2).

Bioinformatics analysis using the Phyre2 program [23] proceeded to distinguish any hazard associated with the GM sequences by informing on similarity with microbial sequences encoding known functions. The principal concept in the genomic section of bioinformatics is homology. Homology between genes is involved in the prediction of gene function. It can be employed to infer that a gene with an unknown function may share the other homologous gene's known function [36]. Sequences falling below a 20% sequence identity will have a completely different structure [31]. The present investigated aadA protein partial sequence has a reasonable sequence identity (48%) to the template protein.

To predict protein structure reliably, homology is also an important concept in the structural section of bioinformatics. Homology is an essential tool to determine which parts of a protein are important in structure formation and interaction with other proteins. Once the structure of a protein is known, the structure of the homologous protein can be predicted. Detectable levels of sequence similarity usually imply significant structure similarity [37].

Results of the current study indicated that homology between the investigated aadA partial protein sequences and the corresponding segment of aminoglycoside adenyltransferase protein is 48% identity. However, the investigated segment represents only 36% of the total length of the protein, a full-length protein sequence is required to estimate reliably its general physical properties [31, 37]. Therefore, the present investigation also highlights the importance of full *aadA* gene detection and studying the whole length of the predicted aadA protein sequence to evaluate its properties and possible functionality. Adding to the risks associated with the random insertion of the horizontally transferred genes, which may induce genetic instability, there are other threats related to the possible functionality of the full-length gene transferred and its transcribed protein in the recipient organism. Therefore, the present study also recommends utilizing genetic markers

other than antibiotic resistance genes during the production of genetically modified crops to avoid any health hazards to the consumer.

Conclusions

The horizontal transfer of a partial sequence of the antibiotic resistance *aadA* gene from the GM diet to microbiota in rats was confirmed by PCR assay. Transferred *aadA* segment and its predicted protein sequence are 100% similar to the corresponding sequences of the *aadA* gene in cloning vectors. Results indicated the possible functionality of the aadA protein only when the full-length gene is transferred to the recipient organism.

Acknowledgments

This work was funded by the National Research Center (NRC) in Egypt under the 11th Research Project Plan, Project ID:11040201. The authors wish to thank Dr. Mahrousa Kandil for providing the experimental animals' large intestines (rectum and caecum).

Funding statement

The authors declare that this work was funded by the National Research Centre in Egypt under the 11th Research Project Plan, Project ID: 11040201.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conflict of Interests

None**.** The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed equally to this work. Accordingly, all authors agree to be accountable for the content of this work. All authors read and approved the final manuscript.

References

1. Chassy, B. Food safety of transgenic rice in: Rice improvement in the genomics era. Datta, S.K., ed., Taylor and Francis (2008). https://doi.org/10.1201/9780367803117
2. Aviv, G., Rahav, G. and Gal-Mor, O. Horizontal transfer of the Salmonella enterica serovar infantis resistance and virulence plasmid pESI to the gut microbiota of warm-blooded hosts. mBio, 7(5), e01395-16 (2016). https://doi.org/10.1128/mBio.01395-16
3. Aminov, R.I., Horizontal gene exchange in environmental microbiota. Front. Microbiol., 22, 158 (2016). https://doi.org/10.3389/fmicb.2011.00158
4. Robinson, K.M., Stieber, K.B. and Hoop, J.C.D. A review of bacteria-animal lateral gene transfer may inform our understanding of diseases like cancer. PLoS Genet, 9(10), e1003877 (2013). https://doi.org/10.1371/journal.pgen.1003877
5. Gyles, C. and Boerlin, P. Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease. Vet. Pathol., 51(2), 328–340 (2014). https://doi.org/10.1177/0300985813511131
6. Rizzi, A., Raddadi, N., Sorlini, C., Nordgard, L., Nielsen, K.M. and Daffonchio, D. The stability and degradation of dietary DNA in the gastrointestinal tract of mammals - implications for horizontal gene transfer and the biosafety of GMOs. Crit. Rev. Food Sci. Nutr., 52(2), 142-61 (2012). https://doi.org/ 10.1080/10408398.2010.499480
7. Oraby, H.A-S., Kandil, M.H., Hassan, A.A.M. and Al-Sharawi, H.A. Addressing the issue of horizontal gene transfer from a diet containing genetically modified components into rats' tissues. Afr. J. Biotechnol., 13(43), 4410- 4418 (2014). https:// doi.org/10.5897/AJB2014.14088
8. Chainark, P., Satoh, S., Hirono, I., Aoki, T. and Endo, M. Availability of genetically modified feed ingredient: investigations of ingested foreign DNA in rainbow trout Oncorhynchus mykiss. Fish Sci., 74, 380-390 (2008). https://doi.org/10.1111/j.1444-2906.2008.01535.x
9. Tudisco, R., Mastellone, V., Cutrignelli, M.I., Lombardi, P., Bovera, F., Mirabella, N., Piccolo, G., Calabrò, S., Avallone, L. and Infascelli, F. Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. Amin., 4(10), 1662-1671 (2010). https://doi.org/10.1017/S1751731110000728

10. Mazza, R., Soave, M., Morlacchini, M., Piva, G. and Marocco, A. Assessing the transfer of genetically modified DNA from feed to animal tissues. Transg. Res., 14(5), 775-784 (2005). https://doi.org/10.1007/s11248-005-0009-5

11. Sharma, R., Damgaard, D., Alexander, T.W., Dugan, M.E., Aalhus, J.L., Stanford, K. and McAllister, T.A. Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. J. Agric. Food Chem., 54(5), 1699-1709 (2006). https://doi.org/10.1021/jf052459o

12. Spisak, S., Solymosi, N., Ittzes, P., Bodor, A., Kondor, D., Vattay, G., Bartak, B.K., Sipos, F., Galamb, O., Tulassay, Z., Szallasi, Z., Rasmussen, S., Sicheritz-ponten, T., Brunk, S., Molnar, B. and Csabai, I. Complete genes may pass from food to human blood. Plos One, 8(7), e69805 (2013). https://doi.org/10.1371/journal.pone.0069805

13. Liu, L., Tong, H. and Dong, X. Function of the pyruvate oxidase-lactate oxidase cascade in interspecies competition between Streptococcus oligofermentans and Streptococcus mutans. Appl. Environ. Microbiol., 78(7), 2120-2127 (2012). https://doi.org/10.1128/AEM.07539-11

14. Nawaz, M.A., Mesnage, R., Tsatsakis, A.M., olokhvast, K.S., Yang, S.H., Antoniou, M.N. and Chung, G. Addressing concerns over the fate of DNA derived from genetically modified food in the human body: A review, Food Chem. Toxicol., 124, 423–430 (2019). https://doi.org/10.1016/j.fct.2018.12.030

15. Netherwood, T., Martín-Orúe, S.M., O'Donnell, A.G., Gockling, S., Graham, J., Mathers, J.C. and Gilbert, H.J. Assessing the Survival of Transgenic Plant DNA in the Human Gastrointestinal Tract. Nat. Biotechnol., 22(2), 204-9 (2004). https://doi.org/10.1038/nbt934

16. Wilcks, A., vanHoek, A.H., Joosten, R.G., Jacobsen, B.B. and Aarts, H.J. Persistence of DNA studied in different ex vivo and in vivo rat models simulating the human gut situation. Food Chem. Toxico., l42(3), 493-502 (2004). https://doi.org/10.1016/j.fct.2003.10.013

17. Wilcks, A. and Jacobsen, B.B. Lack of detectable DNA uptake by transformation of selected recipients in mono-associated rats, BMC Res. Notes, 3, 49 (2010). https://doi.org/10.1186/1756-0500-3-49

18. Oraby, H.A-S, Aboul-Maaty, N.A-F, Al-Sharawi, H.A. and Osman, N.M. Horizontal transfer of antibiotic resistance genes into microflora and blood cells in rats fed on GM-diet, Bull. Natl. Res. Cen., 46, 268 (2022). https://doi.org/10.1186/s42269-022-00956-0

19. Oraby, H.A-S., Aboul-Maaty, N.A-F and Al-Sharawi, H.A. Exploratory and confirmatory molecular approaches to determine genetically modified status in different crops, Bull. Natl. Res. Cen., 45, 196 (2021). https://doi.org/10.1186/s42269-021-00654-3

20. Hemmer, W. Foods derived from genetically modified organisms and detection methods, BATS-Report, 2/97, Basel, Switzerland (1997). Available online at www.bats.ch/abstr/297intro.htm

21. Clark, N.C., Olsvik, O., Swenson, J.M., Spiegel, C.A. and Tenover, F.C. Detection of a streptomycin/spectinomycin adenyltransferase gene (aadA) in Enterococcus faecalis. Antimicrob Agents Chemother., 43, 157-160 (1999). https://doi.org/10.1128/AAC.43.1.157

22. Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D. and Higgins, D.G. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol., 7, 539 (2011). https://doi.org/10.1038/msb.2011.75

23. Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N. and Sternberg, M.J. The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Protoc., 10, 845–858 (2015). https://doi.org/10.1038/nprot.2015.053

24. FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) Safety assessment of food derived from genetically modified microorganisms. Report of a joint FAO/WHO expert consultation on food derived from biotechnology, WHO headquarters, Geneva, Switzerland, 24-28 (2001). https://www.who.int/foodsafety/publications/biotech/en/ec_sept2001.pdf

25. Ji, Y.Y. and Li, Y.Q. The role of secondary structure in protein structure selection. Eur. Phys. J. Soft Matter, 32, 103-7 (2010). https://doi.org/10.1140/epje/i2010-10591-5

26. Kwok, S.C., Mant, C.T. and Hodges, R.S. Importance of secondary structural specificity determinants in protein folding: Insertion of a native β-sheet sequence into an α-helical coiled-coil. Protein Sci: A Publication of the Protein Society, 11(6), 1519-1531(2002). https://doi.org/10.1110/ps.4170102

27. Sun, P.D., Foster and C.E., Boyington, J.C. Overview of protein structural and functional folds. Curr. Protoc. Protein Sci., 35(1), 1711–171189 (2004). https://doi.org/10.1002/0471140864.ps1701s35

28. Uversky, V.N and Dunker, A.K. Understanding protein non-folding. Biochim. Biophys. Acta, 1804(6), 1231-1264 (2010). https://doi.org/10.1016/j.bbapap.2010.01.017

29. Uversky, V.N., Oldfield, C.J. and Dunker, A.K. Intrinsically disordered proteins in human diseases: introducing the D2 concept. Annu. Rev. Biophys., 37, 215–246 (2008). https://doi.org/10.1146/annurev.biophys.37.032807.125924

30. Ward, J.J., Sodhi, J.S., McGuffin, L.J., Buxton, B.F. and Jones, D.T. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J. Mol. Biol., 337(3), 635-645 (2008). https://doi.org/ 10.1016/j.jmb.2004.02.002

31. Chothia, C. and Lesk, A.M. The relation between the divergence of sequence and structure in proteins. EMBO J., 5(5), 823-826 (1986). https://doi.org/ 10.1002/j.1460-2075.1986.tb04288.x

32. Chen, V.B., Arendall 3rd, W.B., Headd, J.J., Keedy, D.A., Immormino, R.M., Kapral, G.J., Murray, L.W., Richardson, J.S. and Richardson, D.C. MolProbity: all-atom structure validation for macromolecular crystallography, Acta Cryst., D66(Pt1), 12–21 (2010). https://doi.org/10.1107/S0907444909042073

33. Ollis, D.L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S.M., Harel, M., Remington, S.J., Silman, I. and Schrag, J. The alpha/beta hydrolase fold. Protein Eng., 5, 197-211(1992). https://doi.org/ 10.1093/protein/5.3.197.

34. Jones, T.A., Zou, J.Y., Cowan, S.W. and Kjeldgaard, M. Improved methods for building protein models in electron density maps and the location of errors in these models, Acta Cryst., A47(Pt 2), 110-119 (1991). http://doi.org/10.1107/s0108767390010224

35. Gennaro, A., Gomes, A., Herman, L., Nogue, F., Papadopoulou, N. and Tebbe, C. Technical report on the explanatory note on DNA sequence similarity searches in the context of the assessment of horizontal gene transfer from plants to microorganisms. European Food Safety Authority (EFSA), EN-1273, 1-11 (2017). https://doi.org/10.2903/sp.efsa.2017.EN-1273

36. Chandonia, J.M. and Brenner, S.E. The impact of structural genomics: expectations and outcomes, Science. 311(5759), 347-351 (2006). https://doi.org/10.1126/science.1121018

37. Marti-Renom, M.A., Stuart, A.C., Fiser, A., Sanchez, R., Melo, F. and Sali, A. Comparative protein structure modeling of genes and genomes. Annu. Rev. Biophys. Biomol. Struct., 29, 291–325 (2000). https://doi.org/10.1146/annurev.biophys.29.1.291

الخطوات الأساسية لتقييم الكفاءة الوظيفية المحتملة للجينات المقاومة للمضادات الحيوية المنقولة أفقياً من النظام الغذائي المعدل وراثياً إلى البكتيريا المعوية للحيوانات

هناء عبد الصادق عرابى[1] ، أمال احمد محمد حسن[1] وناديه أبو الفتوح أبو المعاطي[1]
[1]قسم بيولوجيا الخلية - معهد بحوث التكنولوجيا الحيوية - المركز القومي للبحوث - القاهرة - مصر.

قد يسبب الانتقال الأفقي للجينات المقاومة للمضادات الحيوية من الأعلاف المعدلة وراثياً إلى البكتيريا المعوية في الحيوانات آثار صحية ضارة للمستهلك. يعد هذا العمل دراسة تكاملية لتقييم الكفاءة الوظيفية المحتملة لجين أمينوغليكوزيد أدينيل ترانسفيراز (*aadA*) المنقول أفقيًا من الأعلاف المعدلة وراثيًا إلى ميكروبيوم الجرذان بعد 90 يومًا من مدة التغذية، حيث اقترن كل من الكشف العملي لحدوث ظاهرة الانتقال الأفقي لهذا الجين باستخدام تقنية التفاعل المتسلسل لإنزيم البلمرة مع توظيف التطبيقات المتاحة لتقنية المعلوماتية الحيوية لتقييم الكفاءة الوظيفة له.

تم فحص الأجزاء المنقولة من جين *aadA* إلى الجينوم البكتيري (284 نيوكليوتيدة) وتأكيدها من خلال دراسة سلسلة النيوكليوتيدات الداخلية لأجزاء الحمض النووي المضخمة بناءً على تحديد تسلسلها النيوكليوتيدي وترجمته إلى بروتين ثم مواءمة هذه النتائج مع قاعدة بيانات الجينات ((GenBank.أشارت النتائج إلى أن التسلسل النيوكليوتيدي للجزء الذي تم فحصه لجين *aadA* والبروتين المتنبأ به متشابهان تمامًا مع التسلسلات المقابلة من جين *aadA* في العديد من نواقل الاستنساخ وهذا يؤكد انتقال هذا الجين أفقيا من الغذاء المعدل وراثيا الى جينوم البكتيريا المعوية لهذه الحيوانات. أشارت النتائج أيضًا إلى أن البنية ثلاثية الأبعاد المتوقعة للجزء المنقول من بروتين aadA التي تم فحصها قد تم تصميمها بدرجة ثقة 100٪ وهوية 48٪ بواسطة أعلى تسجيل لنموذج الأمينوغليكوزيد أدينيل ترانسفيراز في قاعدة بيانات البروتين. التقرير الناتج عن PDB X-Ray للتحقق من أو للتصديق على صحة هيكل وفاعلية نموذج ال-aadA بروتين أكد عدم وجود قيم متطرفة أو غير متجانسة لأي من البيانات وإحصائيات النموذج المقترح في بنية جزيء النموذج هذا وتسلط النتائج الضوء أيضًا على أهمية دراسة الطول الكامل لتسلسل البروتين لتقييم خصائصه الفيزيائية العامة وفاعليته الوظيفية.

الكلمات الدالة: المحاصيل المعدلة وراثيا، بكتيريا الأمعاء الدقيقة، جين *aadA*، أدوات تحليل المعلومات الحيوية، التنبؤ بالبروتين.