The Utilization of Rum Slops by Marine Bacteria. II. Characterization of Efficient Strains[1]

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ABSTRACT

Twenty bacterial isolates from selected marine communities were obtained employing solid, modified sea water media containing slops. Thirteen basic morphological, cytological, physiological and biochemical tests were conducted to characterize six of the strains that grew most successfully on the slops media.

The ability to hydrolyze high molecular weight sugars and proteins appears to be a necessary condition for the successful growth of some of these isolates on slops media. Tentative identifications of these bacterial strains were made.

INTRODUCTION

The growth in slops media of 20 bacterial isolates was studied. The bacterial strains that were able to grow (after approximately 24 h) at what turned out to be the highest tolerable concentration of slops (4.04 mg/ml) were characterized morphologically, cytologically, physiologically, and biochemically. The different properties of the strains were compared and the probable roles of each strain in the degradation of slops are discussed. Tentative generic assignments are made where possible.

MATERIALS AND METHODS

The growth studies were conducted in the manner previously reported (7). The tests employed for the identification and characterization of the bacteria were based on established procedures (2, 3, 4, 5, 6). Bergey's Manual (1) was employed to make tentative generic assignments.

RESULTS AND DISCUSSION

GROWTH OF THE BACTERIA ON SLOPS

The results of the growth studies conducted with strains DIALMUD 1 and DIALMUD 2 are presented in table 1. It can be seen that strain DIALMUD 1 was able to grow at a slops concentration of 4.04 mg/ml, though it was able to do so only after at least 5 h of incubation. The substrate utilization efficiency (SUE), which is essentially the degree of growth of the bacterial strain during a given period of incubation divided by the concentration of slops employed (7) also occurred at a slops concentration of 4.04 mg/ml in this bacterial strain. Strain DIALMUD 1

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TABLE 1.—*Growth of two bacterial isolates in slops*

Determination	Crude slops concentration (mg/ml)					
	0.00	0.404	0.808	1.62	4.04	6.06
			Dialmud 1			
OD (5 h)	−0.042	+0.404	+0.138	+0.323	+0.03	−0.02
OD (24 h)	−0.029	+0.048	+0.118	+0.393	+1.3	+0.07
SUE[1]			0.147	0.243	0.322	
			Dialmud 2			
OD (5 h)	−0.26		+0.213	+0.349	+0.015	
OD (24 h)	−0.030		+0.373	+0.654	+0.03	
SUE[1]			0.338	0.404		

[1] Based on the 24 h incubation.

was thus able to overcome the inhibitors present in slops. These data suggest that the bacteria were initially at a steady state in the culture and that they were able to detoxify the medium by either slowly metabolizing the inhibitors and/or inactivating them by the secretion of appropriate extracellular materials. The optimum growth and SUE of strain DIALMUD 2 occurred at the 1.62 mg/ml concentration of slops (table 1).

The 10 isolated strains of bacteria whose growth was characterized by cellular aggregation and "clump" formation (CRRT 1, CRRT 4, CRMRS 1, CRMRS 3, CRSED 1, DIALRT 1, DIALMRS 1, DIALMRS 2, DIALSED 1 and CRMUD 3) were grown at a slops concentration of 4.04 mg/ml for periods of 24 h. Only one of these strains, CRMRS 3, showed any ability to grow at this concentration of the slops. Thus, of the 20 bacterial strains that were able to grow in the slops agar employed in their initial isolation, approximately one-fourth of these were able to grow at slops concentrations as high as 4.04 mg/ml.

CHARACTERIZATION OF THE MARINE BACTERIAL ISOLATES THAT EXHIBITED THE BEST GROWTH IN THE SLOPS

Thirteen basic morphological, cytological, physiological and biochemical tests were conducted with each strain of bacteria in order for the researchers to get some idea of the taxonomical and physiological types of marine bacteria that degrade the slops. The strains examined were CRRT 2, DIALMR 1, CRMUD 2, DIALMUD 1, and CRMRS 3. DIALMUD 1 was found to be impure upon careful examination and was separated into two strains of bacteria designated as DIALMUD 1 W and DIALMUD 1 C.

The test results are presented in table 2. All of the strains were of the gram negative, aerobic or facultatively anaerobic, short rod type of bacteria. None of the six strains tested appeared to be duplicates. DIAL-

MUD 1 W and CRMRS 3 produce pigment when grown on nutrient agar, though they did not appear to do so on the slops agar. Four of the six bacterial strains examined were strict aerobes, the other two being facultative anaerobes.

The two facultative anaerobes (DIALMR 1 and CRMUD 2) showed powerful diastatic as well as proteolytic activity in nutrient gelatin and litmus milk. The aerobe CRMRS 3 grew slowly in nutrient gelatin and litmus milk, but did show proteolytic activity in each. It would be expected that bacteria with such abilities would be likely to degrade polysaccharide or proteinaceous materials found in spent fermentation wastes such as the slops. In addition, DIALMUD 1 C hydrolyzed gelatin readily, but did not attack starch or milk.

CRRT 2 and DIALMUD 1 W did not attack starch, gelatin or milk, however, appeared to grow well on the slops at a concentration of 4.04 mg/ml. Thus the ability to hydrolyze high molecular weight sugars and proteins appeared not to be a necessary condition for the growth of this marine bacterium on the crude slops. Probably the most significant factor controlling the initial proliferation of marine bacteria in a sea water-slops medium is the tolerance of the particular strain to the inhibitors present.

Longer term incubations would likely provide these bacteria with ample time to detoxify the slops and thus be able to degrade it more fully. Evidence presented previously in this report suggests that such a detoxification process does take place. Proper manipulation of such reasonably "resistant" bacteria, especially the ones that have the ability to degrade the polysaccharide and proteinaceous substances, might provide an efficient system for the digestion of the slops. However, the data suggest that this would have to be done at relatively dilute slops concentration over a period exceeding 24 h. The potential harvestable "bacterial protein" could conceivably make such a process commercially viable.

TENTATIVE IDENTIFICATION OF THE SIX BEST-GROWING BACTERIAL STRAINS

Based on the tests performed, tentative identifications of the bacterial strains were attempted. The following tabulation summarizes these results:

Bacterial Strain	*Genera*	
	Likely	*Possible*
CRRT 2		Pseudomonas Alcaligenes
DIALMUD 1 W		Pseudomonas
DIALMUD 1 C	Pseudomonas	
CRMRS 3		Pseudomonas
DIALMR 1		Aeromonas
CRMUD 2	Vibrio	Aeromonas

TABLE 2.—*Characterization of the marine bacterial isolates*

Test	Bacterial strain					
	CRRT 2	DIALMUD 1 W	DIALMUD 1 C	CRMRS 3	DIALMR 1	CRMUD 2
Cell morphology (unstained, oil immersion phase)	(l) (w) Rods 1.6–3.2 × 0.8–1.2 (μm)	(l) (w) Rods 0.8–1.6 × 0.4 (μm)	(l) (w) Rods 1.6–2.4 × 0.8 (μm)	(l) (w) Curved rods 2.4–4.0 × 1–1.5 (μm)	(l) (w) Rods 1.6–2.4 × 0.8 (μm)	(l) (w) Rods 1.6–2.4 × 0.8 (μm)
Gram reaction	Negative	Negative	Negative	Negative	Negative	Negative
Motility (hanging drop)	Sluggishly motile	Motile	Motile	Non-motile	Very motile	Very motile
Temperature of incubation	22–24°C	22–24° C	22–24°C	22–24°C	22–24°C	22–24°C
Starch hydrolysis	Negative	Negative	Negative	Negative	Positive	Positive
Gelatin hydrolysis	Negative	Negative	Positive	Positive (slow, but complete)	Positive	Positive
Carbohydrate fermentations						
glucose	0	A	0	A	0	A
sucrose	0	0	0	0	0	A
lactose	0	0	0	0	0	0
Litmus milk reaction (3% NaCl)	Reduction of litmus; no curd formation, no proteolysis	Reduction of litmus; to red (surface), no curds, no proteolysis after 1.5 weeks	Slow reduction of litmus, no curds, no proteolysis	Reduction of litmus, "sweet" curds, proteolysis in progress, transparent layer becomes blue	Reduction of litmus (reversible); top layer blue; proteolysis in progress (increasing transparency)	Litmus reduced, proteolysis in progress
Oxygen requirement	Aerobe (surface growth only)	Aerobe (surface growth only)	Aerobe (surface growth only)	Aerobe (surface growth only)	Fac. anaerobes; growth throughout, microaerophilic band	Facultative anaerobe (growth throughout)

Growth in nutrient broth (0.3% beef extract, 0.5% peptone)						
membrane	Yes	Yes (peeling)	Slight	Slight	Yes	Yes
pellicle	No	No	No	No	No	No
ring	Slight	No	No	Yes	No	No
sediment	Yes	Slight	Slight	Yes	Yes	Slight
comments	Growth spread surface bottom over 48 hrs	Growth throughout	Growth throughout (48 hrs), small flocs	Growth throughout	Growth throughout (24 hours)	Growth throughout
Agar slope	Filiform	Filiform	Filiform	Filiform	Filiform-arborescent	Effuse
Catalase test	Strong positive	Positive	Positive	Positive	Positive	Negative
Colony morphology (0.3% beef extract, 0.5% peptone, 1.5% agar)						
color	Off-white	Light orange	White	Yellow	Off-white	Off-white
shape	Circular	Punctiform-circular	Circular	Circular	Irregular	Irregular, colonies not formed
elevation	Convex	Convex	Convex	Convex	Convex	Convex
edge	Entire	Entire	Entire	Entire	Entire	Entire-undulate
surface	Smooth, glistening	Smooth, glistening	Smooth, glistening	Smooth, glistening	Smooth, glistening	Smooth, glistening
optical	Mildly opaque	Opaque	Mildly opaque	Opaque, clear area around	Reasonably opaque	Opaque

[1] Natural seawater was used as the base in all of the culture media except that of the litmus milk. Fermentations were done with Durham tubes in Trypticase-Yeast Extract based media.

RESUMEN

Veinte cepas bacterianas de comunidades marinas seleccionadas se aislaron en un medio de cultivo sólido modificado que contenía agua de mar y mosto.

Trece pruebas morfológicas, citológicas, fisiológicas y bioquímicas se realizaron para caracterizar seis de las cepas que crecieron mejor en el medio de cultivo con mosto.

Estas cepas bacterianas han sido identificadas tentativamente.

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