دراسة الصفات المرفولوجية والأحتياجات الغذائية لكائنات الأرثروباكتر اللبنية

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أجرى هذا البحث لدراسة الصفات المرفولوجية والاحتياجات الغذائية لـ ١١٩ سلالة من كائنات الاثروباكتر المعزولة من الفلورا السطحية لبعض أنواع الجبن المحلية والمستورده (الدمياطى - الرأس - الادام - الجواد - الجروبير). وفيما يلى ملخصا لأهم نتائج هذه الدراسة :

١ - قسمت السلالات المعزولة الى خمسة مجاميع هى :

Arthrobacter, Arthrobacter like, Intermediate, Coryneform like, Coryneform.

٢ - أمكن لافراد الـ Arthrobacter like, Arthorbacter استهلاك النتروجين غير العضوى فى وجود أو عدم وجود الفيتامينات .

٣ - جميع السلالات المختبرة مستهلكة للجلوكوز واللاكتات كمصدر وحيد للكربون .

٤ - كان أهم الصفات المميزة للخمسة مجاميع المصنفة سابقة الذكر هى اختلافها فى شكل الخلية .

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MORPHOLOGICAL CHARACTERS AND NUTRITIONAL REQUIREMENTS OF ISOLATED DAIRY ARTHROBACTERS

(With One Table and 2 Figures)

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(Received at 8/4/1977)

SUMMARY

The investigations studied the morphological characters and nutritional requirements of isolated Arthrobacter like organisms isolated from the surface of some locally made & imported cheeses namely Domiatti, Ras, Edam, Gouda and Gruyere cheese. 119 isolated strains were included in the study. The obtained result are summarized as follows:

1. The isolated strains were classified into five groups, namely Arthrobacter, Arthrobecter like, Intermediate,Coryneform like and Coryneform.
2. Members of both the Arthrobacter and Arthrobacter like groups were able to utilize inorganic nitrogen either with or without vitamins. The intermediates were equally divided to a vitamin dependant and inorganic nitrogen utilizing in the abcense of vitamin.
3. All strains utilized both glucose and lactate as the only carbon source.
4. The most important differential character in classifying the isolated strains to groups was their cell form.

INTRODUCTION

Athrobacter is an aerobic, pleomorphic micro-organism belonging to the corynebacteriacese. In young cultures the cells appear as rods which

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may vary in size and shape, from straight to bent,swollen or club-shaped forms; sometimes developing filaments and true branching. Snapping division may give rise to angular cell arrangement. Upon ageing, the rod-shaped cells turn into coccoid forms. The cocoid cells are persistent as the predominant from in older cultures. It can be generally said that bacteria of this genus are charaterized mainly by their cell morphology.

Extensive investigations concerning the morphology and physiology of bacteria of the genus Arthrobacter have been carried out by several workers. Morphological studies on these micro-orgainsms have been reported by (TOPPING 1937). (TAYLOR 1938), (SACKS 1954), (SGUROS 1955, 1957), (CHAPLIN 1957), (SUNDMAN 1958),(BLANKENSHIP and DOETSEH 1961),(STEVENSON 1961, 1962, 1963), (STARR and KUHN 1962), (ENSIGN and WOLFE 1964), (SIEBURTH 1964), (MULDER and ANTHEUNISSF 1963),(MULDER 1964) and (MULDER et al.1966). Nutritonal studies have been made by (CAMPBELL and WILLIAMS 1951), (MULDER 1957), (MORRIS 1960), (LOCHHEAD and his COLLABORATORS 1953, 1955, 1957, 1958) as well as by (MULDER et al. 1962, 1966).

Brevibacterium linens was found to be usually present in large numbers in the slimy orange or orange-brow growth developing on the surface of many soft cheeses, and was thought to contribute to the ripening process of such cheeses (WOLFF,1909,1910; WELGMAN,1911; STEINFATT , 1930; GRIMMER and SCHMIAL 1936, KELLEY 1937, KELLEY and MARQUARDT,1939 and ALBERT, LONG and HAMMER, 1944). (MULDER et al. 1966) and (EL-ERIAN 1969) showed the close resemblance between Br. linens and isolated Dairy Anthrobacters. (EL-ERIAN 1969) found that Arthrobacter played an important role in the a ripening of lemburger cheese.

(EL-SISSI 1977) used Arthrobacter like bacteria to produce an early ripened good quality Domiaitti cheese throughout the use of special surface flora and adjusting the way of manufacturing and ripening of the cheese to suit the growth of such flora.

The objective of this investigations was to study the morphological characters and nutritional requiremints of isolated Arthrobacter like

Organisms isolated from the surface of some locally made and imported cheeses namely Domiatti, Ras, Edam Gouda and Gruyére cheese.

MATERIALS AND METHODS

Material:

Arthrobacter-like bacteria which used in this study were isolated from the surface of Five types of cheeeses namiely, Domiatti cheese,Ras, Edam Gouda and Guryére Cheese.

Isolation and identification of Arthrobacter-like bacteria were carried out according to (MULDER 1964),(MULODER et al.1966) and(EL-ERIAN 1969). 119 isolated strains were used in this study (Table 1).

Methods:

1. Replica method:

Strains isolated were tested for proteolytic activity, catalase reaction, utilization of carbon compounds and for Arthrobacter characteristics with the replica method (MALING 1960).

2. Gram's Stain:

The Gram's stain was carried out according to Hucker's modification described in the Manual of Microbiological Methods (1957).

3. Media:

Oxoidtryptone glucose extract agar (T.G.E.A.).This medium contains: beef extract, 3g; tryptone, 5g; glucose, 1 g; agar, 12 g; tap water, 1000 ml; pH: 7.0.

Casein agar:

This medium is composed of: casein, 1 g; yeast extract, 0.7 g; glucose, 1 g; K_2HPO_4, 1 g; Ca (H_2PO) 2, 0.25 g; Mg SO_4. $7H_2O$, 0.25 g; (NH_4) $_2SO_4$, 0.25 g; agar, 10 g; tap water, 1000 ml; pH : 70.

Yeast extract glucose agar:

This medium had the following composition: yeast extract, 7,9; glucose, 10 g; agar, 12 g; tap water, 1000 ml; pH : 70.

The mineral nutrient medium:

The mineral nutrient medium compolyed for certain nutritional tests had the following composition: K_2HPO_4, 1; $MgSO_4 . 7H_2O$, 0.3; $CaCl_2 . 2H_2O$, 0.05; $FaCl_2 . 6H_2O$, 0.01 g per litre; $CuSO_4 . 5H_2O$, 0.1; $ZnSO_4 . 7H_2O$, 0.1; $MnSO_4 . 7H_2O$, 1.0; Na_2MoO_4, 0.01 and H_3BO_3. 0.01 mg per litre of medium. The pH of medium was 7.0.

The vitamin mixutre:

The vitamin mixture used, condsisted of: biotin, 2; folic acid,20; riboflavin, 100; thiamin, 100; pyrixoxine, 100; nicotinic acid, 100; pantothenic acid, 100; p-amino-benzoic acid, 100 and Vitamin B_{12}, 1 ug per litre of medium.

4. Nutritional requirements:

a) Utlization of carbon compounds:

The effect do different carbon compounds was tested on agar media containing 0.15 percent yeast extract and 0.5 percent of compound to be tested.

b) Nitrogen and vitamin requirements:

In this investigation, use was made of the mineral nutrient medium, supplied with 0.25 gram nitrogen in the form of ammonium sulphate or Casamino acids, and 5 g glucose per litre of medium; pH:7.0. The vitamin mixture was added in this experiment.

RESULTS AND DISCUTIONS

According to 'CONN and DIMMICK 1947), the soil Arthrobacter represent those which can utilize inorganic nitrogen in the absence of

vitamins but this observation has not been substantiated by other investigators (TAYLOR 1938,TENSEN 1952, VEDDKAMP et al.1963 and MULDER 1963).

(MULDER 1964) tested the nitrogen and vitamin requirements of a large number of coryneform bacteria from different suources. He found that nearly all the Arthrobacter strains isolated from soil were able to utilize inorganic nitrogen, either in the presence or absence of vitamine. Arthrobacter strains isolated from activated sludge reacted like the soil arthobacters. Of 46 cheese strains, he found 13 to have the same nitrogen and vitamin requirements as the soil strains, 11 utilized ammonium nitrate when a vitamin mixture had been added and 22: required Casamino acids either with or without vitamins.

After keeping their cheese arthrobacters for a number of years at room temperature on yeast extract glucose agar slopes, (MULDER et al. 1966) found that several strains had lost the ability to utilize ammonium nitrate and had to be supplied with a mixture of an ammonium salt and glutamic acid.

In the present investigation out of the 19 true arthrobacter strains only two required the presence of Casamino acids. The rest of the strains were able to utilize inorganic nitrogen either with or without vitamine.

All of the 28 Arthrobacter-like strains were able to utilize inorganic nitrogen. The 17 intermediates were equally divided according to its vitamin requirements (Table 1). Sixteen out of 42 coryneform-like strains both Casamino acids and vitamins. The 13 coryneform strains were vitamin dependant either for inorganic nitrogen or Casamino acids. These results were found to be in close agreement with the findings or(EL-ERIAN 1969).

All the isolated strains were found to be catalase positive. As for the utilization of carbon compounds, all the strains utilized both glucose and lactate as the only carbon source. This was not true of Sucrose

and lactose which were utilized by only few strains. These results agreed with those found by (MULDER 1964).

A large difference in proteolytic activity of the various groups was found. The intermidiate and coryneform-like strains (59 strains) were highly proteolytic and able to liquefay gelatin. The rest of the strains were nonproteolytic. Only 16 strains (green colored) blonging to the coryneform-like group were able to coagulate milk.

Thirteen coryneform strains were able to grow on casein as the sole nitrogen source.

The most important differential character in classifying the isolated strains to groups was their cell form. The group Arthrobacter is casily and quickly transformed from the rod into the coccus form upon ageing (mostly after 24-48 hr.). The rods of the Arthrobacter group were mostly short and thick. Coccoids were found to be the predominant cells in ageing cultures (Fig. 2). The rods of the corynefor-like groups were slender, relatively long and sometimes branched. The transformation of rods into cocci was found to be a much retarded process. In the group of coryneforms this process was even more retarded or entriely absent.

All the isolated straine showed, in the rod stage, the morphological implications of coryneform bacteria: straight, slightly bent, swollen or club - shaped cells, sometimes developing filaments and true branching. Snapping division and angular cell arrangement (palisade arrangement) are regular features (Figures 1 and 2).

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Table (1): Utilization of different carbom and nitrogem carbon compounds by Dairy Arthrobacters

Group		No. of checked strain in the total	Carbon Comp.				Gelatin liquefation	Tweens						Inorganic Nitr.		Casamino-A		Gram Stain		Catalase test	Coagulation of Milk	Casein	Colour on Yeast Extract Agar
			Glucose	Sucrose	Lactose	Lactate		20	40	60	65	80	85	-	+ Vitamins	-	+ Vitamins	Rods	Cocci				
Arthrobacter	1	2	+	+	+	+	-	+	+	-	-	+	-	-	-	+	+	+	+	+	-	-	grey
	2	6	+	-	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	cream
	3	11	+	-	-	+	-	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	white
Arthrobacter like	4	3	+	-	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	cream
	5	14	+	-	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	white
	6	4	+	-	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	cream
	7	7	+	-	+	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	-	-	red
Intermediate	8	8	+	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	red
	9	9	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	-	orange
Coryneform like	10	10	+	-	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	orange
	11	15	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	orange
	12	16	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	green
	13	1	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	white
Coryneform	14	13	+	-	-	+	-	+	+	-	-	-	-	-	+	-	+	+	+	+	-	+	green

Dairy Arthrobacter

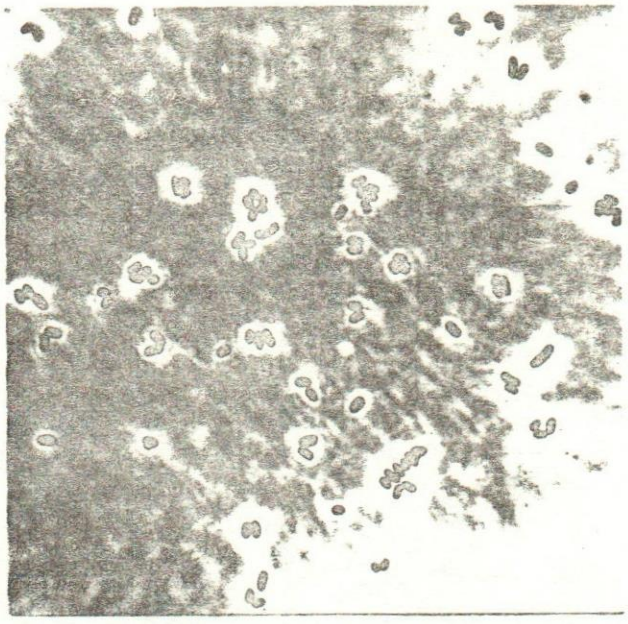

Figure 1 : Arthrobacter strain at the rod stage

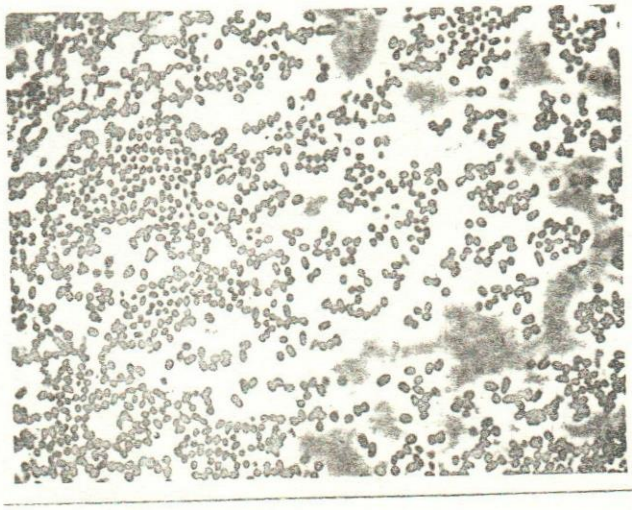

Figure 2 : Arthrobacter strain at the cocci stage