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Jackielyn R Carpio
Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela, Philippines

Mary Joy L Asprec
Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela, Philippines

Isagani P Angeles Jr
Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela, Philippines

Marites R Castro
Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela, Philippines

Evelyn C Ame
DA-Bureau of Fisheries and Aquatic Resources, Tuguegarao City, Cagayan, Philippines

Emma L Ballad
DA-Bureau of Fisheries and Aquatic Resources, Tuguegarao City, Cagayan, Philippines

Corresponding Author:
Jackielyn R Carpio
Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela, Philippines

Characterization, isolation and identification of bacteria and parasites in glass eel *(Anguilla spp.)* in cagayan, Philippines

Jackielyn R Carpio, Mary Joy L Asprec, Isagani P Angeles Jr, Marites R Castro, Evelyn C Ame and Emma L Ballad

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Abstract
The research focused on identifying bacteria and parasites found in glass eels collected from the province of Cagayan. The characterization, isolation, and identification of bacteria were conducted through morphological analysis involving size, elevation, margin, and pigmentation characteristics, as well as the API 20E test, while parasites were assessed using morphological examination or gross inspection. The prevalence of the identified bacterial and parasitic species was calculated, obtaining percentages of: *Elizabethkingia meningoseptica* (43.24%), *Pseudomonas luteola* (32.43%), and *Pseudomonas oryzihabitans* (16.21%). The bacteria identified from the glass eels included *Elizabethkingia meningoseptica, Pseudomonas luteola, Pseudomonas oryzihabitans*, and *Pseudomonas aeruginosa*. The parasites identified were *Trichodina spp.* and *Dactylogyrus spp*. Additional confirmation regarding the epizootiology features of the identified bacteria and parasites is required, along with an evaluation of their presence in the water. Moreover, it is essential to investigate the impact of water quality on the occurrence of pathogenic agents in future studies.

Keywords: glass eel, *Trichodina spp., Dactylogyrus spp., Pseudomonas spp., Elizabethkingia spp.*

Introduction

The glass eel stage of *Anguilla spp.* represents a critical transitional phase in the life cycle of eels, where larvae migrate from oceanic spawning grounds to freshwater or brackish environments (Dorst, 2024) [5]. In the Philippines, the province of Cagayan is a vital hub for glass eel fisheries, contributing significantly to local livelihoods and the aquaculture industry (Crook, 2014) [4]. Despite their economic and ecological importance, glass eels face significant challenges, including exposure to a variety of bacterial pathogens and parasites. These factors can compromise their survival, impact aquaculture productivity, and pose risks to natural populations. Fish diseases caused by bacteria and parasites are major concerns in aquaculture, as they can lead to mass mortality, reduced growth rates, and poor-quality stock. Understanding the microbial and parasitic communities associated with glass eels is, therefore, essential for effective management strategies (Septyan et al., 2021) [16]. However, limited studies exist regarding the microbiological and parasitological profiles of glass eels in the Philippines, particularly in the Cagayan region. This gap in knowledge underscores the need for targeted investigations to ensure the health and sustainability of these valuable resources. The present study aims to characterize, isolate, and identify the bacteria and parasites associated with glass eels in Cagayan. By employing microbiological and parasitological techniques, this research sought to determine the morphological characteristics of isolated bacteria, isolated external parasites, and the isolated microorganisms through biochemical test, and to identify the different microorganisms present in the glass eel samples collected using Analytical Profile Index (API) 20E test. This study's findings will not only enhance the understanding of disease dynamics in glass eels but also support the sustainable management of *Anguilla spp.* populations in the Philippines.

Materials and Methods: Characterization and Site Description: This study was conducted on the month of March 2023. Eel samples were gathered at three sampling sites in the municipalities of Aparri, Abulug, and Sta. Ana, Cagayan. The entire procedure was performed at the Bureau of Fisheries and Aquatic Resources-Fisheries Integrated Laboratory Services Regional Office No. 2 in Tuguegarao City, Cagayan.

There were three identified sampling areas, specifically Site 1-Aparri, Cagayan, Site 2-Abulug, Cagayan and Site 3-Sta Ana, Cagayan. Each site employed three sample collection schemes which (1) used stationary fishing gear, (2) offshore collection, and (3) consolidators having three replicate samples secured with each scheme.

In this study, isolation of bacteria in a culture media was done to observe bacterial growth. Recognizing and differentiating the growth characteristics of bacteria on the agar plate is important in its identification. Colony morphology which are the different growth characteristics of bacteria may not appear to be important initially, but it is necessary in order to identify it. The colony features aided in determining the bacteria's identity.

The collected live samples glass eel approximately 4.1 centimeters (cm) to and weighed from 0.05 g (gram) to 0.21 g (Nieves, et. al., 2019) [10] were placed in plastic bag/fingerling bag. Thereafter, samples were transported to BFAR RO2 - Fisheries Integrated Laboratory Section (FILS) for isolation, characterization and identification.

Procedures

(Bureau of Fisheries and Aquatic Resources QMS Manual)

Each collected samples from different sampling sites were properly labelled. The following the procedures on bacteria and parasites identification were based on the BFAR Quality Management System (QMS) Manual as shown in the figure 4.

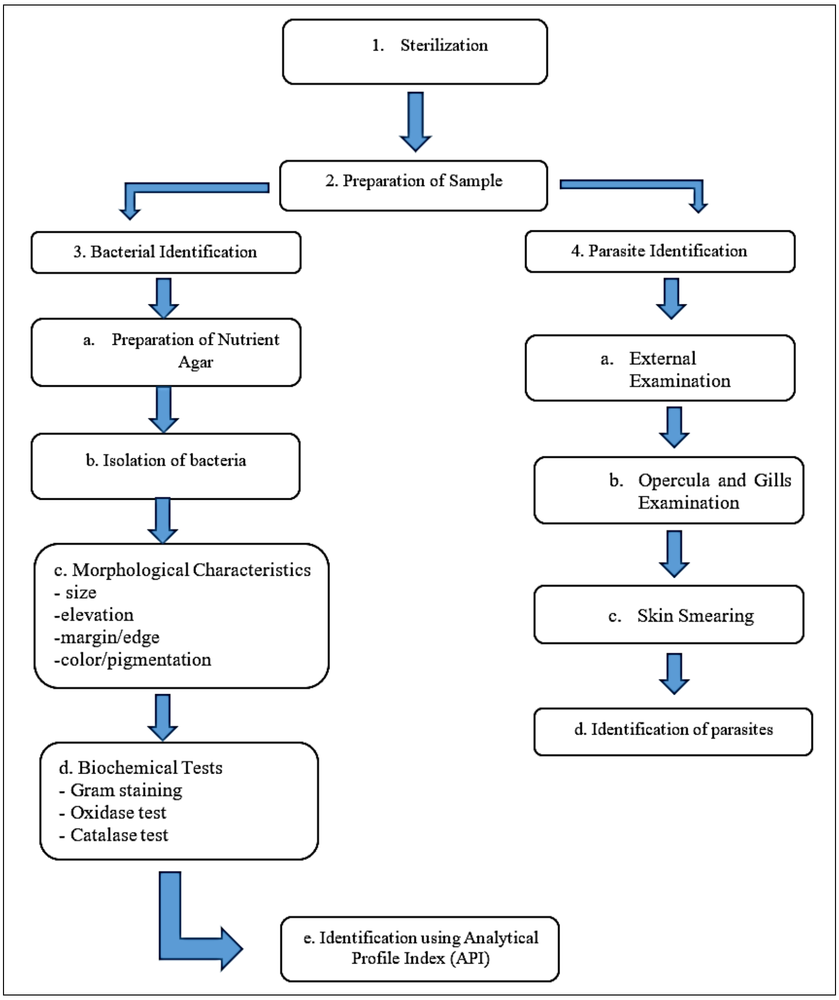

Fig 1: Process flow of characterization, isolation and identification of isolated bacteria and parasites in glass eel

Bacterial Identification

Each collected sample were subjected to direct skin scrapping and swabbing, and used for plate method and undergone incubation thereafter, the plates were checked for growth of colony. After 18-24 hours incubation of the bacterial pure culture, the morphological characteristics in terms of size, color, elevation and margin of the colony were observed macroscopically. Each plate was subjected to Biochemical test (Gram straining, Oxidase Test, Catalase test). The Identification of bacteria were done using the API 20 E based on BioMerieux, API 20 E Manual. The prevalence of bacteria was calculated using the following formula:

$$Prevalence\ (\%) = \frac{Number\ of\ fish\ host}{Total\ number\ of\ fish\ examined} \times 100$$

Parasites Identification

Parasite examination, preparation, and observation procedures were carried out according to the standard work instructions for the Parasite Examination Method (Roberts, et. al., 2012) [12] at FILS. The parasite species found was placed on a glass object, observed meristically-morphometrically using a microscope, and identified. Analysis was carried out on the prevalence of parasitic species calculated according to the previous formula.

Results and Discussion: Morphological Characterization of isolate bacteria in agar plate colonies, in terms of size, margin or edge, elevation and color/pigmentation.

The glass eels most commonly come from the wild, so they are more prone to carry parasites or diseases. It is important to quarantine the glass eels before introducing them to tanks with other fish. The change from salt to fresh water also helps to eliminate many parasites naturally. However, there are some parasites and diseases that need further attention (Starmer, 2015) [17]. Causative agents were not specifically determined but most of the diseases are caused by bacterial and parasites infection. Listed on the table below are common diseases detected on glass eels showing clinical signs and treatments.

Table 1: Common diseases of glass eel

Common Diseases	Clinical Signs	Treatments
Physical damage of collected glass eels as experienced by eel collectors/ gathers Disease/ Damage Nature of disease	Cuts Physical Slightly cuts or incomplete parts such as fin or scratches	Methylene blue or ox tetracycline
Red Fin Bacterial	Rotting of caudal fin	Salt solution
Red Eel Pest Bacterial Swelling	red spots, and ulcerated lesions on skin	Antibiotics such as ox tetracycline
Parasites Parasitic Mucus	frayed fins, respiratory distress, white patches on skin and rubbing off parasites on the side of the tub	Salt solution or formaldehyde

Source: Nieves, et al., 2019 [10]

Based on the results of this study, in the five selected bacteria isolates subjected to identification using the API 20E test kit, there were four identified bacteria, *Pseudomonas oryzihabitans* 98 %, *Elizabethkingia meningoseptica* 98.4 %, *Pseudomonas luteola* 99.9 %, and *Pseudomonas aeruginosa* 99.9 %. These four bacteria having high percentage occurred as reflected in the result of the study.

The percentage of the frequency distribution of the morphological characteristics of the isolated bacteria based on size, elevation, margin, and pigmentation were used as basis for the selection of colony. Biochemical testing composed of gram staining, oxidase test and catalase test also considered to support in the identification of the bacteria.

After 18 to 24 hours incubation of the bacterial pure culture, the morphological characteristics of the colony were observed. The frequency distribution of morphological characterization in terms of size is shown on the table 2. Based on the results, most percentage (56.76 %) of the size of isolated bacteria from different sampling sites were medium. Bacterial colony size is measured in millimeters (mm). Small colonies are less than one mm in diameter, medium colonies are one mm in diameter, and large colonies are greater than one mm in diameter (Manupalli, 2023) [9]. The shape, size, and pigmentation of the colonies are the characteristics that determine colony morphology. The colony features may aid in determining the bacteria's identity the sizes vary due to the competition of nutrients available that shows some bacteria absorb low nutrients while the others have more nutrients uptake (Chacon, et. al, 2018) [3].

Table 2: Frequency distribution of morphological characteristics of isolated bacteria in terms of size

Sampling Site	Number of Samples	Size					
		Small (<1mm)		Medium (1mm)		Large (>1mm)	
		Frequency	%	Frequency	%	Frequency	%
Aparri	10	3	30	7	70	0	0
Abulug	10	4	40	6	60	0	0
Sta. Ana	17	8	47.06	8	47.06	1	5.89
Grand Total	37	15	40.54	21	56.76	1	2.7

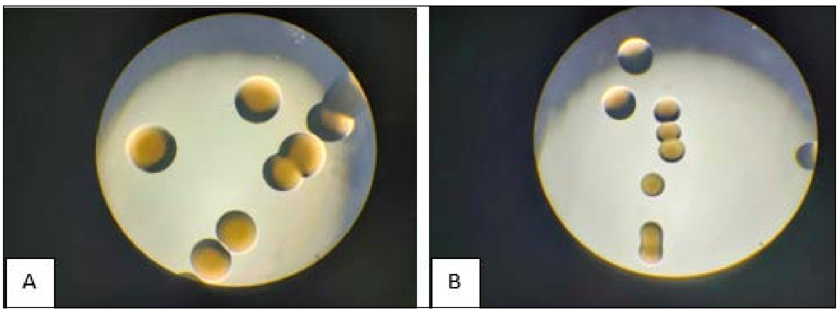

Fig 2: Morphological characteristics of isolated bacteria in terms of size viewed under microscope. Large and medium size isolated bacteria colonies (*A*) medium and small size isolated bacteria colonies (*B*).

The frequency distribution of morphological characteristics of isolated bacteria in terms of margin is shown on table 3. Mostly entire type of margin/edge were observed with the 94.59 %. This type of margin/edge were observed in three different sampling sites. In terms of margin of the isolated bacteria, filamentous bacteria are those bacteria or microorganism that grow through filaments and not supported by cell division. The variance in colony yields depends on the species and environment (Kevan, 2011) [8].

Table 3: Frequency distribution of morphological characteristics in terms of margin or edge

Sampling Site	Number of Samples	Margin/Edge					
		Entire		Undulate		Filamentous	
		Frequency	%	Frequency	%	Frequency	%
Aparri	10	9	90	1	10	0	0
Abulug	10	10	100	0	0	0	0
Sta. Ana	17	16	94.12	0	0	1	5.88
Grand Total	37	35	94.59	1	2.70	1	2. 70

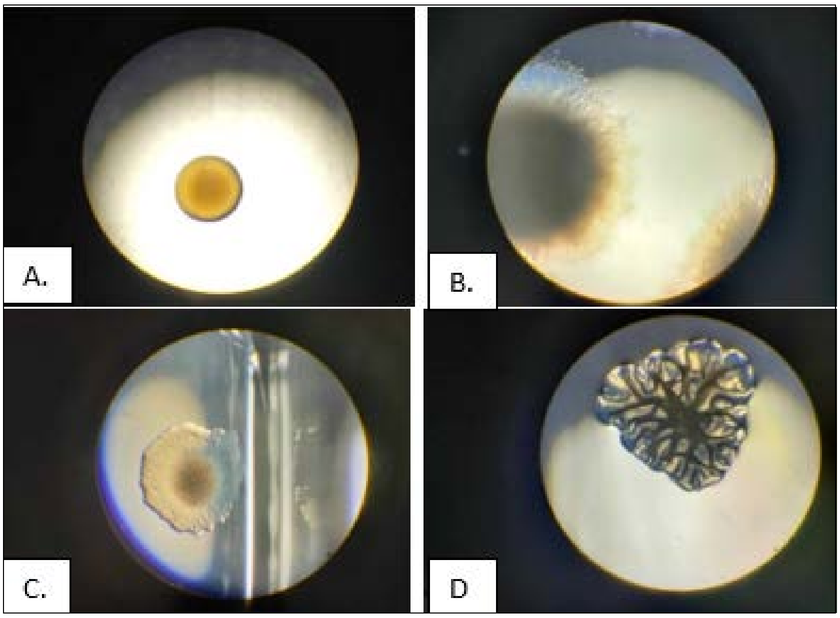

Fig 3: Morphological Characteristics of isolated bacteria in terms of margin or edge. Entire (*A.*), filamentous (*B.*), (*C.*), and undulate (*D.*).

For the elevation, factors considered were pH and nutrient requirements on the medium. Table 4 shows the frequency distribution of the morphological characteristics of the colony bacterial growth in terms of elevation. The two identified types of elevation were flat and raised. Based on the results below, out of 37 samples flat type of elevation has the higher percentage of 97.30 % compared to raise type of colony with a percentage of 2.70 % which implied that there are common bacteria found in glass eel from different sampling sites?

Table 4: Frequency distribution of morphological characteristics in terms of elevation

Sampling Site	Number of Samples	Elevation			
		Flat		Raised	
		Frequency	%	Frequency	%
Aparri	10	9	90	1	10
Abulug	10	10	100	0	0
Sta. Ana	17	17	100	0	0
Grand Total	37	36	97.30	1	2.70

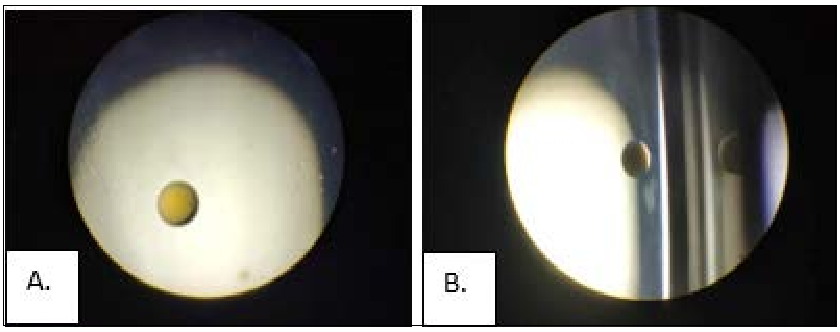

Fig 4: Morphological Characteristics of isolated bacteria in terms of elevation. Flat *(A)* raised *(B).*

The frequency distribution of morphological characteristics in terms of pigmentation was presented in table 5 which shows higher percentage of 70.27 % in white pigmentation. Different types of isolated bacteria from different sites gave rise to different colonies. A color, or the absence of color in a biological material culture, is of fundamental importance in guiding microbiologists in the vast microbial universe, leading them along the path of diagnosis (Fiscarelli, 2019) [7].

Table 5: Frequency distribution of morphological characteristics in terms of Pigmentation

Sampling Site	Number of Samples	Pigmentation							
		Dull		White		Yellow		Orange	
		Freq.	%	Freq.	%	Freq.	%	Freq.	%
Aparri	10	3	30	6	60	1	10	0	0
Abulug	10	0	0	9	90	1	10	0	0
Sta. Ana	17	0	0	11	64.71	4	23.53	2	11.77
Grand Total	37	3	8.11	26	70.27	6	16.22	2	5.41

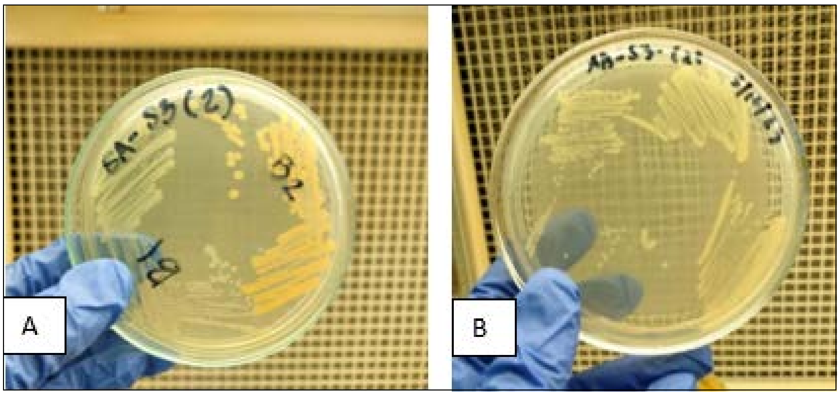

Fig 5: Morphological characteristics of isolated bacteria in terms of pigmentation. White (B1) and yellow (B2) (*A*), and white (*B*)

Morphological Characterization and Identification of the isolated external parasites: There were 54 prepared samples of skin smearing (27) and gills (27) from live glass eel that were characterized morphologically. Total length of samples used in skin smearing were recorded and also subjected to gill examination. The results for skin smearing of glass eel were negative with a percentage of 100 % which indicates that environment has a great impact in the presence of parasites in an organism as shown in the frequency distribution of morphological characterization and identification of isolated external parasites found in skin and gills of glass eel on the table 6.

Table 6. Frequency distribution of morphological characterization and identification of the isolated external parasites in skin of glass eel

Sampling Site	Number of Samples	SKIN			
		Negative		Positive	
		Frequency	%	Frequency	%
Aparri	9	9	100	0	0
Abulug	9	9	100	0	0
Sta. Ana	9	9	100	0	0
Total	27	27	100%	0	
SD		.00000			

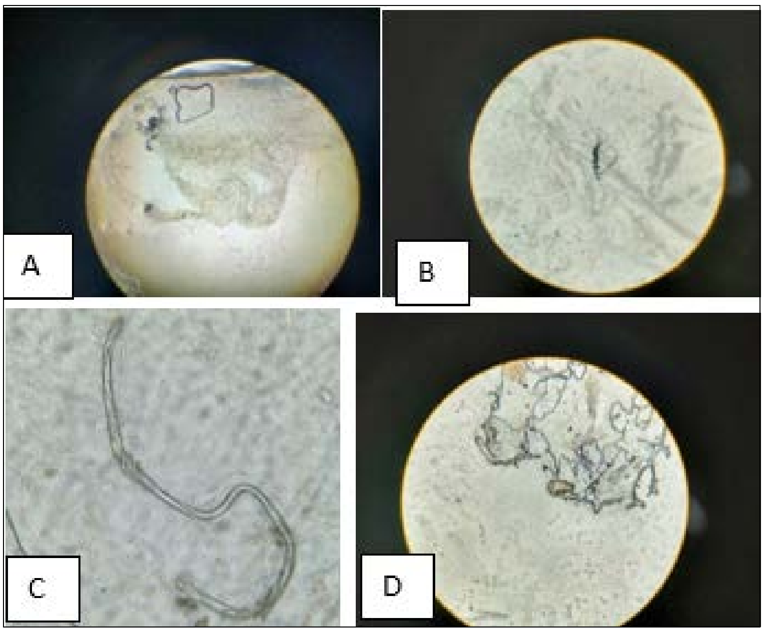

Fig 6: Morphological characterization and identification of the isolated external parasites in skin of glass eel. Negative result (*A, B, C, and D*).

Fish gills are also the preferred habitat of many external parasites, attached to the gill but living out of it. The most common are *monogeneans* and certain groups of parasitic *copepods*, which can be extremely numerous (Reed, et al., 2012) [11]. Results of gill examination of glass eel were presented on the table below and based on the frequency distribution of morphological characterization and identification of isolated external parasites in glass eel, there were 25.93 % of samples that were recorded positive. Most of the parasites were observed in the gills sample and identified as Trichodina spp. and Dactylogyrus spp. as shown in figure 7.

Table 7: Frequency distribution of morphological characterization and identification of the isolated external parasites in gills of glass eel

Sampling Site	Number of Samples	GILLS			
		Negative		Positive	
		Frequency	%	Frequency	%
Aparri	9	5	55.5	4	44.4
Abulug	9	8	88.9	1	11.1
Sta. Ana	9	7	77.8	2	22.2
Total	27	20	74.07	7	25.93
SD				1.52753	

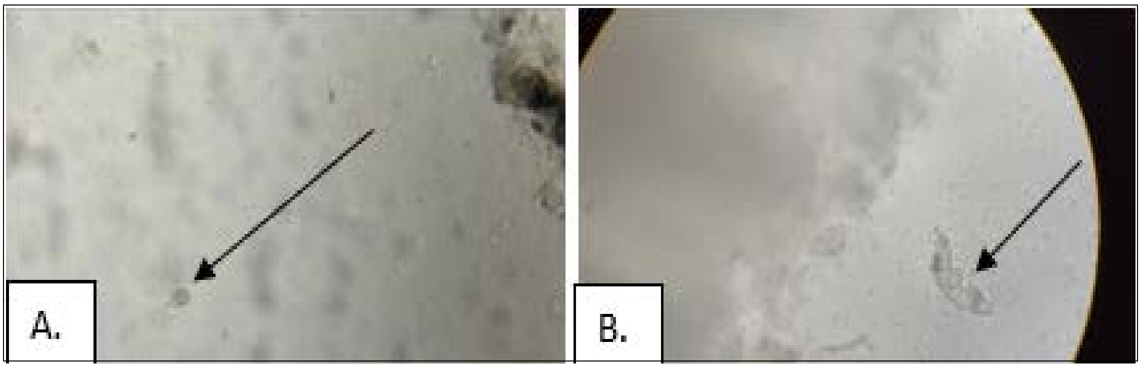

Fig 7: Morphological characterization and identification of the isolated external parasites in gills of glass eel. *Trichodina spp.* (*A*), *Dactylogyrus spp* (*B*).

Isolated microorganisms through conventional method

The isolated bacteria were then subjected to biochemical testing which includes, gram staining, oxidase test and catalase test to determine what type of microorganisms were isolated from glass eel samples. Frequency distribution of isolated bacteria in terms of biochemical test were presented in the table 8. Most of the samples gram-stained tested negative with a percentage of 94.59% and tested positive with the catalase test with a percentage of 64.86 % while most of the samples tested with the oxidase test is positive with the percentage of 40.54%. The positive results indicated the presence of catalase enzyme of pathogenic organisms as it protects the organism from oxidative damage from reactive oxygen species or against the lethal effect of hydrogen peroxide accumulated at the end of aerobic metabolism (Sapkota, 2022) [15]. The results served as a basis to support the results of the identification through API 20E kit.

Different microorganisms present from the sample collected using API 20E test: There were five selected isolates which were all gram-negative type of bacteria with different distinguishing characteristics subjected to bacteria identification using the API 20E test. Gram negative bacteria was identified through gram staining while the API 20E Test results identified by the kit itself and provides the code. The codes were input to the database and the database produced the ID and percent of the selected sample. Table 9 showed the type of bacteria identified in the database and the percent ID recorded.

Table 8. Frequency distribution of isolated bacteria in terms of biochemical test

Sampling Site	Number of Samples	Gram Staining				Catalase test				Oxidase Test			
		positive (+)		negative (-)		positive (+)		negative (-)		positive (+)		negative (-)	
		F	%	F	%	F	%	F	%	F	%	F	%
Aparri	10	1	10	9	90	7	70	2	20	7	70	3	30
Abulug	10	0	0	10	100	5	50	5	50	7	70	3	30
Sta. Ana	17	1	5.88	16	94.12	12	70.59	5	29.41	10	58.82	7	41.18
Grand Total	37	2	5.41	35	94.59	24	64.86	12	32.43	15	40.54	13	35.14

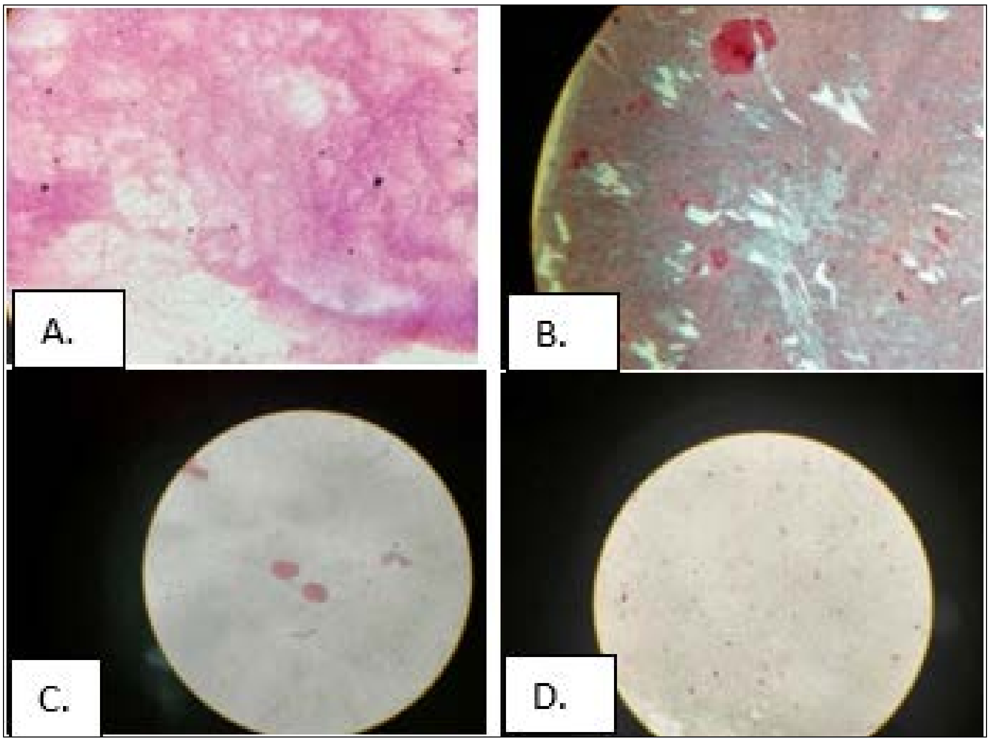

Fig 8: Results of biochemical test on isolated bacteria. Positive (*A and B*) and negative (*C and D*) on gram staining.

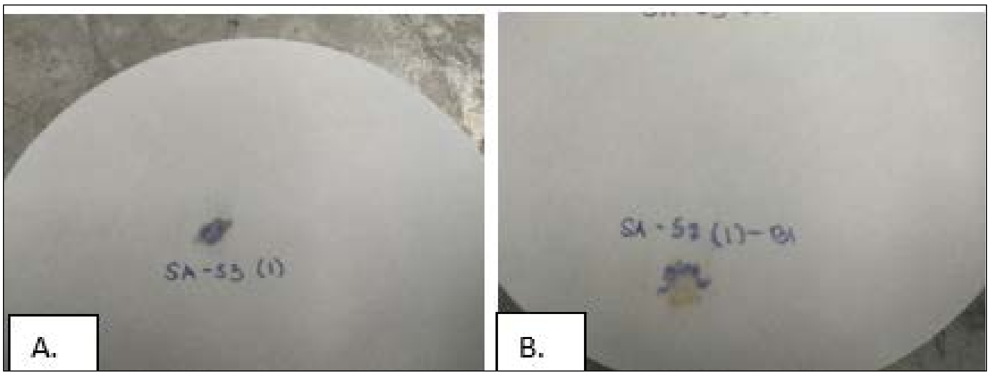

Fig 9: Results of biochemical test on isolated bacteria. Positive (*A*) negative (*B*) on oxidase test

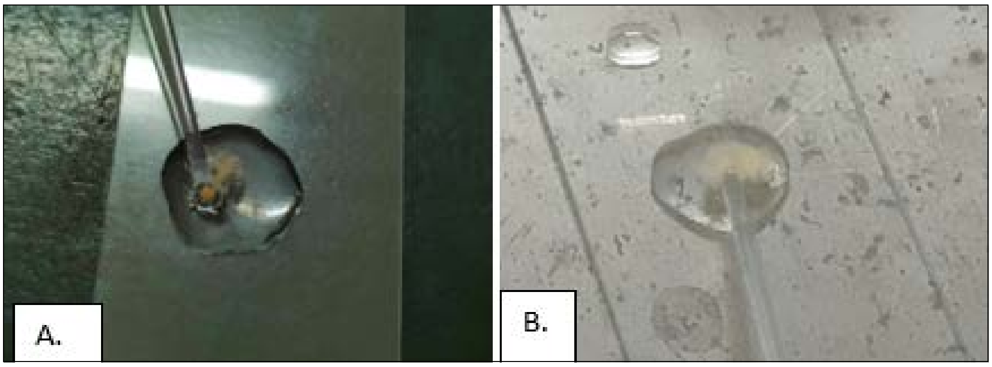

Fig 10: Results of biochemical test on isolated bacteria. Positive (*A*) negative (*B)* on catalase test

Table 9: Identified various microorganisms present from sample collected using API 20E.

Selected Isolates	Type of Bacteria	Characteristics	ID	% ID
AP - S2 - 1	Gram Negative	Small/yellow/entire	*Pseudomonas oryzihabitans*	98%
AB - S3 - 3	Gram Negative	Medium/dull/entire	*Elizabethkingia meningoseptica*	98.4%
SA - S2 – 1 (White)	Gram Negative	Small/white/entire	*Pseudomonas luteola*	99.9%
SA - S2 – 2	Gram Negative	Filamentous	*Pseudomonas aeruginosa*	99.9%
SA - S3 – 2 (Yellow)	Gram Negative	Yellow/Orange	*Elizabethkingia meningoseptica*	98.4%

Table 10 presented the prevalence (%) of the bacteria and parasites identified using the API 20E test under the El gohanry formula. Based on this, only three bacteria have high % prevalence with *Elizabethkingia meningoseptica* the highest prevalence of 43.24%. The observed morphological characteristics were medium in size, dull in color, with flat elevation and entire margin. Additionally, other characteristics such as yellow/orange in color with a prevalence of 5.4% were also identified as *E. meningoseptica*. It is a non-motile, non-fermentative, oxidase positive, and opportunistic Gram-negative bacterium, normally found in water and soil (Sahu et al., 2019) [14] first described by Elizabeth O. King in 1959. It is not known to reside in human hosts but notorious for causing nosocomial infections including meningitis, pneumonia and bacteremia in extremes of ages and immunocompromised hosts. Followed by *Pseudomonas luteola* with 32.43% prevalence, its morphological characteristics were small in shape, white in color, flat in elevation with entire margin. It is a gram-negative bacillus, testing positive with both oxidase test and catalase test that rarely causes human disease and are often isolated from internal organs of fish. *Pseudomonas oryzihabitans* with 16. 21% prevalence was observed to be small in shape, yellow in color, flat elevation and with entire margin morphologically. It also resulted positive with catalase and oxidase biochemical tests. *P. oryzihabitans* is a rod-shaped, Gram-negative bacterium known to cause infections in both immunocompromised and healthy individuals. In nature, *P. oryzihabitans* can be found in moist environments such as soil, rice paddies, running or standing water, and groundwater.

Among the identified parasites, *Trichodina spp*. was the most dominant species with 9.8% prevalence. *Trichodina* is a saucer-shaped parasite that attacks fish skin and gills. The typical signs of the disease include skin and gill damage, respiratory distress, loss of appetite and loss of scales. Skin and gill damage caused by this parasite may lead to the entry of other pathogens such as bacteria and fungus (El-Sayed, 2020) [6].

Dactylogyrus spp. was the next dominant species identified with a prevalence of 3.9%. It is a genus of monogeneans in the Dactylogyridae family. Like other monogeneans, species of *Dactylogyrus* only require one host to complete their life cycle. *Dactylogyrus* predominantly affect the gills, whereas *Gyrodactylus* is more commonly found on the skin (Stoskopf, 2015) [18]. Its symptoms in fish are flashing, tiny red spots or yellow dusting, excessive production of mucus, shedding of the slime coat, clumped fins. Bacteria and parasites identified in glass eel needs further verification on the epizootiology characteristic of each pathogenic agent.

Table 10: Prevalence (%) of Bacteria and Parasites Identified using the El Gohary Formula

Total number of Samples	Bacteria				Parasites		
	Species	Total number (based on morphology)	Preva-lence (%)	Total no. of Samples	Species	Total no.	Preva-lence (%)
37	*Pseudomonas oryzihabitans*	6	16.21	51	*Trichodina spp.*	5	9.8
	Elizabethkingia meningoseptica	16	43.24		*Dactylogyrus spp.*	2	3.9
	Pseudomonas luteola	12	32.43				
	Pseudomonas aeruginosa	1	2.7				
	Elizabethkingia meningoseptica	2	5.4				

Conclusion

The bacteria identified on glass eel samples were *E. meningoseptica*, *P. luteola*, *P. oryzihabitans*, and *P. aeruginosa* using the API 20E kit. The observed morphological characterization was consistent with that of the literature of the determined microorganisms which was mainly medium in size, flat in elevation, with entire margin and with white pigmentation. Employed biochemical tests, primarily Gram staining, oxidase and catalase tests, revealed that were gram–negative and indicated positive results with both oxidase and catalase tests. Skin smearing and gill examination were used to identify external parasites in glass eel. From the results, most of the parasites thrived on the gill part of the glass eel. The parasites identified were Trichodina *spp* and *Dactylogyrus spp*.

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