Characterization of fecal coliform contaminants in seawater and seafood samples collected from Namibe, Angola

Beatriz L Calado[1+], Inês C Leal [1+], Eunice Cassoma[2], Isaac Bumba[2], Carmen dos Santos[2], Adelino VM Canário[1], Deborah M Power[1], João CR Cardoso[1]

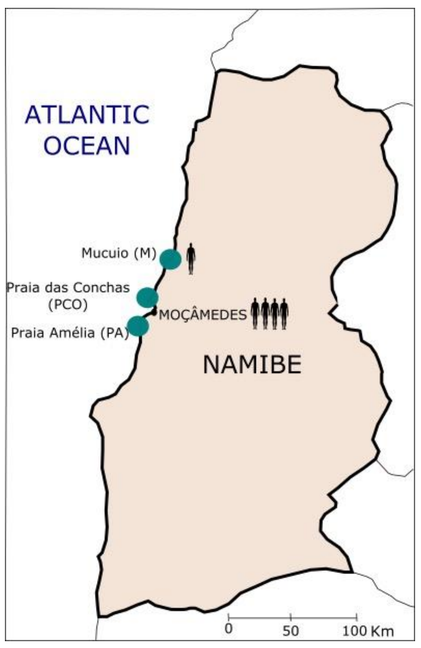
[1] Comparative Endocrinology and Integrative Biology, Algarve Centre of Marine Sciences (CCMAR-Algarve), Universidade do Algarve, Faro, Portugal
[2] Universidade do Namibe, Campus Farol de Noronha, Moçâmedes, Angola
+ shared first authorship

INTRODUCTION

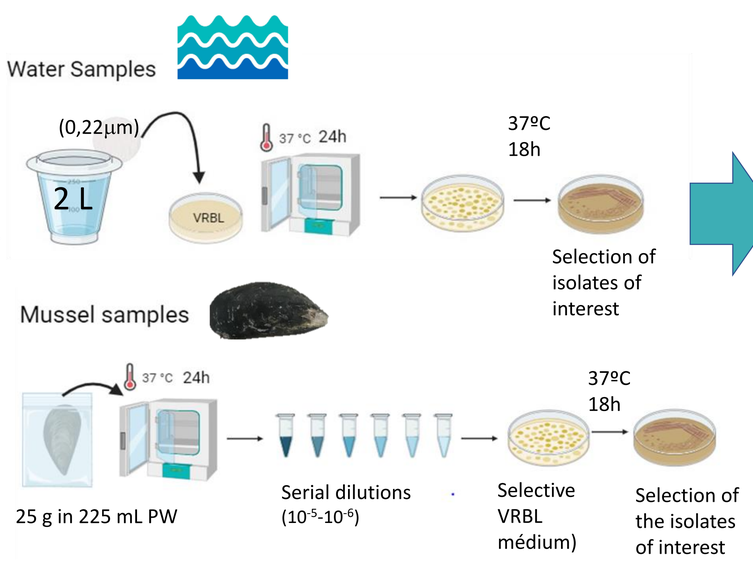
Contamination of aquatic environments by fecal coliform bacteria is a standard indicator of water quality and safety. The Enterobacteriaceae family can be a major contaminant in aquatic environments receiving untreated sewage, urban water run-offs and waste and represents a major Public Health risk. The aim of the present study was to characterize fecal coliform bacterial contaminants in seawater or seafood collected from coastal regions of the Namibe Provence in Angola to determine if they represent a potential risk for human health. Seawater and bivalves, the brown mussel (*Perna perna*), were collected from 4 different sampling sites located near urban areas and bacteria were isolated by conventional microbiological approaches and by quantification using 16S rRNA PCR. The study identified for the first time the diversity and type of fecal bacteria contaminants present in coastal areas and mussels of Namibe and highlights a potential public health risk that has not previously been identified, particularly in relation to water-borne infections since most of the Namibe population depends on marine resources for their livelihood.

METHODS

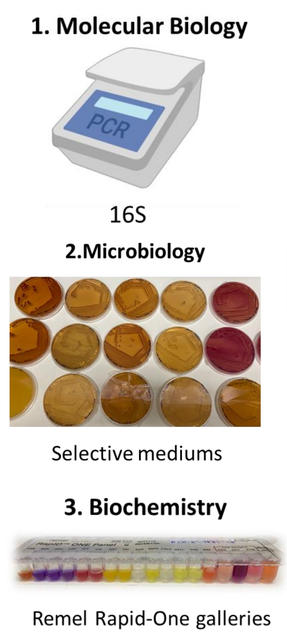
ENVIRONMENTAL SITES



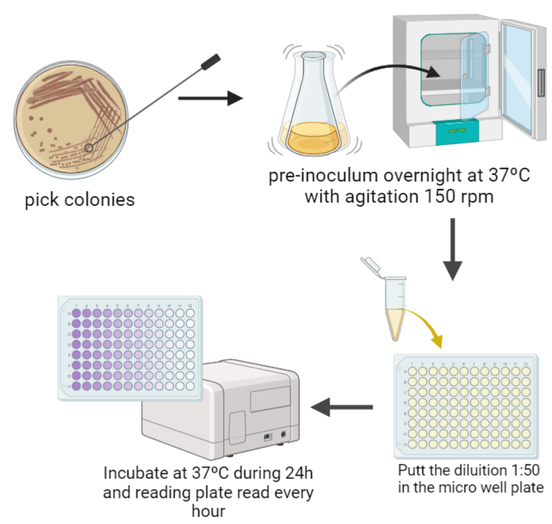
SAMPLE PREPARATION



ISOLATE CHARACTERIZATION



GROWTH KINETICS OF SELECTED ISOLATES



1 MOLECULAR IDENTIFICATION OF ISOLATES

Phylogenetic analysis of the 16SrRNA sequences of the selected isolates from the seawater (blue) and mussel tissue (black) revealed that they belong to the *Enterobacteriaceae* family. Some of the isolates cluster with known bacterial reference species but other isolates are unknown. Clustering of isolates within the same branch revealed that potential novel strains were isolated.

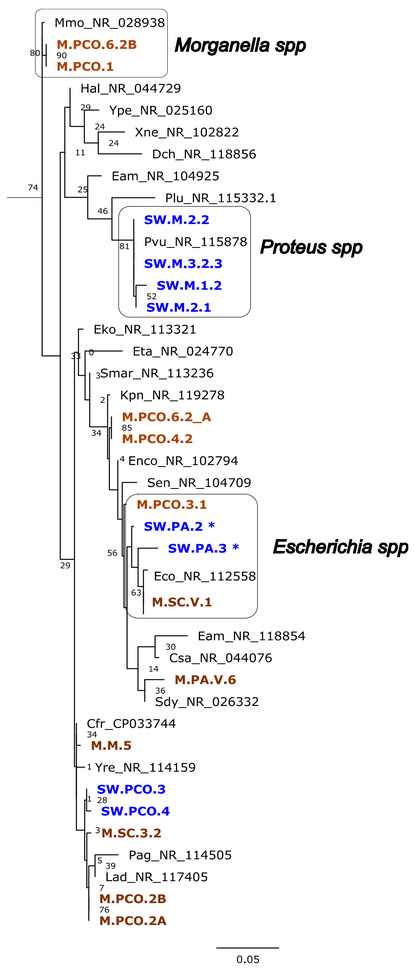


Fig 1: Evolutionary analysis of the *Enterobacterecea* family isolated from the seawater (SW, in blue) and mussel tissues (M, black) based on 16S rRNA sequence analysis. The tree was constructed using the 16S rRNA aligned sequences according to the Maximum Likelihood Method in the PHYML platform with the HKY85 substitution model and 100 boostrap replicates. PCR products of the isolates were obtained with 16S_27F and 16S_1492R primers. The 16S rRNA sequence of other bacteria were obtained from the NCBI database. The tree was rooted using *Vibrio parahaemolyticus* and *Pseudomonas aeuruginosa*. Other species included in the analysis are: *Eco-Escherichia coli, Csa- Cronobacter sakazakii, Eamy- Erwinia amylovora, Sdy- Shigella dysenteriae, Kpe- Klebsiella pneuminae, Enco- Enterobacter cloacae, Sen- Salmonella Enterica, Yre- Yokenella regensburgei, Cfe- Citrobacter freundii, Lad- Leclercia adecarboxylata, Pag-Pantoea agglomerans, Eta- Edwardsiella tarda, Sma-Serratia marcescens, Pvu- Proteus vulgaris, Olu- Photorhabdus luminescens, Eam- Ewingella americana, Xne-Xenorhabdus nematophila, Dch- Dickeya chrysanthemi, Ype-Yersinia pestis, Hal-Hafnei alvei, Mmo-Morganella morganii, Eko-Enterobacter kobei** Were selected from biochemical and microbiological analysis.

2 BIOCHEMICAL CHARACTERIZATION OF *ESCHERICHIA* SPP. ISOLATES

Two *Escherichia spp.* isolates (SW.PA.2 and SW.PA.3) were selected for biochemical characterization. The results revealed that they have a similar biochemical profile to the reference strain *E. coli* strain (ATCC 25922) but differ in urea, arginine, glycoside and arylamide hydrolysis activities and in the utilization of aldehyde. Selective screening by growth in medium revealed both isolates are lactase positive in MacConkey agar and had characteristic blue colonies in CHROMOGENIC COLIFORM agar. Two characteristics that are specific to *E. coli*.

Isolates	URE	ADH	ODC	LDC	TET	LIP	KSF	SBL	GUR	ONPG	βGLU	βXYL	NAG	MAL	PRO	CGT	PYR	ADON	IND	OXI
E. coli ATCC 25922	+	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-
SWPA2	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-	+	-	-	+	-
SWPA3	-	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-

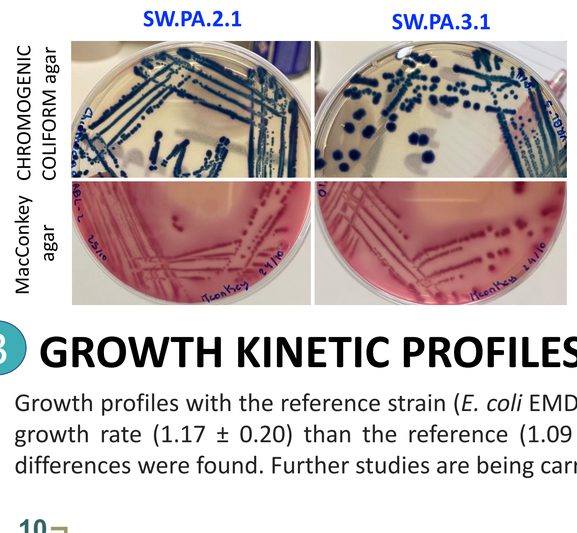


Table 1: Biochemical results of the two putative *Escherichia* isolates. The profile of the reference *E. coli* ATCC 25922 is represented**.** Tests were performed using the Rapid One System (ThermoFisher)**,** biochemical test in the gallery: URE- urease, ADH-arginine, ODC-ornithine, LDC-Lysine, TET-Aliphatic thiol, LIP-Fatty acid ester, KSF-Sugar aldehyde, SBL- Sorbitol, GUR- ρ-Nitrophenyl-β, D-glucuronide, ONPG- σ-Nitrophenyl-β, D-galactoside, BGLU-ρ-Nitrophenyl-β,D-glucoside, BXYL- ρ-Nitrophenyl-β,D-xyloside, NAG-ρ-Nitrophenyl-n-acetyl-β,D-glucosaminide, MAL-malonate, PRO-Proline-β-naphthylamide, GGT- ɣ-Glutamyl- β- naphthylamide, PYR-Pyrrolidonyl- β- naphthylamide, ADON- Adonitol, IND- Tryptophane.

Fig 2. Digital photographs of the isolates on MacConkey medium and CHROMOGENIC COLIFORM agar (Oxoid CM1205) plates at 37 °C. Each isolate was incubated overnight. Colonies on MacConkey agar turned pink revealing lactase fermentation activity and in CHROMOGENIC COLIFORM agar are blue. Both phenotypes are characteristic of *E.coli*.

3 GROWTH KINETIC PROFILES OF THE *ESCHERICHIA* SPP. ISOLATES

Growth profiles with the reference strain (*E. coli* EMDB 1077, donated by Prof Leonor Faleiro). Data revealed that SW.PA.2.1 had a higher growth rate (1.17 ± 0.20) than the reference (1.09 ± 0.14) and SW.PA.3.1 had a lower growth rate (1.02 ± 0.15) but no significant differences were found. Further studies are being carried out to fully characterize the two isolates.

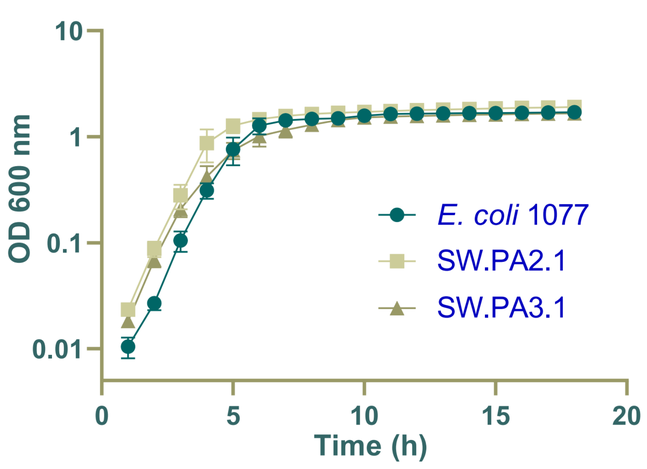


Table 2: Growth kinetics of the the isolates and reference strain *E. coli* EMDB 1077. Growth rate (m, h^{-1}) and duplication time (dt, h) were calculated at 37ºC. One-way ANOVA with Multiple comparisons was used to detect significant differences between strains.

Isolates	Growth rate (µ, h^{-1})	Duplication Time (dt, h)
***E. coli* 1077**	1,09 ± 0,14	0,65 ± 0,09
SW.PA.2.1	1,17 ± 0,20	0,61 ± 0,12
SW.PA.3.1	1,02 ± 0,15	0,70 ± 0,12

Fig 3: Growth curves of the isolated *E. coli* strains and of the reference strain (EMDB 1077). Each curve was constructed using the mean values of three replicates per time-points. The OD 600nm were transformed to a logarithmic scale.

CONCLUSION

- → *Enterobacteriaceae* were isolated from seawater and mussel samples, which indicates the presence of fecal contamination in the coastal waters of Namibe.
- → 16S rRNA molecular analysis revealed that some isolates clustered with characterised bacterial species but others require further studies for identification.
- → Molecular, biochemical and microbiological analysis confirmed the isolation of two novel *E. coli* strains, but other novel bacterial strains may exist, and studies are on-going to isolate them.

Acknowledgements: We would like to thank Prof Leonor Faleiro (Universidade do Algarve) for providing the *E. coli* EMDB 1077 and for the help in isolating and characterizing the colonies. This study received Portuguese national funds from FCT - Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121 from FCT, IP and Aga Khan Development Network (AKDN) project FCT/AKDN/541666287/2019 (HealthyBi4Namibe project).