Reverse Vaccinology approach for antigen candidates prioritization to develop a vaccine against a poultry pathogen

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Background

Avibacterium paragallinarum (AvP) is the causative agent of infectious coryza, an acute disease that affects the upper respiratory system of chickens. This Gram-negative pathogen is widely distributed in poultry production systems all over the world, causing significant economic losses. Despite vaccination being the main form of prevention, commercially available vaccines show incomplete protection against strains not included in the formulation.

Methods

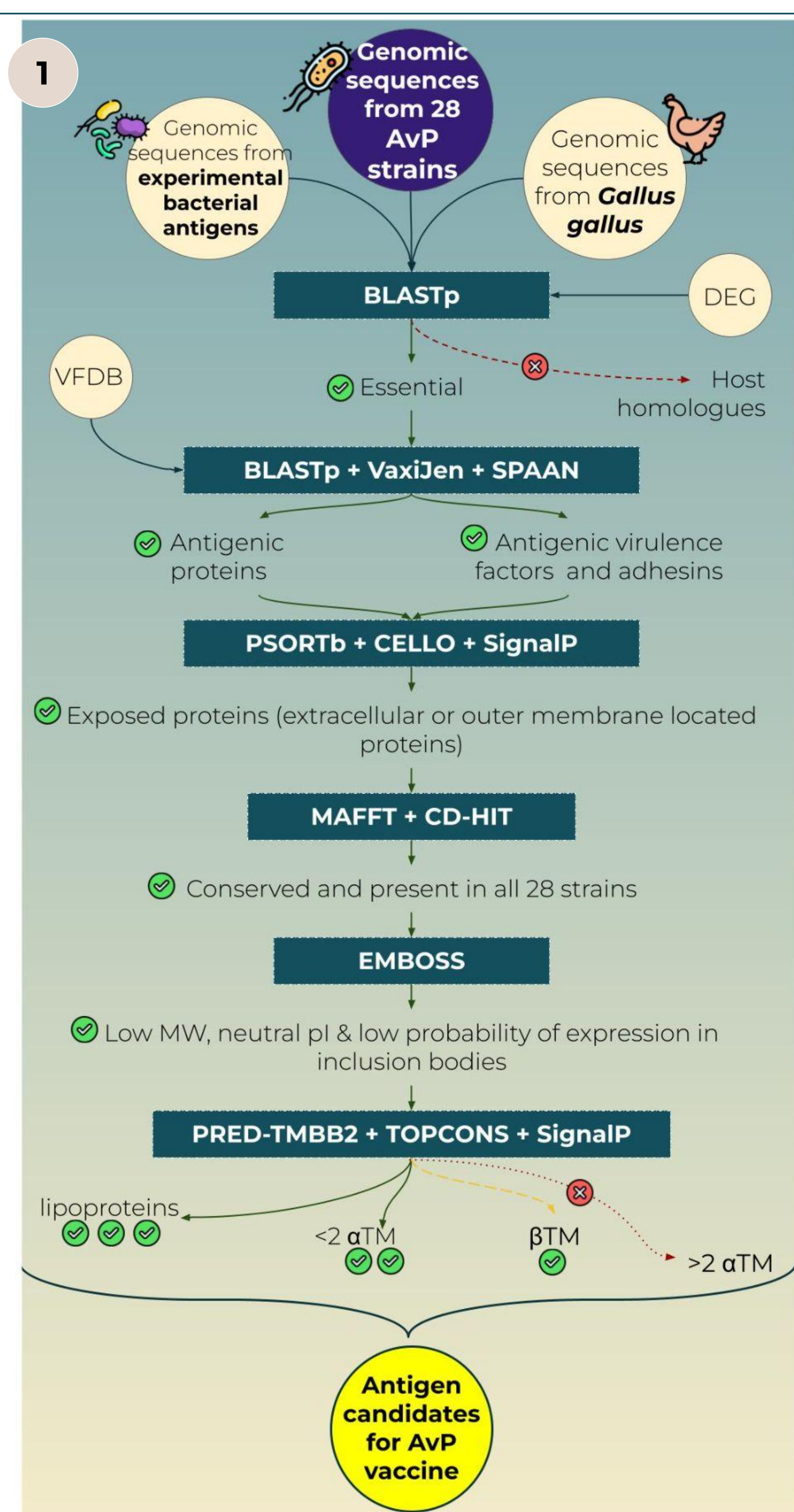
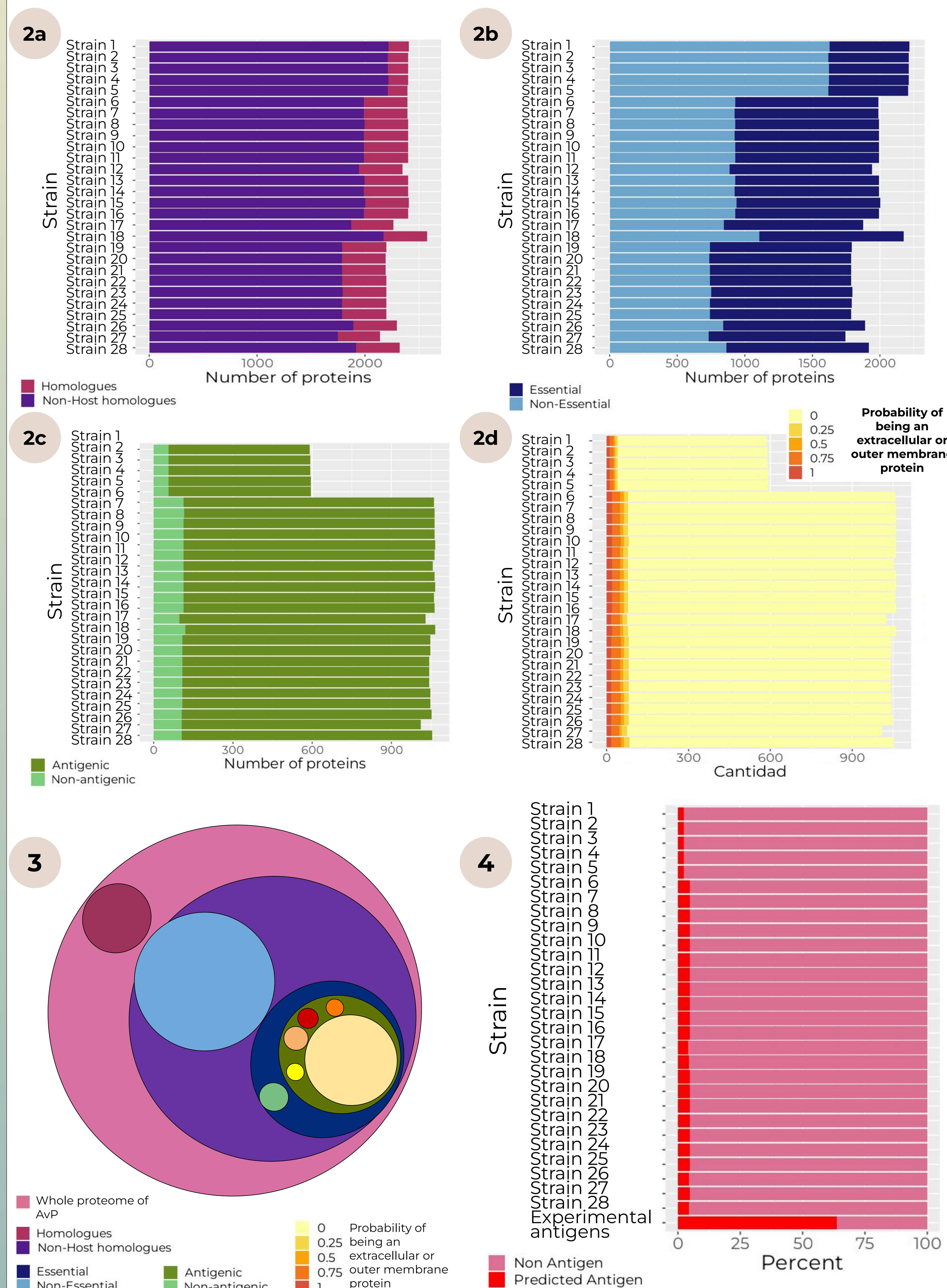


***Figure 1*. Comparative and subtractive genomics and prediction of several protein attributes were used to prioritize proteins with ease of expression and protection potential. Proteins were discarded or selected according to the following criteria: no homology to the host** (E-value < 1x10^{-15}, Percent Identity > 25% and Bit-score > 50); **essentiality** (defined as protein homology against DEG); **antigenicity** (according to VaxiJen3), **subcellular localization** (outer membrane or extracellular according to CELLO or PSORTb) and **sequence conservation** (Conservation Score: mean of 1 - Shannon Entropy for each amino acid). **To validate the results, the same workflow was applied to a set of 2271 experimental antigens.**

Results



***Figures 2a-d*: Discarded and selected proteins according to the set cut offs: no homology to the host (*Figure 2a*), essentiality (*Figure 2b*); antigenicity (*Figure 2c*) and subcellular localization (*Figure 2d*:** probability that the protein is being exposed outside of the cell, from 0 -probably cytoplasmic or inner membrane protein- to 1 -probably outer membrane or extracellular protein-).

***Figure 3*. Schematic representation of the groups and subgroups of selected and discarded proteins throughout the computational workflow.** From the whole proteome of AvP (light pink), only the sequence conservation of the candidates (red and orange) that fulfilled the set criteria were further studied.

***Figure 4*. Proportion of proteins catalogued as antigen candidates or non-antigens after the *in silico* analysis. The same computational pipeline was applied to a dataset of 2271 proteins with experimentally known antigenicity, collected manually from published literature and various antigen and/or epitope databases** (IEDB, VaxiJenDB, AntigenDB, Protegen, and DNAVaxDB). **Between 2 and 4% of the whole proteome of the 28 AvP strains were classified as antigens. On the other hand, 63.8% of the experimental antigens were accurately classified as potential antigens.**

***Table 1*. Physico-chemical properties and conservation score of the best antigen candidates.** The final step of the workflow consisted in selecting antigen candidates that belong to families of proteins previously shown to be antigens or used in experimental vaccines, according to the literature.

Protein Name	Length / MW (kDa) / pI	Signal peptide according to SignalP	Number of TM α Helix / Number of TM β sheet	Beta Barrel (BB) / BB Family	Adhesin probability	Probability of expression inside inclusion bodies	Conservation Score (among strains)
Outer membrane protein assembly factor BamA	790 / 88,2 / 5.99	SP(Sec/SPI)	0 / 20	Yes / The Outer Membrane Protein Insertion Porin (OmpIP/Omp85) Family	0.428	0.523	0.997
Porin OmpA / hemagglutinin antigen	345 / 36,9 / 8.98	SP(Sec/SPI)	0 / 8	Yes / The OmpA Family	0.455	0.358	0.978
Peptidoglycan-associated lipoprotein Pal	150 / 16.3 / 7.27	SP(Sec/SPI)	0 / 0	No / -	0.513	0.380	0.999
Translocation - assembly module TamB	1317 / 143.7 / 5.78	OTHER	1 / 32	Yes / The Aggregatibacter actinomycetemcomitans omp67/ morC Family	0.443	0.428	0.976
Tol-Pal system beta propeller repeat protein TolB	429 / 45.9 / 6.50	SP(Sec/SPI)	0 / 0	No / -	0.392	0.472	0.993
TolC family protein	456 / 50.6 / 9.36	SP(Sec/SPI)	0 / 14	Yes / The Outer Membrane Factor (OMF) Family	0.595	0.339	0.990
LPS assembly protein LptD	781 / 90.1 / 6.94	SP(Sec/SPI)	0 / 28	Yes / The Imp/OstA Family	0.410	0.521	0.992

Discussion

The proposed workflow allowed us to identify 25 antigenic candidates from the 28 proteomes analyzed. Moreover, 7 of these putative antigens belong to protein families which have been previously identified as experimental antigens in other bacteria. The thresholds used in our workflow lead to discarding some experimentally validated antigens. However, the majority of them were successfully classified as potential antigens, which highlights the predictive capability of our workflow.

Conclusions

The applied workflow proved useful to achieve our goal of protein prioritization. Although more *in silico* and *in vivo* analyses are still needed, this study provides a basis for the development of a novel subunit vaccine against AvP.