

SCREENING OF SPORE-FORMING BACTERIA WITH PROBIOTIC POTENTIAL IN PRISTINE ALGERIAN CAVES

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Why cave bacteria ?

- Extremes of near-starvation or oligotrophy (less than 2mg of total organic carbon, TOC, per liter)
- Only one study in Algeria about cave microbiology.
- Probiotics isolated from other ecosystems than human origin were poorly studied

Why *Bacillus* ?

- Most diverse and commercially useful group of microorganisms
- Wide range of industrial processes
- Used as probiotics for at least 50 years

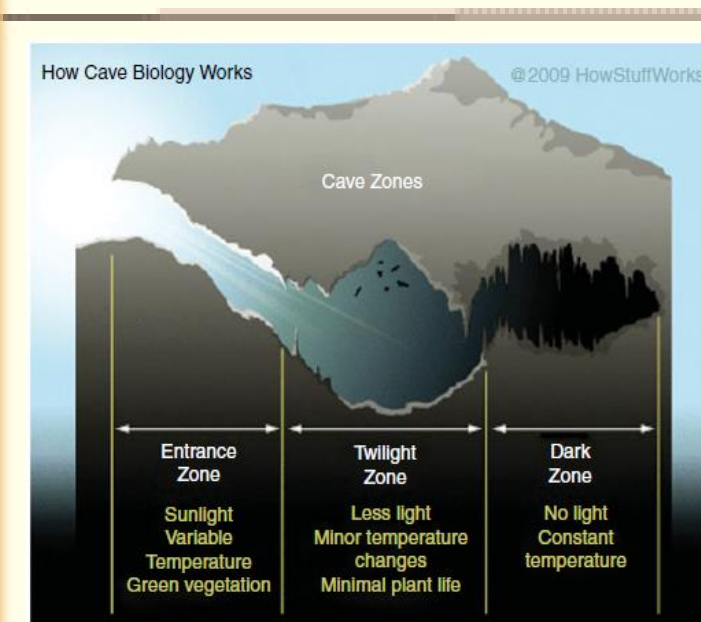
Keywords: Probiotics, beta-galactosidase, gliadinase, *Bacillus*, *paenibacillus*



Sampled caves

- Mchounech 1
- Mchounech 2
- Ain Smara 1
- Ain Smara 2
- Blanche
- Anou Bousouil
- Ghar Dbaa
- Ghar Djmaa
- Pirates
- Bouakkous

Sampling



The three zones (entrance, twilight, dark) of a cave (graph from www.howstuffworks.com)

PS: all the samples were taken from the dark zone



Summary of general characteristic of studied caves

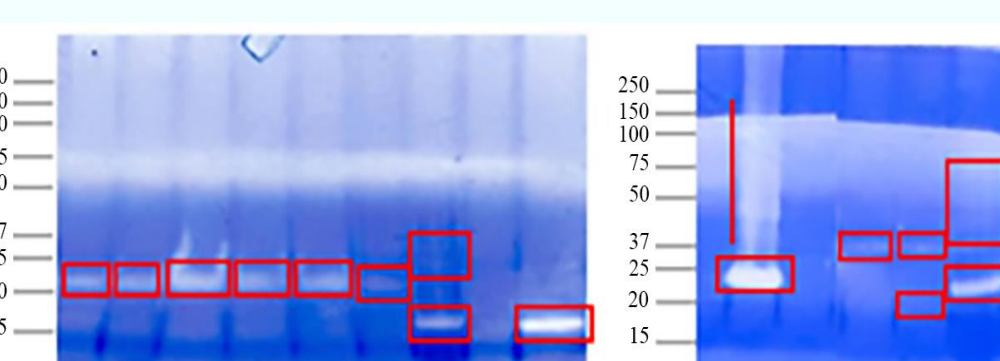
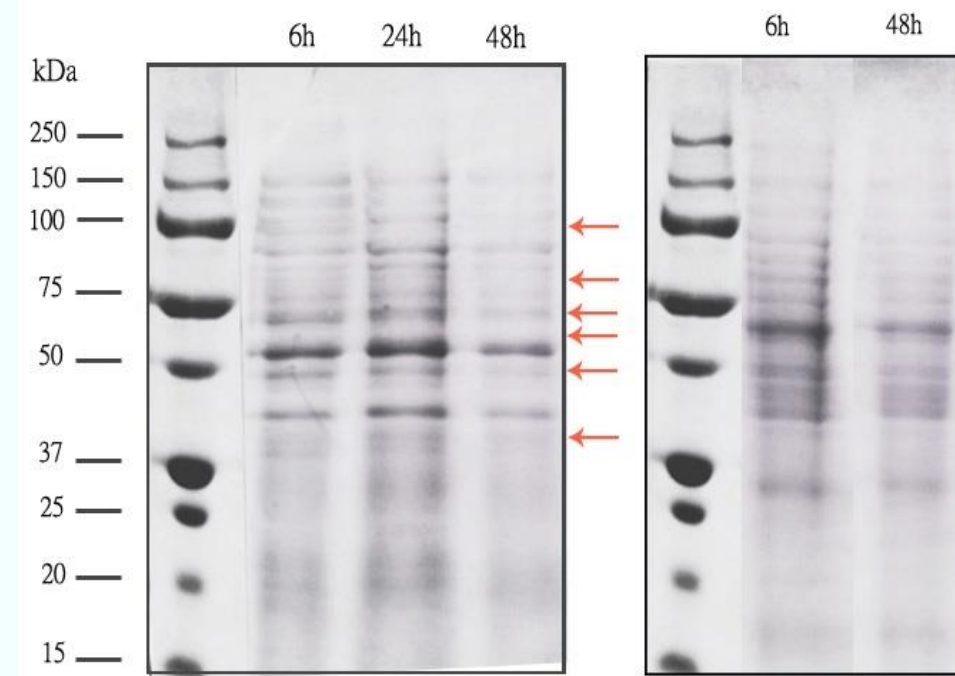
Caves	Codes for the caves	GPS coordinates	Region	Depth (m)	Annual precipitation n*
Ain smara 1	AS1	36°15'34.85"N 06°31'25.78" E	Constantine	-10	630 mm
Ain smara 2	AS2	36°15'34.85"N 06°31'25.78" E	Constantine	-10	630 mm
Blanche	BL	36°46'12.5"N 5°04'42.9"E	Bejaia	NA	830 mm
Bouakkous	BOU	35°25'04.4"N 7°57'49.2"E	Tébessa	-50	830mm
Bousouil	ANOU	36°28'09.4"N 4°11'29.5"E	Bouira	-805	650 mm
Ghar dbaa	GD	36°21'40.8"N 6°28'29.7"E	Constantine	NA	630 mm
Ghar djmaa	GG	36°27'27.2"N 7°23'08.9"E	Guelma	-129	557 mm
Mchounech 1	MB1	34°57'23.4"N 6°24'1.6"E	Biskra	NA	141 mm
Mchounech 2	MB2	34°57'41.6"N 6°00'48.6"E	Biskra	NA	141 mm
Pirate	PR	36°46'19.4"N 5°05'53.9"E	Bejaia	NA	830mm

NA= not applicable Algerian national meteorological office

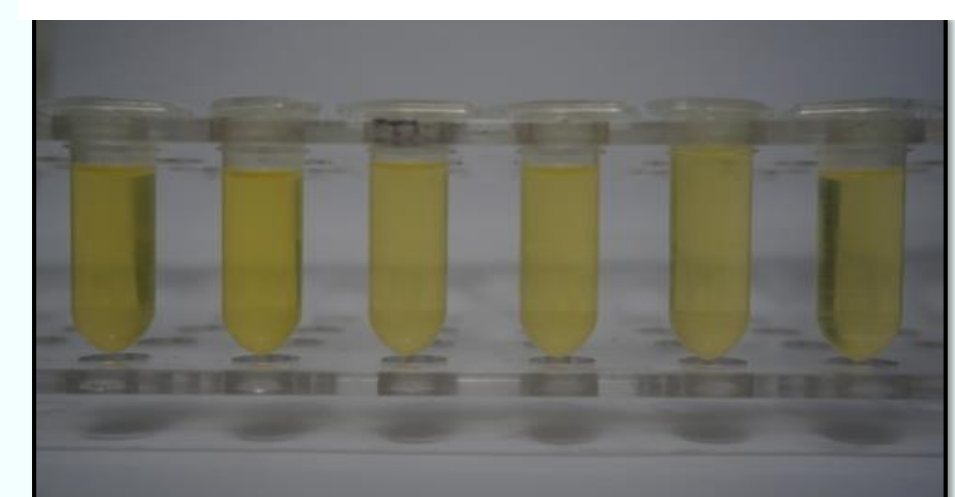
Physico-chemical- biological characteristics of the sediments in investigated caves.

Caves codes	Macro-biodiversity (recorded so far)	Sampling area depth (m)	Distance from the entrance	pH	Organic matter (%)	Total carbonate (%)	Temperature (°C)	Humidity (%)
AS1	Spiders	-10m	15m	8,6 ± 0,02	1,56 ± 0,98	90	13	60,0
AS2	Spiders	-10m	20m	7,7 ± 0,02	0,83 ± 0,18	100	14	61,0
BL	-	-10m	48m	9,3 ± 0,00	0,46 ± 0,25	100	13	61,3
BOU	-	-50	27m	7,1 ± 0,01	0,37 ± 0,31	80	10	72,2
ANOU	Non identified insects	-50m		8,0 ± 0,02	0,00	80	7,3	99,99
		-100m		7,15 ± 0,03	0,00	80	4,5	99,99
		-450m		6,91 ± 0,03	0,00	80	3,2	99,99
GD	Bats	-	45m	7,5 ± 0,00	2,12 ± 0,31	10	13,5	84,0
GG	Moths	-	68m	8,3 ± 0,00	0,18 ± 0,12	0	14,1	73,3
MB1	Reptiles	-	9m	8,1 ± 0,00	0,09 ± 0,06	40	23	43,2
MB2	Reptiles	-	11m	7,4 ± 0,12	0,00	60	25	42,2
PR	Bats	-	39m	8,0 ± 0,00	4,69 ± 0,18	40	19	63,3

Enzymatic potentiality in solution



SDS-PAGE and *in gel* zymography to detect Gliadinase activity in solution and degradation patterns



Beta-galactosidase activity in solution using Miller method.

Screening for digestive enzymatic activity

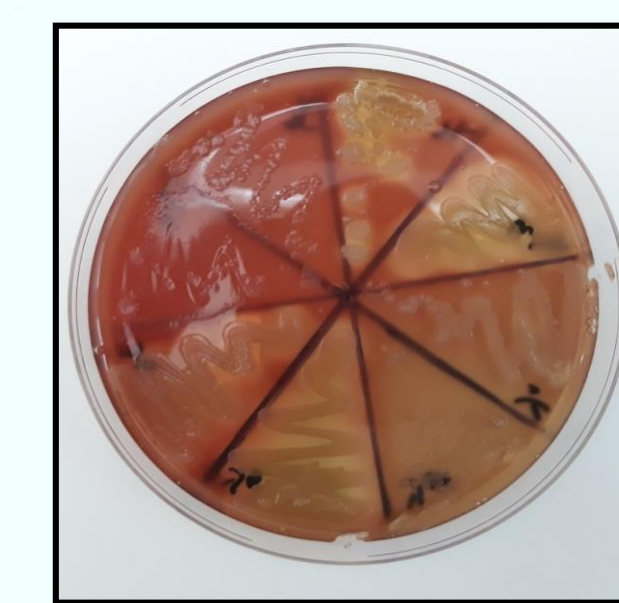


Lactose degradation in ONPG agar

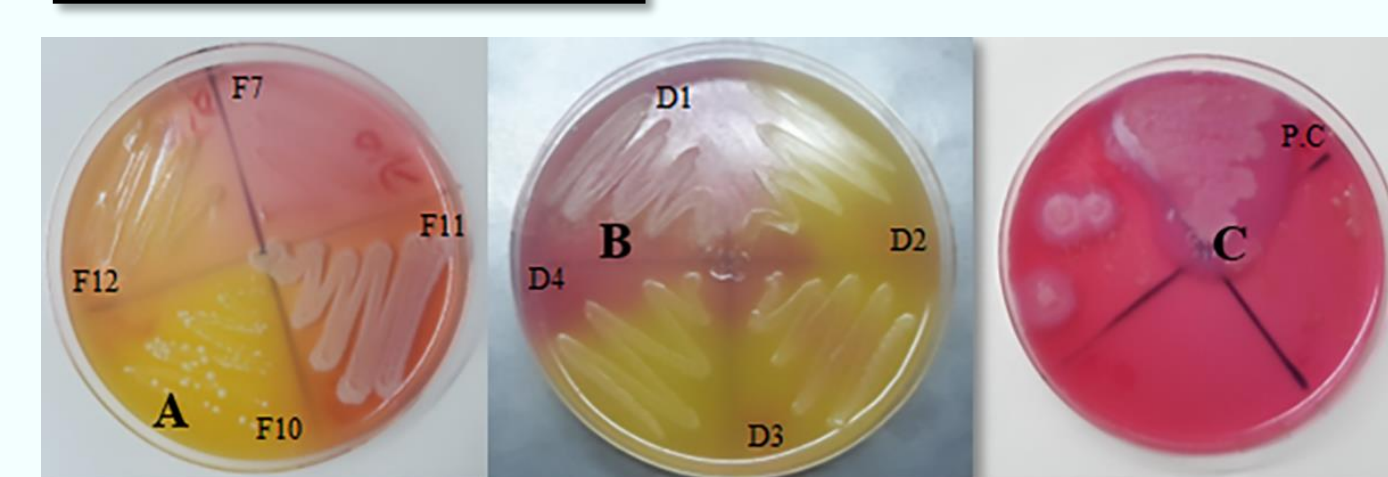


Gliadin degradation in Gliadin based agar

Safety assessment



Hemolysis patterns using Columbia Blood Agar



Lecithinase using Egg-Yolk agar

Enzymatic potential



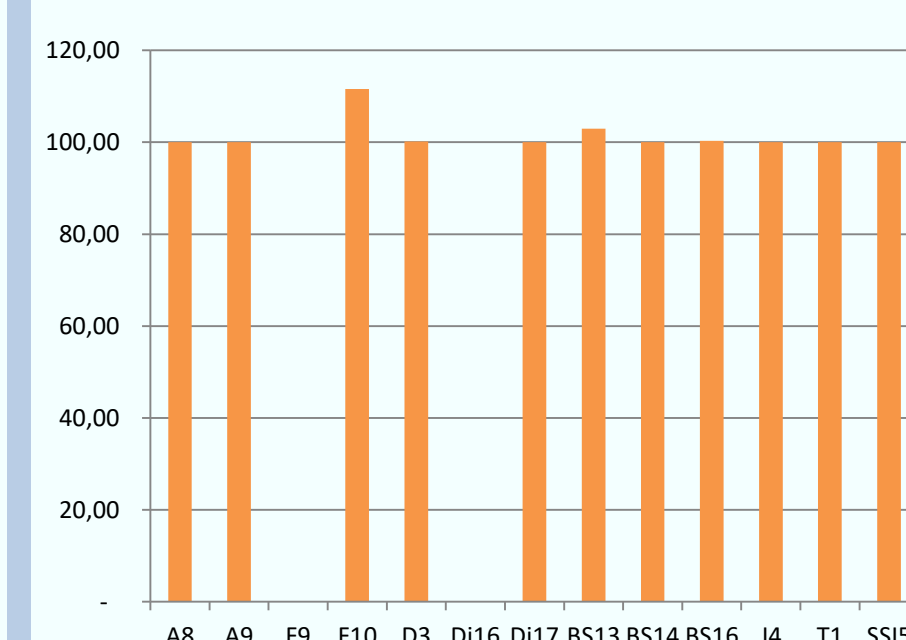
Antibiotic susceptibility

According EFSA guidelines for testing antimicrobial susceptibility of *Bacillus* species (EFSA, 2012)

Antibiotics	A8	A9	D3	F9	F10	BS1	BS1	DJ1	DJ17	BS16	T1	SS15	J4
Amikacine 30ug	R	S	R	S	R	S	S	R	R	R	R	R	R
Ampicilline 2ug	S	R	S	R	R	S	R	R	S	R	R	R	S
Amoxycillin 10ug	R	R	R	R	R	R	R	R	S	R	R	R	S
Ciprofloxacin 1ug	S	S	S	S	S	S	S	R	S	S	S	S	S
Erythromycin 10ug	S	S	S	S	R	R	R	S	S	R	R	R	R
Gentamicin 10ug	R	R	R	S	R	R	R	R	R	R	R	S	R
Rifampicin 15ug	R	S	R	R	R	R	R	R	S	R	S	S	S
Vancomycin 5ug	S	S	S	S	R	S	S	R	R	R	S	R	R
Imipenem 10ug	S	S	S	S	S	S	S	S	S	S	S	S	S

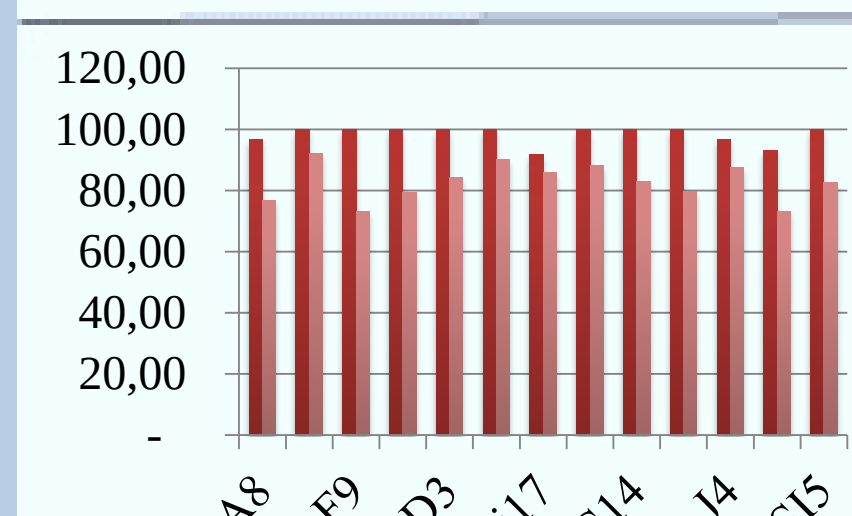
Survival in GIT

In vitro survival in simulated intestinal fluid

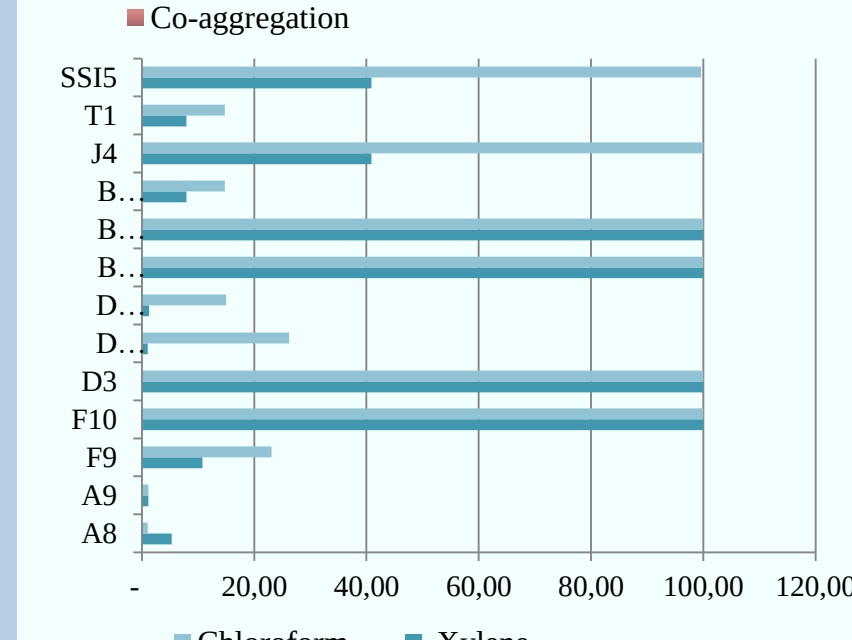


In vitro survival in simulated intestinal fluid

Cell surface characteristic

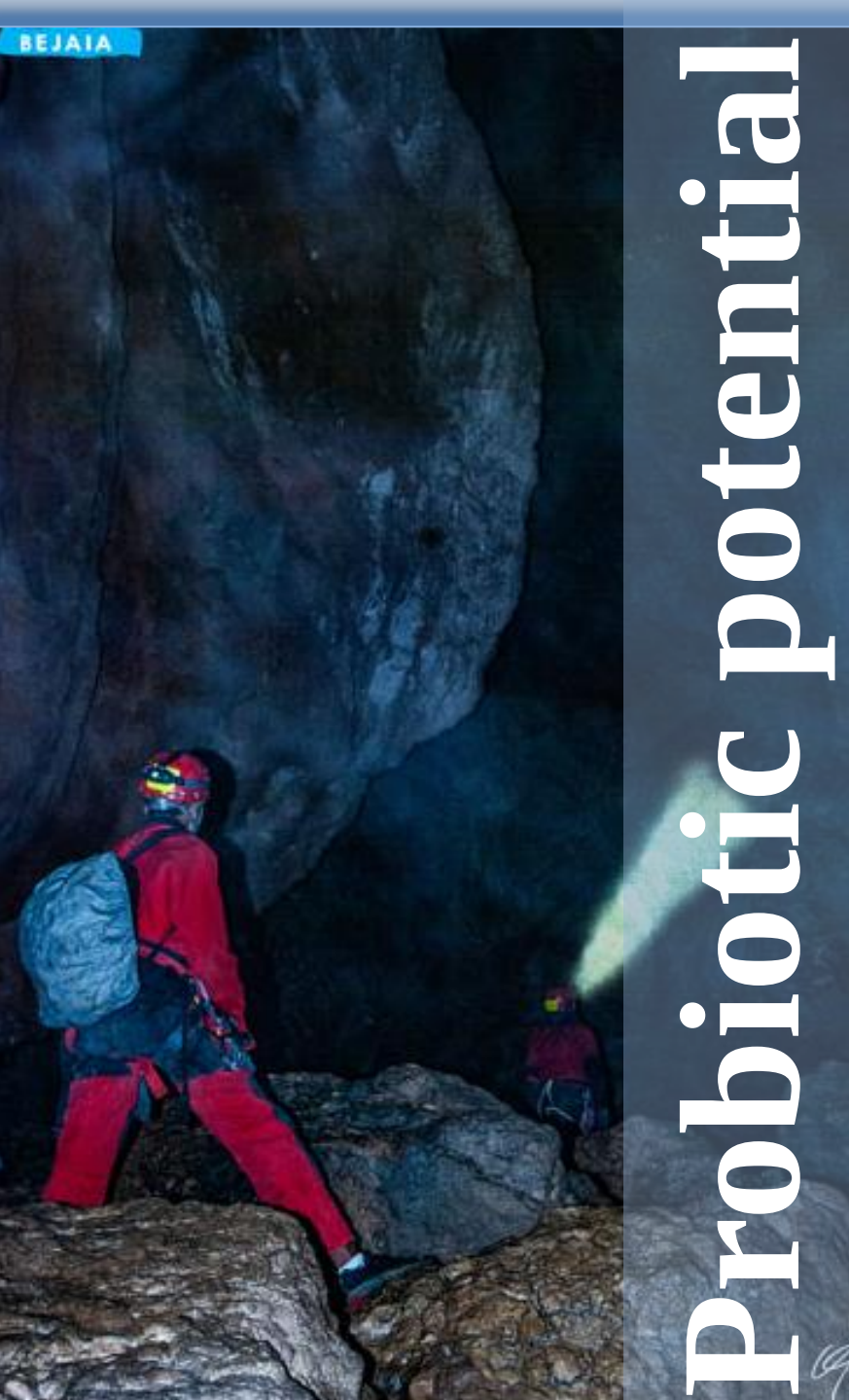


Auto-aggregation and co-aggregation play a role in colonization effectiveness



Hydrophobicity and electron receptor as adhesion ability parameter

Probiotic potential



Phylogenetic tree (unrooted) showing the tentative 16S rRNA gene relationships between the 13 isolates retrieved in this study and their closest relationships to 15 other sequences from culturable organisms within the LTP database, version 132.

Molecular biology



Conclusion

Cave microorganisms studies may lead to

- ❖ Microbes could be isolated from deep, pristine caves in Algeria.
- ❖ 13 out of 250 cave isolates of heat tolerant, spore-forming bacteria showed probiotic potential.
- ❖ However, the age and role of these isolates in their natural cave environment is still unknown.

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