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Mutation in Transglycosylase Domain of Penicillin Binding Protein 1A (PBP1A) and *Helicobacter pylori*

David Guindo[1, 2, *], Alpha Seydou Yaro[1, 2, 3], Astan Traore[1, 2], Yaya Bouare[1, 4], Bernard Sodio[1, 2]

[1]Department of Biology, Faculty of Sciences and Techniques, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali

[2]Entomology and Parasitology Laboratory, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali

[3]International Center for Excellence in Research, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali

[4]Applied Microbiology Laboratory, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali

Email address:

davidguindo@yahoo.com (David Guindo)

[*]Corresponding author

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Abstract: *Helicobacter pylori* (*Hp*) is associated in human gastric diseases. It touches more than 50% of the world's population. Clinically, amoxicillin is one of the antibiotics used to treat the pathogenic agent. It inhibits bacterial wall synthesis, by blocking the transpeptidase domain of penicillin binding protein 1A (PBP1A). Mutations in this domain are responsible for antimicrobial resistance (AMR). The transglycosylase domain is necessary to activate the transpeptidase. But its part in the resistance remains little documented. The objective of this study was to analyze the protein sequence of this domain in samples of Malian patients. Therefore, the PCR product was sequenced from five *Hp* positive samples. After alignment with the *Helicobacter pylori* 26695 sequence (reference), several amino acid substitutions were identified: T30N / F67S / I79V / I101V / F125L / I148L for sample 1 (PBP1A-ML1); G44S / I101V / F125L for PBP1A-ML2 and PBP1A-ML5 and A36V / F125L / I148L for PBP1A-ML3 and PBP1A-ML4. The last two groups of mutations were also observed in *Hp* PBP1A from other continents. Their existence shows the distribution of two or more *Hp* strains in Mali and worldwide. Although their direct implications for AMR have not been demonstrated, but their presence is supposed to modify the affinity of amoxicillin for its target. Considering the importance of transglycosylase in the activation of the transpeptidase domain, substitutions would allow *Hp* to adapt to a change in its environment. Additional research is needed to identify the role of observed substitutions.

Keywords: *Helicobacter pylori*, Amoxicillin, PBP1A, Transglycosylase Domain, Diversity, Mali

1. Introduction

The pathogenic agent *Helicobacter pylori* (*Hp*) is a spiral-shaped Gram-negative organism that was initially isolated from the human stomach. It is responsible for gastric disease varying from chronic gastritis to gastric cancer (GC) [1-3]. The presence of this pathogen in humans precedes the first wave of migration from Africa to the rest of the world (-60,000 years). During the process, the genetic diversity and virulence of *Hp* evolved, due to mutations and the acquisition of new genes [4, 5]. The worldwide average prevalence of infection is over 50% and may increase to 90% in some developing countries [6]. In general, triple or quadruple therapy with a proton pump inhibitor (PPI) and antibiotics can eradicate the pathogen and restore the tissue damage it causes [7]. The PBP1A transglycosylase (TG) and transpeptidase (TP) domains contribute to bacterial wall synthesis and homeostasis [8]. Amoxicillin is one of the first-choice antibiotics in the clinic. This beta-lactam is a bactericide that prevents the neosynthesis of bacterial peptidoglycans by inhibiting the activity of the PBP1A TP subunit. The predictive data

indicate that amoxicillin has a non-covalent interaction with amino acids at 366-371, 414-416, 433, 435, 468-471 and 558-560 positions of the same domain [9]. But like many other bacteria, *Hp* is developing multi-drug resistance to clinical antibiotics. These adaptations are due to mutations in the target sequences of antibiotics or other genes that prevent their bactericidal or bacteriostatic action [7, 10-12]. With regard to amoxicillin, mutations in the interacting amino acids in the TP domain are recognized as responsible for resistance [13, 14]. However, few studies mention the implication of TG domain in resistance.

The objective of this study is to identify the presence of possible mutations in the TG domain of *Hp* strains identified in Mali and to determine their role if they exist.

2. Methods

2.1. DNA Extraction from Gastric Biopsy

Sixteen gastric biopsies were isolated from patients with symptoms of gastritis in two medical clinics in Bamako - MALI «clinique *LES ANGÉVINS* of Bagadadji and polyclinique *GUINDO* of Badala-Est». DNA extraction was realized following the protocol recommended by the kit manufacturer (Wizard ® Genomic DNA Purification Kit, Promega). It can be briefly summarized as follows: addition of 20-30 mg of the biopsy in 600 µl of nucleic lysis solution, incubation 30 min at 65°C in the water bath for cell lysis; addition of 3 µl of RNase in the cell lysate, incubation 30 min at 37°C for denaturation of RNA; addition of 200 µl of protein precipitation solution, vortexed for 20 s and incubated for 5 min at 4°C; centrifugation at 14,000 × g for 3 min; recuperated the DNA-containing supernatant in 600 µl of isopropanol; centrifuged at the same number of revolutions as the precedent for 1 min; suspended the DNA-containing pellet in 600 µl of 70% ethanol to remove the isopropanol using the same centrifugation program as the precedent; rehydrated the pellet in 100 µl of DNA rehydration solution for 60 min at 65°C in the water bath. The resulting DNA was stored at 2-8°C before use for PCR.

2.2. Amplification of the pbp1A Gene by PCR (Polymerase Chain Reaction)

The following reaction mixture was used in a final volume of 25 µl for the amplification of *pbp1A* TG domain: 25-50 ng of DNA template, 0.5 ng of *pbp1AF* primer (5'-TAGCCATTCTTATCGCTC), 0.5 ng of *pbp1AR* primer (5'-CGACTAGCATGGTGATTT), 12.5 µl of the mixed (GoTaqR G2 Hot Start Green Master Mix) from Promega, and the volume of water needed to have the 25 µl. The PCR amplification was done according to the following program: pre-denaturation 94°C for 5 min, 32 cycles (denaturation 94°C 1 min, hybridization 53°C 1 min, elongation 72°C 1 min) and a final elongation 72°C for 7 min. The resulting PCR product was migrated for 75 min at 90 volts on a 1% agarose gel [Agarose LE, Molecular Biology Grade, Thermo Fisher Ultrapure Thermo Scientific; 0.5X TBE buffer (Tris-borate-EDTA 10X, Thermo Fisher]; 0.5 µg/ml ethidium bromide, Promega). After UV light exposure, the size of the *pbp1A* fragment (530 bp) was determined using the BenchTop pGEM® DNA Markers (36 to 2645 bp).

2.3. Sequencing and Data Analysis

Sequencing of PCR products was realized by Inquaba Biotec laboratory, Pretoria-South Africa, according to their internal protocols [minimum 10 µl of primers with 10 µM concentration (+1 µl for each reaction), minimum 15 µl of PCR product]. Sanger technique was used employing ABI 3500XL genetic analyzer, POP7™, BrilliantDye™ Terminator v3.1.

The resulting sequences were visualised and cleaned with SnapGene 6.0.2 software. They are accessible in the NCBI (National Center for Biotechnology Information) database with the respective access codes: OP574217-OP574220. These ML sequences were then aligned with others' PBP1A sequences including: Gambia (EMG96538.1, EMG92274.1, EMJ42817.1, EMJ39048.1, EMH44117.1, EMH17520.1, EMH02810.1), Nigeria (QKW90261.1, QKW90267.1, QKW90266.1), China (QKW90261.1, QKW90267.1, QKW90266.1), Cambodia (TPH90784.1, TPH82730.1, TPH47395.1), Korea (ACX97926.1), Argentina (AMX23354.1, AMT85902.1), Peru (QQX48136.1, QQW86317.1, QQW80554.1, QQX45343.1), Mexico (QEF43243.1, QEF42326.1, QEF20800.1), Switzerland (RVZ97764.1, RVZ87164.1, RVZ68877.1), Holland (AAW69866.1, AAW69867.1, AAW69865.1), Poland (TLR92100.1) and *Helicobacter pylori* 26695 (AAD07660.1) as reference. The sequences were aligned via the following site: https://ngphylogeny.fr/ [15].

3. Results

After amplifying the *pbp1A* gene fragment, the migration profile of the PCR product was photographed. The image in Figure 1 shows the presence of a signal of approximately 530 bp for all samples (1–16). The size of the signal corresponds to that theoretically expected. This shows the presence of the *pbp1A* gene in the DNA samples.

PCR products from samples 1-5 were sequenced and aligned with isolated PBP1A sequences from other countries (sequences available in the NCBI database) to identify possible differences. The sequences obtained were transcribed into amino acids and respectively called PBP1A-ML1-PBP1A-ML5. Substitution of multiple amino acids was noted after alignment with PBP1A of *Hp* strains from Asia, America, Europe and other African countries. Mutations in PBP1A-ML are summarized in Table 1. For the PBP1A-ML1 sample, a substitution of amino acids T30N, F67S, I79V, I101V, F125L and I148L was noted. The PBP1A-ML2 and PBP1A-ML5 samples have the same mutations, substituting G44S, I101V and F125L. In addition to A36V in PBP1A-ML3, an F125L and I148L substitution appears in PBP1A-ML3 and PBP1A-ML4's TG. F125L appears

in all strains, both from Mali and other countries, with reference to the *Hp* 26695 strain. The majority of mutations observed in ML strains are also present in the TG sequence of *Hp* PBP1A around the world. Consequently, there is a distribution of at least two strains depending on the nature of these mutations (Appendix).

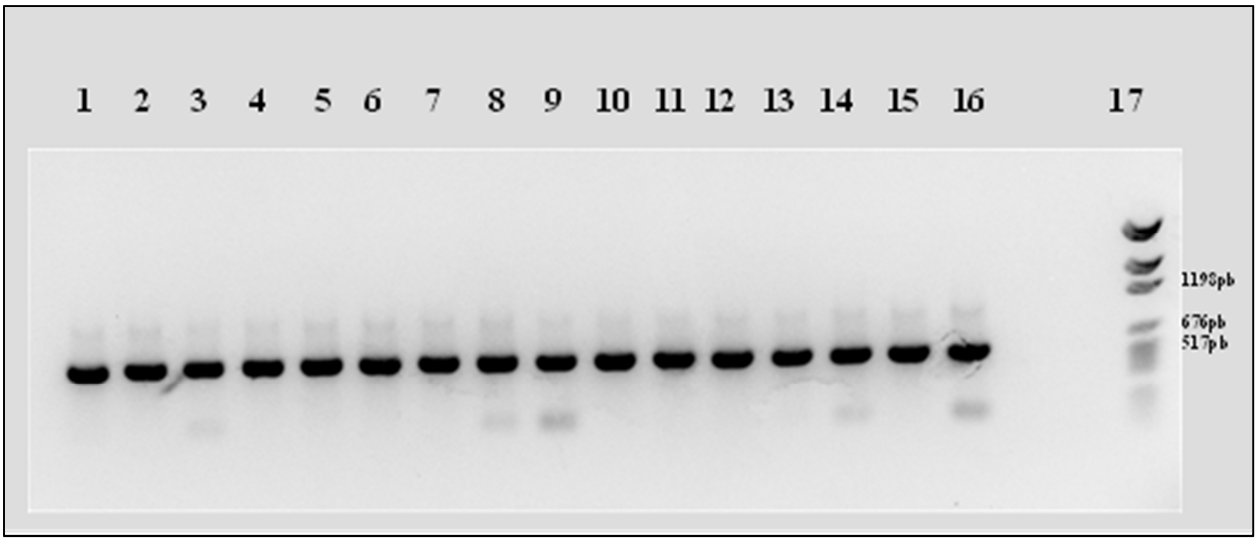

Figure 1. *Migration profile of pbp1A PCR product (530 bp). Lane 17: pGEM DNA size markers. Lanes 1-16: Gastric biopsy samples.*

Table 1. *Amino acid substitution of PBP 1A transglycosylase subunit of Helicobacter pylori strains from Mali. Reference strain: Helicobacter pylori 26695. PBP1A "Penicillin Biding Protein type 1A". The number 1 to 5 corresponds to the sample number, ML (Mali). The first amino acid is the same as in the reference, followed by its position and the amino acid of substitution. A (Alanine), F (Phenylalanine), G (Glycine), I (Isoleucine), L (Leucine), N (Asparagine), T (Threonine) and V (Valine). X indicates the presence of the substitution and empty boxes for similarity with the reference.*

	PBP1A-ML1	PBP1A-ML2	PBP1A-ML3	PBP1A-ML4	PBP1A-ML5
T*30*N	X				
A*36*V			X		
G*44*S		X			X
F*67*S	X				
I*79*V	X				
I*101*V	X	X			X
F*125*L	X	X	X	X	X
I*148*L	X		X	X	

4. Discussion

Amoxicillin is one of the first-line antibiotics used for controlling *Hp*. It targets the PBP1A protein within its transpeptidase (TP) domain. The latter, and the transglycosylase (TG) domain of the same protein, are essential for bacterial wall synthesis [8]. Indeed, amoxicillin interacts with certain amino acids from the tunnel where the key amino acid of the TP domain is found. Substitution of one or more of these amino acids causes the bacterial resistance observed clinically [9, 14]. Very few studies report variability in the TG sequence and its effect on resistance. The alignment of the TG/PBP1A sequence of five samples of Malian patients with sequences from other countries shows two strains with similar mutations around the world. The first shows a serine instead of glycine in position 44 (G44S), isoleucine 101 by valine (I101V) and phenylalanine 125 by leucine (F125L). In addition to F125L, isoleucine 148 was changed to leucine (I148L) for the second. Furthermore, the comparison with the sequences of amoxicillin-resistant (clinically established) strains is inconclusive. Because substitutions are available in PBP1A of sensitive and resistant strains. The same observation was reported in a recent study that the F125L mutation is only found in sensitive strains. Mutations in TP were the only mutations associated with antibiotic resistance [16]. Another study suggests that I79V and F125L are the consequences of their proximity to the active site of the transpeptidation enzyme (SKN368-371, SNN433-435 and KTG555-557). This proximity and the mutation of the major amino acids in the TP domain would result in a loss of the antibiotic's affinity for its target [17]. In addition, another beta-lactam antibiotic (ampicillin) covalently interacts with the PBP1A TG domain in *Escherichia coli*, not with TP [8, 18]. It is one of the most common drugs used in self-medication [19, 20]. Therefore, the substitutions seen could be the effect of *Hp's* adaptation to this antibiotic. The function of the TG domain in the stability and activation of the TP domain has been described for certain species of bacteria like *Escherichia coli* [8]. It also plays an important role in the division of bacterial cells [21]. It can be supposed, that the substitutions allowed *Hp* to adapt to a change (aggression) in its environment. More study will be needed to justify the presence of these mutations and

determine their role.

5. Conclusions

In conclusion, we have demonstrated the existence of mutations in the *Hp* transglycosylase domain in Mali and around the world. These substitutions revealed that there were two or more distinct circulating strains. Their role in the resistance was unclear. Given the absence of data on amoxicillin resistance in Mali, establishing a prevalence by identifying mutations in the TP domain, will facilitate the understanding of the role of mutations observed in the TG. A study on the resistance of *Hp* strains to anti-*Hp* drugs used in the country will optimize the treatment with the most effective molecules.

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Appendix

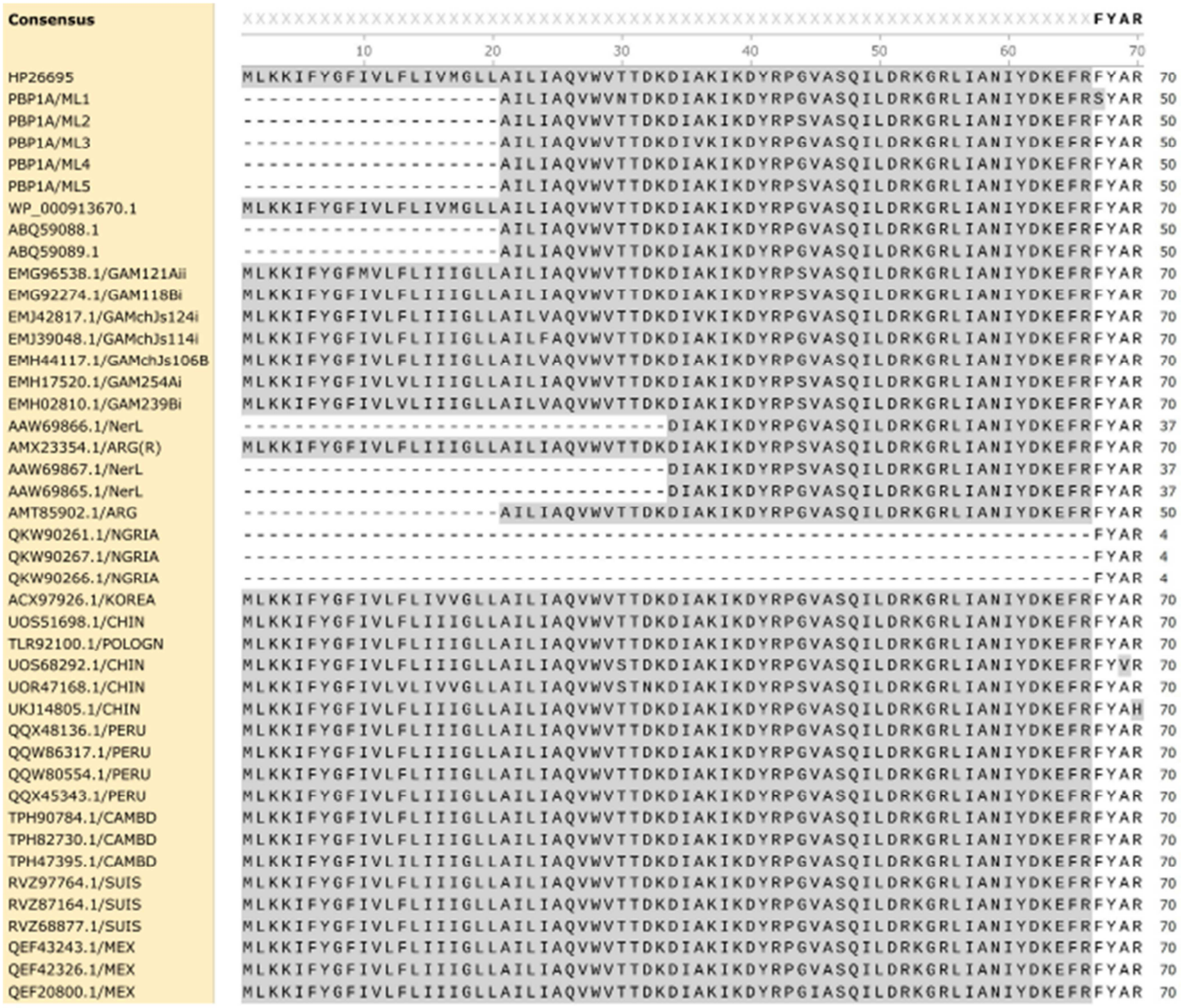

Figure A1. *Comparison of PBP1A transglycosylase domain sequences of Malian strains with those of other existing strains (amino acid 1-70). ML (Mali), GAM (Gambia), NerL (Netherlands), ARG (Argentina), NGRIA (Nigeria), CHIN (China), POLAND (Poland), CAMBD (Cambodia), SUIS (Switzerland), MEX (Mexico). NCBI (National Center for Biotechnology Information) acces codes: Mali (OP574217-OP574220), Gambia (EMG96538.1, EMG92274.1, EMJ42817.1, EMJ39048.1, EMH44117.1, EMH17520.1, EMH02810.1), Nigeria (QKW90261.1, QKW90267.1, QKW90266.1), China (QKW90261.1, QKW90267.1, QKW90266.1), Cambodia (TPH90784.1, TPH82730.1, TPH47395.1), Korea (ACX97926.1), Argentina (AMX23354.1, AMT85902.1), Peru (QQX48136.1, QQW86317.1, QQW80554.1, QQX45343.1), Mexico (QEF43243.1, QEF42326.1, QEF20800.1), Switzerland (RVZ97764.1, RVZ87164.1, RVZ68877.1), Holland (AAW69866.1, AAW69867.1, AAW69865.1), Poland (TLR92100.1) and Helicobacter pylori 26695 (AAD07660.1).*

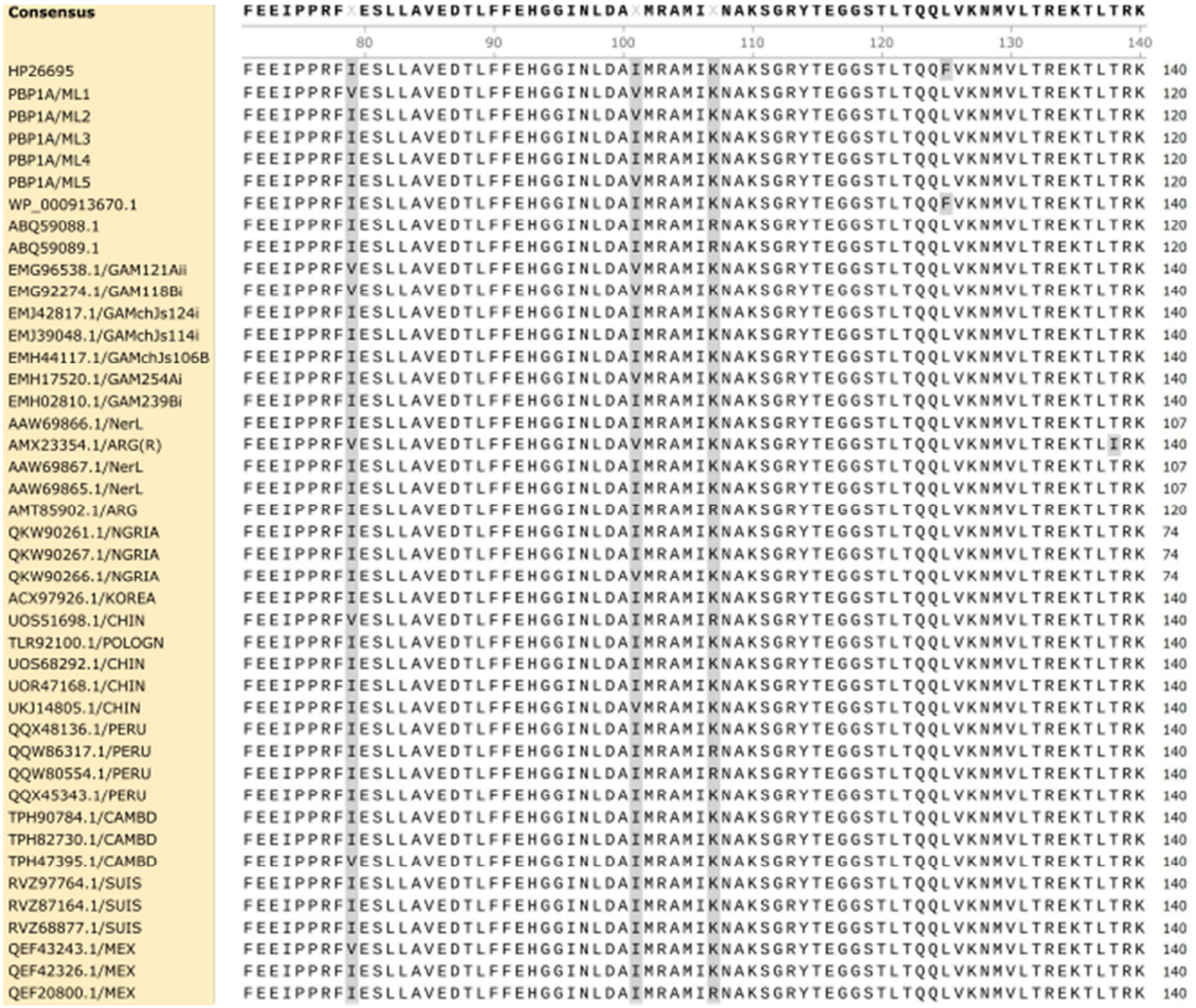

Figure A2. *Comparison of PBP1A transglycosylase domain sequences of Malian strains with those of other existing strains (amino acid 71-140). ML (Mali), GAM (Gambia), NerL (Netherlands), ARG (Argentina), NGRIA (Nigeria), CHIN (China), POLAND (Poland), CAMBD (Cambodia), SUIS (Switzerland), MEX (Mexico). NCBI (National Center for Biotechnology Information) acces codes: Mali (OP574217-OP574220), Gambia (EMG96538.1, EMG92274.1, EMJ42817.1, EMJ39048.1, EMH44117.1, EMH17520.1, EMH02810.1), Nigeria (QKW90261.1, QKW90267.1, QKW90266.1), China (QKW90261.1, QKW90267.1, QKW90266.1), Cambodia (TPH90784.1, TPH82730.1, TPH47395.1), Korea (ACX97926.1), Argentina (AMX23354.1, AMT85902.1), Peru (QQX48136.1, QQW86317.1, QQW80554.1, QQX45343.1), Mexico (QEF43243.1, QEF42326.1, QEF20800.1), Switzerland (RVZ97764.1, RVZ87164.1, RVZ68877.1), Holland (AAW69866.1, AAW69867.1, AAW69865.1), Poland (TLR92100.1) and Helicobacter pylori 26695 (AAD07660.1).*

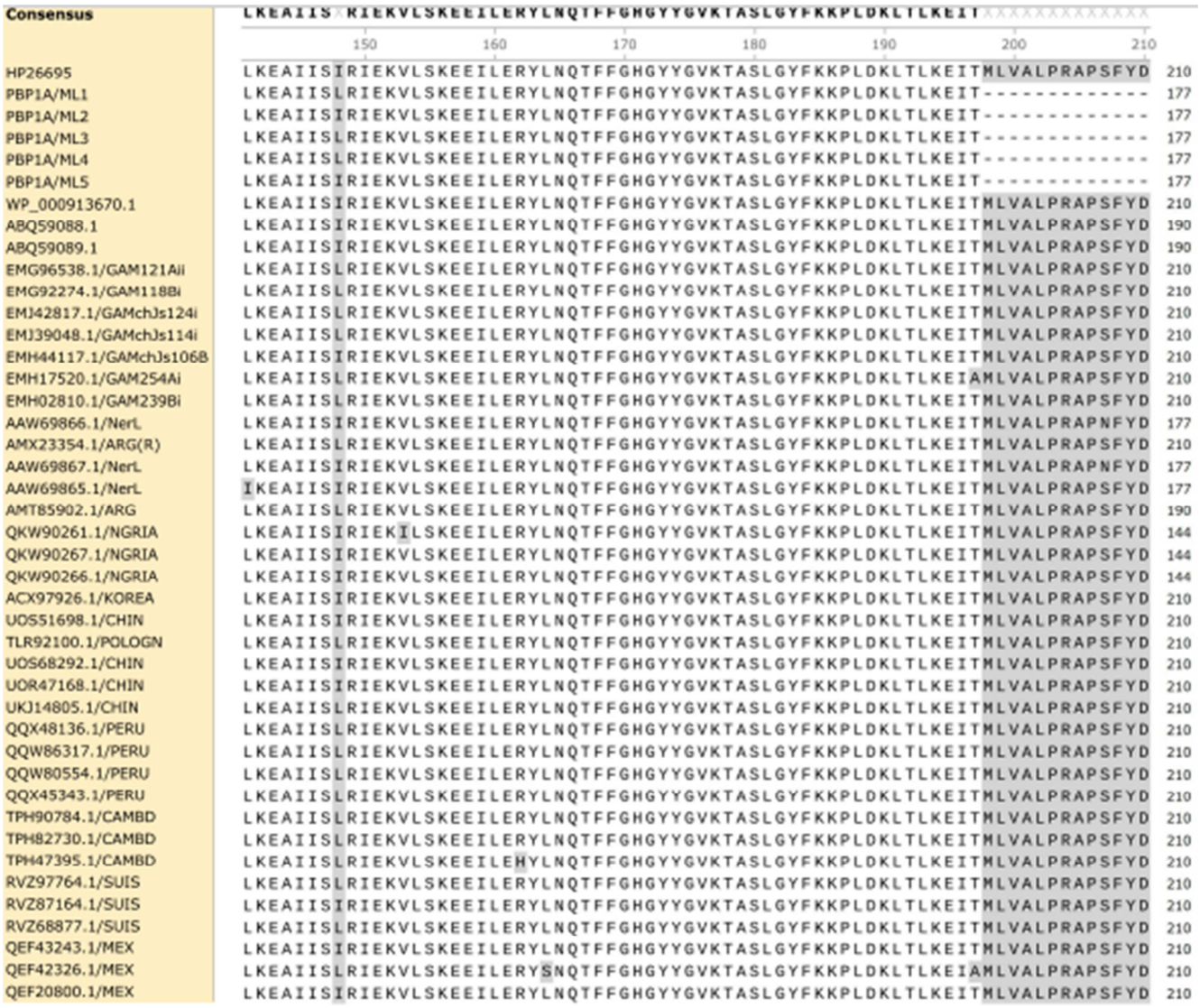

Figure A3. *Comparison of PBP1A transglycosylase domain sequences of Malian strains with those of other existing strains (amino acid 141-210). ML (Mali), GAM (Gambia), NerL (Netherlands), ARG (Argentina), NGRIA (Nigeria), CHIN (China), POLAND (Poland), CAMBD (Cambodia), SUIS (Switzerland), MEX (Mexico). NCBI (National Center for Biotechnology Information) acces codes: Mali (OP574217-OP574220), Gambia (EMG96538.1, EMG92274.1, EMJ42817.1, EMJ39048.1, EMH44117.1, EMH17520.1, EMH02810.1), Nigeria (QKW90261.1, QKW90267.1, QKW90266.1), China (QKW90261.1, QKW90267.1, QKW90266.1), Cambodia (TPH90784.1, TPH82730.1, TPH47395.1), Korea (ACX97926.1), Argentina (AMX23354.1, AMT85902.1), Peru (QQX48136.1, QQW86317.1, QQW80554.1, QQX45343.1), Mexico (QEF43243.1, QEF42326.1, QEF20800.1), Switzerland (RVZ97764.1, RVZ87164.1, RVZ68877.1), Holland (AAW69866.1, AAW69867.1, AAW69865.1), Poland (TLR92100.1) and Helicobacter pylori 26695 (AAD07660.1).*

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