# Putative role of the YbhFSR efflux pump in resistance of *Aliarcobacter butzleri* to several antimicrobials

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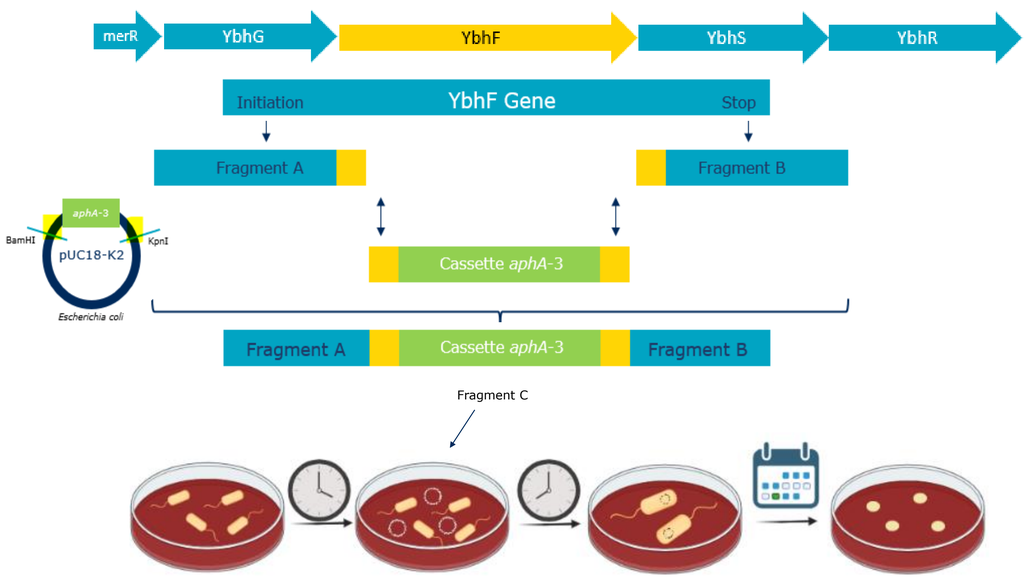
## Introduction

- *Aliarcobacter butzleri* is considered the fourth *Campylobacter-like* microorganism most frequently found in human diarrheal stool samples. Increasing rates of multidrug resistance to different antimicrobials have been observed in isolates of this microorganism[1], with efflux pumps being one of the described resistance mechanisms[2]. Efflux pumps of the *ATP-binding cassette* (ABC) family are known to export a wide variety of substances and are ubiquitous in almost all organisms[3]. Several genes coding for efflux pumps of this family have been observed in the *A. butzleri* genome[2].
- Despite the resistance associated with this specie being widely described, the research on the mechanisms involved in this process is scarce. Therefore, the **aim** of this work was to evaluate the role of an ABC family efflux pump system in the resistance of *A. butzleri*.

## Experimental Design

**A Construction of mutant strain by insertional mutagenesis and natural transformation**




**B Growth curves of parental and mutant strains**

**C Determination of the minimum inibitory concentration (MIC) of antimicrobials by the agar dilution method**

## Results

### Confirmation of Mutagenesis

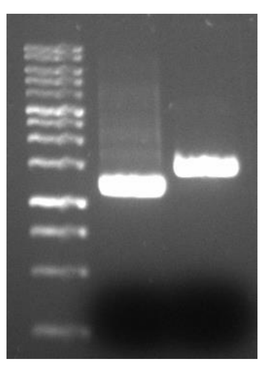


**Fig 1.** Electrophoresis agarose gel of the PCR products corresponding to the *ybhF* gene amplification in the parental and mutant strains.

### Growth Curves

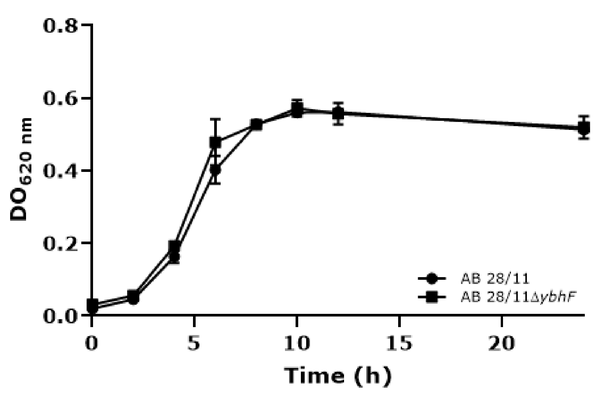



**Fig 2.** Growth curves of *A. butzleri* parental AB 28/11 strain and derived mutant.

- The transformation and mutant construction was confirmed by PCR and sequencing.
- Similar bacterial growth of both strains proves that **inactivation of the *ybhF*** gene **does not affect bacterial growth**.

### Antimicrobial Resistance Profile

**Table 1.** Minimum inhibitory concentration of heavy metals for the parental and mutant strains of *A. butzleri*.

| Strains | MIC (mM) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ag | Cd | Co | Cr | Cu | Hg | Li | Na | Ni | Mn | Mo | Pb | Zn |
| **AB 28/11** | 0.01 | **0.25** | 1 | 0.01 | **0.5** | 0.003 | 32 | **256** | 1 | **4** | 64 | 8 | **1** |
| **AB 28/11Δ*ybhF*** | 0.01 | **0.03** | 1 | 0.01 | **0.25** | 0.003 | 32 | **128** | 1 | **2** | 64 | 8 | **0.5** |

(Ag: Silver; Cd: Cadmium; Co: Cobalt; Cr: Chrome; Cu: Copper; Hg: Mercury; Li: Lithium; Mn: Manganese; Mo: Molybdenium; Na: Sodium; Ni: Nickel; Pb: Lead; Zn: Zinc)

**Table 2.** Minimum inhibitory concentration of disinfectants and antibiotics, germicide and substrate of efflux pumps for the parental and mutant strains of *A. butzleri*.

| Strains | MIC (µg/ mL) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CHX | CLBZ | AMP | CFX | CHL | CIP | ERY | GEN | KAN | TET | ACR | EtBr |
| **AB 28/11** | 1 | **64** | **64** | 32 | **128** | 16 | **32** | 0.5 | **1** | **8** | **32** | **64** |
| **AB 28/11Δ*ybhF*** | 1 | **16** | **32** | 32 | **64** | 16 | **16** | 0.5 | **64** | **16** | **16** | **32** |

(CHX: Chlorhexidine; CLBZ: Benzalkonium chloride; AMP: Ampicillin; CFX: Cefotaxime; GEN: Gentamycin; KAN: Kanamycin; CIP: Ciprofloxacin; TET: Tetracycline; ERY: Erythromycin; CHL: Chloramphenicol; ACR: Acriflavine; EtBr: Ethidium Bromide)

- Inactivation of *ybhF* gene resulted in a **reduction of the MIC** to some **heavy metals** and **antibiotics**, as well as to **benzalkonium chloride**, **acriflavine** and **ethidium bromide**.

## Conclusion

- Although it is likely that other efflux pumps are involved in tolerance or resistance in *Aliarcobacter butzleri*, **the inactivation of *ybhF* gene supports the role of YbhFSR efflux pump in intrinsic resistance of this bacterium.**

## References

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