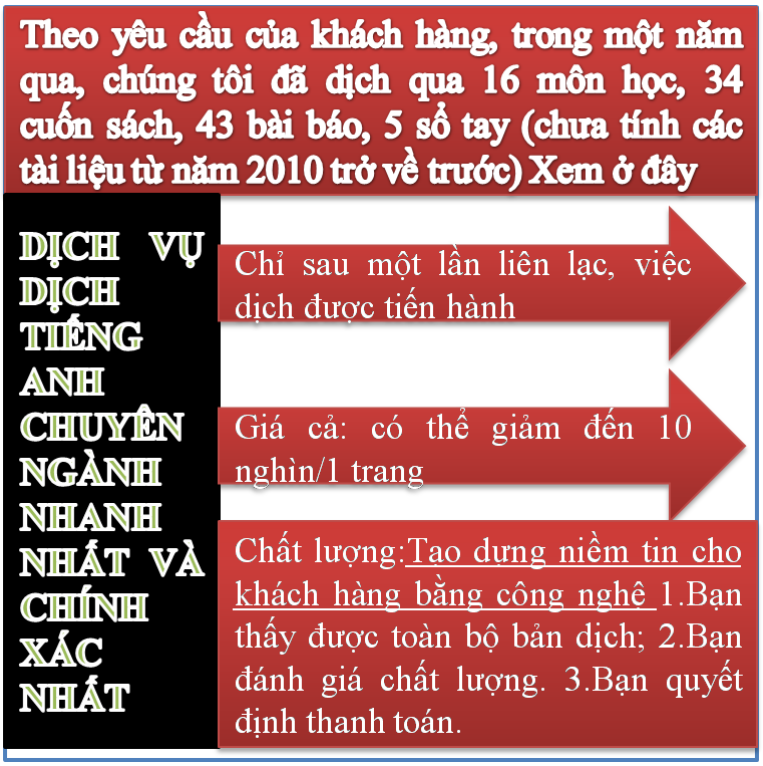

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Organic Nutrition of Beggiatoa sp. Culture OH-75-B of Beggiatoa sp. differed significantly from any described previously in its utilization of organic carbon and reduced sulfur compounds. It deposited internal sulfur granules characteristic of Beggiatoa sp. with either sulfide or thiosulfate in the medium. This strain (OH-75-B, clone 2a) could be grown in agitated liquid cultures on mineral medium with acetate as the only source of organic carbon. The resultant growth yields and rates were comparable to those for typical heterotrophsệ Of the other simple organic compounds tested, only pyruvate, lactate, or ethanol could singly support the growth of this strain. Single sugars or amino acids neither supported growth nor enhanced it when added to acetate-containing medium. In contrast, compounds of the tricarboxylic acid cycle enhanced growth yields when tested in concert with acetate. These and fluoroacetate inhibition results indicate that Beggiatoa sp. possesses a functional tricarboxylic acid cycle. Poor yields characterized the	Dinh dưỡng hữu cơ của Beggiatoa sp. Phương pháp nuôi Beggiatoa sp. OH-75-B khác biệt rất nhiều so với bất kỳ phương pháp nào được mô tả nào trước đây ở việc sử dụng cacbon hữu cơ và các hợp chất lưu huỳnh khử. Nó lắng tụ các hạt lưu huỳnh nội tại đặc trưng của Beggiatoa sp. với sunfua hoặc thiosulfat trong môi trường. Chủng này (OH-75-B, dòng vô tính 2a) có thể phát triển bằng phương pháp nuôi khuấy lỏng trong môi trường khoáng chất với acetat là nguồn cacbon hữu cơ duy nhất. Năng suất tăng trưởng và tốc độ tăng trưởng toàn phần cũng vào cỡ các phương pháp nuôi dị dưỡng thông thường. Trong số các hợp chất hữu cơ đơn giản được thử nghiệm, chỉ có pyruvate, lactat và ethanol có thể hỗ trợ sự tăng trưởng của chủng này. Chỉ sử dụng đường hoặc axit amin sẽ không thể hỗ trợ sự phát triển cũng như tăng cường nó khi thêm vào môi trường chứa acetat. Ngược lại, các hợp chất của chu trình axit xyclic lại tăng cường sản lượng tăng trưởng khi được thử nghiệm với acetat. Những điều này và các kết quả ức chế fluoroacetat chỉ ra rằng Beggiatoa sp. có một chu trình axit xyclic chức năng. [redacted]	

growth of this strain on dilute yeast extract medium, and higher concentrations of yeast extract proved inhibitory. The enzyme catalase, contrary to the findings of others, had no synergistic influence on growth yields when added to medium containing yeast extract or acetate or both.

[redacted]

Previous studies of the nutrition of Beggiatoa species have been characterized by uncertainties and contradictions. Since the early studies of Winogradsky (31, 32), investigations of this genus have been linked with the notion that Beg- giatoa species possesses chemoautotrophic capabilities dependent on the oxidation of sulfide or elemental sulfur (S°) or both. Whether there are strains capable of obligate or facultative chemoautotrophic growth is still in doubt (20, 30).

[redacted]

A strain of Beggiatoa sp. (culture OH-75-B; clo~~nes 1, 2a, 2b, 2c, and 3) that deposits the internal s° granules characteristic of Beggiatoa speci~~es from thiosulfate or sulfide ions has been isolated. This strain, which corresponds morphologically to B. leptomitiformis, was used in attempts to determine the autotrophic abilities and the

[redacted]

nature of sulfur metabolism in Beggia- toa species. The results of these studies are reported in the accompanying paper (20).

The state of knowledge on heterotrophic nu-trition in Beggiatoa species is no more certain than is our knowledge of its sulfur metabolism or autotrophic potential. Winogradsky (31) and Keil (13) believed that heterotrophic nutrition was not possible, but the concentrations of or-ganic compounds used in theù studies were quite high. More recently, heterotrophic nutrition has become a well-established fact for Beggiatoa species (4, 7, 10, 23, 26), but various studies of the extent of growth on particular organic media have frequently yielded disparate results. Faust and Woúe (10) and Scotten and Stokes (26), working with several Beggiatoa sp. strains each, characterized growth yields on media containing dilute yeast extract as "very poor" or "far from luxuriant." Burton and Morita (3), on the other hand, found that growth on media containing acetate and yeast extract or only yeast extract was enhanced by the addition of the enzyme catalase to the point that yields were "on the same order as other heterotrophs." Theù strain of

Beggiatoa sp. did not produce catalase, and this finding has recently been extended to 32 additional Beggiatoa sp. strains by Strohl and Larkin (30).

Acetate is the simple carbon compound most frequently tested in defined media. The question of whether Beggiatoa species can grow with acetate as the sole carbon and energy source in an otherwise mineral medium also seems to have had different answers in different studies.

Pringsheim (23) found that 12 of the 14 strains which he tested grew well on acetate without the addition of complex organic nutrients or sulfide. Others (3, 26, 30) generally found that growth was poor or nonexistent in mineral medium supplemented with acetate. Kowallik and Pringsheim (16) and Pringsheim (22-24) have at various times also obtained growth of some strains in media with each of the following as the sole source of organic carbon: aspartate, glutamate, succinate, malate, lactate, and pyruvate.

Previous studies of the nutrition of Beggiatoa species have used, almost exclusively, only quiil-itative measures of growth rates

and yielc sẵ Thus, if a particular medium is judged "good" or "best," it is only relative to other media used in the same study. Comparisons are difficult, and generalizations are tenuous. Because of this, a quantitative investigation was undertaken of the growth of strain OH-75-B under various conditions.

Given the obvious diversity of previous qualitative observations on Beggiatoa species metabolism, the danger of generalization from the results of a single strain is obviousề However, quantitative results, though more costly in time to obtain, have the distinct advantage of being dừectly comparable with future studies on other strains. It was deemed worthwhile to gain precision at the expense of generality; hence, a rather thorough survey was undertaken of the utilization of simple, defined, organic carbon compounds by strain OH-75-B. In addition, growth on yeast extract was studied, and the influence of catalase on various complex media was also quantified.

MATERIALS AND METHODS

In January 1975, Beggiatoa spế

was isolated on NT medium (6) containing 1.5% agar. NT medium is D medium (5) modified by the addition of thiosulfate, ammonium sulfate, and powdered travertine. D medium is a defined mineral medium used for the culture of blue-green algae (cyanobacteria), and it contains nitrilotriacetatic acid (a chelator) as its only organic ingredient.

The isolation was accomplished through the gliding of filaments from a tuft of Beggiatoa sp. placed in the center of a petri plate containing new medium. The tufts of Beggiatoa sp. utilized in this isolation were obtained for the marginal region (about 40° C) of a thermal spring at Hunter's Hot Springs (lat. 42°12'50" N; long. 120°22'00" W), located 2 miles (ca. 3.2 km) north of Lakeview, Oregon. After a tuft was washed several times in sterile distilled water, it was placed on the center of an agar plate, and within a few hours single filaments had begun to glide away from the central inoculum. The more advanced single filaments were cut out on small blocks of agar, using watchmaker's forceps, and transferred to fresh agar plates of the same medium. Transfers of this type established clones 75-1, 75-2, and 75-3 (culture OH-75-

B), all from the same tuft of field material. In March of that year, three subclones of 75-2 were established, including 75- 2a, which is the basis for the research reported here.

A slight modification of NT medium resulted in a defined basal medium (lacking thiosulfate) which was designated medium A (Table 1). Medium B is the designation given to this medium with 0.1 g of yeast extract added per liter. When supplemented, these media were used in liquid cultures (50 ml in a 125-ml Erlenmeyer flask) or in agar plates as indicated (Table 1). Since 1975, clone 75-2a has been maintained in quadruplicate on medium B supplemented with 0.8% agaré These stock cultures were maintained in the dark at room temperature and transferred once a month. Periodically, the stock cultures were tested for contaminants on PG, NB, and PC media (Table 1).

The latter two media were used at full strength and approximately 10-fold dilutions. These test media were employed as liquids and with 1.0% agar. Test incubations were in the light and in the dark at room temperature. No recloning has been necessary since 1975.

For quantitative growth experiments, liquid stock cultures in 125-ml Erlenmeyer flasks with 50 ml of medium A were employed. The medium was supple-mented with the desùed carbon source (s), and some-times with sodium thiosulfate or catalase or both, then inoculated with a population of Beggiatoa sp. on a small agar block from the margin of a stock culture plate. It will be specifically stated whenever thiosulfate or catalase was included in a medium. These liquid cultures were grown in a shaker water bath (New Brunswick Scientific Co, Gyrotory, model G-76) at 32°c and 150 rpm in dim light (less than 100 lx) unless otherwise stated. After good growth in this first liquid transfer, it was possible to decant some of the Beggia- toa tufts and medium asepticaily into a sterile blender unit (Eberbach, semimicro, stainless steel, model 8580), leaving the piece of agar behind. The tufts were then fragmented with three 10-s bursts of blending. The material could then be pipetted quantitatively and aseptically into other flasks of sterile medium as a means of setting up replicate flasks (up to about 40) for various growth rate and growth yield experiments.

It was necessary to employ this replicate technique in growth rate determinations because the habit of Beg- giatoa species of growing in large tufts made repeated sampling from a single large vessel unreliable.

For growth rate and yield experiments, dry weight was determined by filtering the contents of a flask through a tared membrane filter (47-mm diameter, Nuclepore) with a pore diameter of 0.6 /xm unless otherwise noted. Filters containing cell material were dried at 40°c for at least 6 h, placed in petri plates (with internal desiccant) inside a desiccator, allowed to equilibrate for at least 2 h, and then weighed on a microbalance (Mettler model M5) to the nearest microgram. The filters were returned to the desiccator for at least 6 more h and then reweighed. This process was repeated until weights were obtained which differed by less than 10 ịig. Tare weights of the filters had previously been determined in the same manner.

For the determination of growth yields, at least eight flasks were employed for a given medium. When visual inspection indicated that the culture was near the end of the exponential growth phase,

dry weight determinations were initiated and were continued at 8- to 12-h intervals. After eliminating any values which were obviously from the exponential or declining phases of the growth curve, the remaining values were averaged to compute a yield. With very few exceptions, the duration of the stationary phase was at least 2 days, making a minimum of four values available for averaging.

Catalase (Sigma brand, C-10, from bovine liver) was added to the medium in some experiments at a final concentration of 20 Sigma units per ml from stock solutions of 100 times that strength. The enzymatic activity was determined in accordance with procedures detailed by the supplier (27).

Organic compounds and thiosulfate were added to liquid medium A (after it had been autoclaved) from sterile stocks which were usually 5% (wt/vol). Pyru-vate, oxaloacetate, a-ketoglutarate, glyoxylate, glyco-late, sucrose, glucose, and catalase were filter sterilized with Nuclepore filters (0.2-jtim pore size) before addition, and all other compounds added to liquid medium A were autoclaved as concentrated stocks before addition. Organic acids were adjusted to pH 6 by

the addition of sodium hydroxide before sterilization.

The equations or slopes for all linear relationships (e.g., growth yield versus substrate concentration or logarithm of dry weight versus time) were determined by the method of least-squares linear regression (29). The specific growth rate constant in') was defined according to Meynell and Meynell (18) and was calculated from the slope of the linear portion of a plot of the natural logarithm (loge) of dry weight versus time. All confidence limits in this paper (shown graphically as error bars or listed numerically after a mean as ± x) represent the 95% confidence limits to the mean value under consideration (29).

RESULTS

Since cell width has been the taxonomic cri-terion for the designation of species in Beggiatoa (17), the influence of different organic substrates (3.7 mM acetate, 5.1 mM lactate, 0.20 or 0.25% yeast extract) on cell dimensions was investigated. The results suggest that cell width generally changes little in response to the carbon source utilized. Except for 0.25% yeast extract, all of the substrates tested gave cell widths

ranging from 1.8 to 2.2 Ịim. The medium containing 0.25% yeast extract gave cell widths of 3.2 ± 0.4 jum (n — 10), but these cells, when viewed micro-scopically, appeared distended and somewhat like a string of sausages, unlike normal Beggia-toa cells. The inclusion of thiosulfate in media did not appear to alter cell widths.

Filaments of clone 75-2a grown on medium containing thiosulfate lacked visible cross walls and showed the characteristic internal sulfur granules (Fig. 1). These s° granules were highly refractile under phase microscopy (Fig. 1A) and had a dark ring around the periphery when viewed under bright-field illumination (Fig. lB). The second type of inclusion visible in cells (Fig. 1C) stained as poly-/?-hydroxybutyrate (PHB) granules (18). PHB granules have previously been noted in Beggiatoa species (23, 30). Beg-giatoa species found in the field is frequently in large, almost unispecific aggregations (tufts) which may be several millimeters in diameter. Growth of clone 75-2a in liquid medium in a shaker bath produced similar tufts (Fig. ID).

Beggiatoa sp. grown on acetate or yeast ex-tract medium (without the addition of reduced

Thể vùi

thuộc dòng vô tính

ngang

bên trong

pha

mạnh thể vùi

vô tính

Người ta thấy rằng

sulfur compounds) was found to be catalase negative. Addition of 3% H202 to masses of whole cells failed to produce bubbles. Addition of whole or sonically disrupted cells from late exponential growth phase on acetate medium to more dilute (0.06%) H2O2 solutions failed to decrease the peroxide optical density monitored at 240 nm for 2 h. Control additions of 20 Sigma units of catalase per ml caused rapid declines. Growth on acetate. Clone 75-2a grew well on a shaker bath in liquid medium containing simple carbon sources in the presence of aừ. Acetate is the most widely referenced carbon compound for Beggiatoa growth and was the one tested most thoroughly in this study. Figure 2 shows the growth yield for Beggiatoa sp. grown in liquid medium A supplemented with various concentrations of acetate. This figure indicates a yield of 21.2 mg (dry weight) per mM acetate, and this yield appears to be linear up to 8 mM acetate. Liquid medium A has a relatively weak buffering capacity, and the falling off of growth yields at higher acetate concentrations (Fig. 2) may well reflect the fact that consump-tion of acetate (presumably as the undissociated acid) tends to raise	giai đoạn có kiểm soát vô tính là một	

the pH of the mediumộ Although the final pH was not measured at the highest acetate concentrations, growth in medium containing 3.7 mM acetate raised the pH of the medium from 6ả9 to 8.2. No systematic study of pH influence on growth was conducted with acetate, but a preliminary study with yeast

Fig. Growth yield of clone 75-2a as a function of acetate concentration. Growth was in liquid medium A at 32°c. Error bars represent 95% confidence limits to the mean. Straight lines represent linear regression equation. Larger graph shows yields at acetate concentrations of 37 mM and less. Inset shows yields at acetate concentrations of 3.7 mM and less. Dashed line m larger graph represents linear regression equation from inset graph.

extract indicates that maximal growth yields are obtained between pH 7 and 8 and that yields fall off sharply above and below these values.

The specific growth rate constant, n\ was de-termined at different temperatures in liquid medium A containing 3.7 mM sodium acetate and 2.0 mM sodium thiosulfate (FỉgẼ 3). The rate constant has a maximum value of 0.16 per h at about 37°c. The value of … falls off

extremely rapidly at temperatures above 37° c to the point at which there is no growth above 40.5°c. As is frequently the case, suboptimal temperatures resulted in a more gradual decline in growth rates.

Figure 3 also shows growth yield as a function of temperature. As with growth rate, growth yield declined drastically above 38° c. Although yield appeared to be fairly constant between 28 and 38°c, there was some indication of a maximum yield at about 32 to 33°C; hence, 32°c was used for most of the experiments in this study, in spite of the fact that growth rate changes rapidly as a function of temperature in that region.

Other simple organic media. A number of other organic carbon compounds were tested singly and in concert with sodium acetate to determine whether they were capable of sup-porting growth alone or enhancing yields of clone 75-2a. Of the compounds tested singly (Table 2), only ethanol and lactate were also able to serve as the sole organic carbon source for Beggiatoa growth. Yield was linear up to at least 7 mM with ethanol but became nonlinear above about 2 mM with lactate. Pyruvate also appeared to support growth of clone 75-2a, but there was a reqmrement for a

hay không

trace of yeast extract in the medium (Table 2). When clone 75- 2a was grown on various concentrations of yeast extract and pyruvate in liquid medium A, growth yield increased linearly with increasing pyruvate concentration if a constant trace amount of yeast extract was included. Conversely, for a constant amount of pyruvate, growth yields increased (up to a certain point) as a linear function of the amount of yeast extract in the medium (Fig. 4). There is a ratio of yeast extract to pyruvate concentration beyond which the addition of more yeast extract did not result in increased growth yield unless more pyruvate was also added. This ratio is approximately 40:1 (pyru- vate-yeast extract, by weight). It is clear that yeast extract is requùred as more than a primer for some process related to growth on pyruvate. That is, it is consumed (although at a low rate) in some process related to pyruvate-supported growth. The beneficial influence of yeast extract in trace quantities cannot be mimicked by acetate, lactate, Casamino Acids, or thiosulfate. The

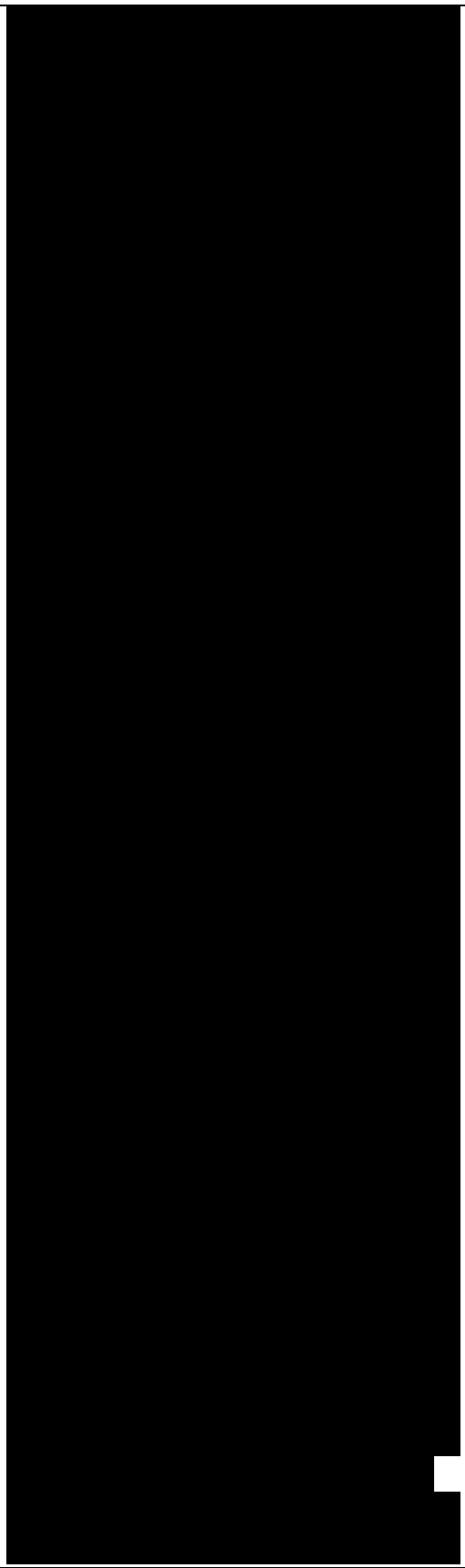


Fig. 3. Specific growth rates (circles) and yields (trmngles) of

clone 75-2a as a function of temperature. Medium contained 3.7 mM acetate and 2.0 mM thio- sulfate. Growth was from equal inocula in replicate Erlenmeyer flasks containing supplemented medium A on shaker baths. Curves were fitted to the data by eye. Error bars represent 95% confidence limits to the mean. At each temperature, dry weights were determined several times during the exponential growth phase, and the value of the specific growth rate (n') was determined by linear regression. For yields, several flasks were harvested during stationary phase at each temperature, and the dry weights were averaged (n > 3).

Table Growth yields for single substrates and substrates together with acetate

Except for acetate, all substrates were at 0.05% (wt/vol). Acids were added as sodium salts. Medium A was used.

Corrected for control flasks lacking substrate but given equivalent inoculum. c Yield in medium containing substrate plus 3.7 mM acetate less yield in 3.7 mM acetate medium. From reference 21.

Values calculated from regression data in linear portion of yield versus concentration. Candida utilis.

đặc trưng

khớp với dữ liệu bằng mắt

nền

trọng

Hydrogenomonas eutropa. h Pseudomonas sp. strain C12B. Pseudomonas aeruginosa. Pseudomonas fluorescens. k 0.01 g of yeast extract added per liter. Average of two separate values from regression calculations on two experiments. N, Negative values. The average yield of experimental flasks was less than that of control flasks, due to experimental uncertainty or inhibition of growth by the organic substrate. inability of clone 75-2a to grow on glucose (see below) is not alleviated by the same trace amount of yeast extract. Other organic compounds were tested singly and together with acetate for theừ effect on growth yields. The compounds tested (Table 2) were selected because some were mentioned as enhancing or supporting growth of Beggiatoa species in previous nonquantitative studies or because they were in the same categories. The first four compounds in Table 2 are those pre-viously discussed, which are capable of serving as the sole source of energy and cell material (except for the trace of yeast extract necessary with pyruvate). The next four are compounds which, though not capable of supporting growth singly, nevertheless had a strong	[redacted] [redacted] đến không định lượng Sản lượng	

synergistic ef-fect on growth yield in conjunction with acetate. The additional growth yield attributable to the inclusion of any one of these compounds in acetate medium is of the same magnitude as the molar yield on acetate alone. The remaining compounds had essentially no influence or a slightly negative influence on growth yields. Influence of catalase on growth yields. The yields of clone 75-2a grown on various con-centrations of yeast extract as the only substrate addition were consistently higher with catalase. This catalase-induced yield increment persisted even without yeast extract or other organic sub-strates in the medium (Fig. 5). This apparently additive interaction between yeast extract and catalase is in contrast to the pronounced multi-plicative influence of catalase on yields found by FIG. 4. Influence of a trace of yeast extract on pyruvate-supported growth of clone 75-2a. Experiments were performed with Erlenmeyer flasks containing liquid medium A supplemented with pyruvate or yeast extract or both. Temperature was maintained at 32°c in a shaker bath. Each point is the average of dry weights of stationary-phase cells from three flasks, (a) Fixed yeast extract	tăng lên vào cỡ sản lượng tính theo mol đến	

concentration (10 mg/liter) and variable pyruvate, (b) Fixed pyruvate concentration (2.0 mM) and variable yeast extract

others (3). The possibility of synergistic inter-actions between catalase and organic substrates was farther tested by determining growth yields of Beggiatoa sp. on two complex media and on some separate components of the media (Table 3) . Growth yields on the complex media (Table 3, column 5) were closely approximated by sums of yields on the organic constituents of the media (Table 3, columns 2 and 3). Yeast extract from 5% autoclaved stock solutions was usually used in experiments; however, filter-sterilized yeast extract gave comparable results when tested.

DISCUSSION

Characterization of Beggmtoa sp. Strohl and Larkin (30) list 20 characteristics shared by all 32 of theừ Beggiatoa spp. strains. Although the Oregon strain has not been tested for all 20 attributes, it matches well where examined. Like theừ strains, clone 75-2a is capable of gliding motility, grows on medium containing acetate and dilute complex organics and on medium containing acetate and sulfide, and deposits internal granules of s° from sulfide. Clone 75-2a and their strains are

[redacted]

kiểm tra thêm

[redacted]

Nghiên cứu

[redacted]

chung của 32 chủng Beggiatoa của họ

kiểm tra

dòng vô tính

lắng tụ các hạt lưu huỳnh

also alike in that both deposit PHB granules when grown on media containing moderate amounts of acetate and

chúng

tăng

trưởng trong môi trường

vừa phải

FIG. 5. Influence of catalase on yeast extract-supported growth yields.

Cultures were grown in replicate flasks in supplemented liquid medium A under standard conditions. Growth media contained no thwsulfate. Circles represent yields in media containing catalase (final concentration, 20 Sigma units per ml). Triangles represent yields in equivalent medium lacking catalase. A minimum of five dry weights (separate flasks) were averaged to obtain each data point. Error bars are 95% confidence limits to the mean.

trong

môi trường lỏng A bổ sung

Môi trường

biểu diễn sản lượng

trong môi trường

nồng độ cuối cùng

biểu diễn sản lượng trong môi trường tương đương

Các thanh

sai số có giới hạn tin cậy 95% so với giá trị trung bình

TABLE 3. Actual and theoretical yields on complex media and components thereof1

All additions are to liquid medium A. Data are in milli-grams per liter ± confidence limit. Yeast extract concentration as indicated in first column. Catalase concentration is 20 Sigma units per ml.

This medium is similar to the best growth medium of Burton and Morita (3). The only differences are in the inorganic constituents and the fact that their catalase concentration was

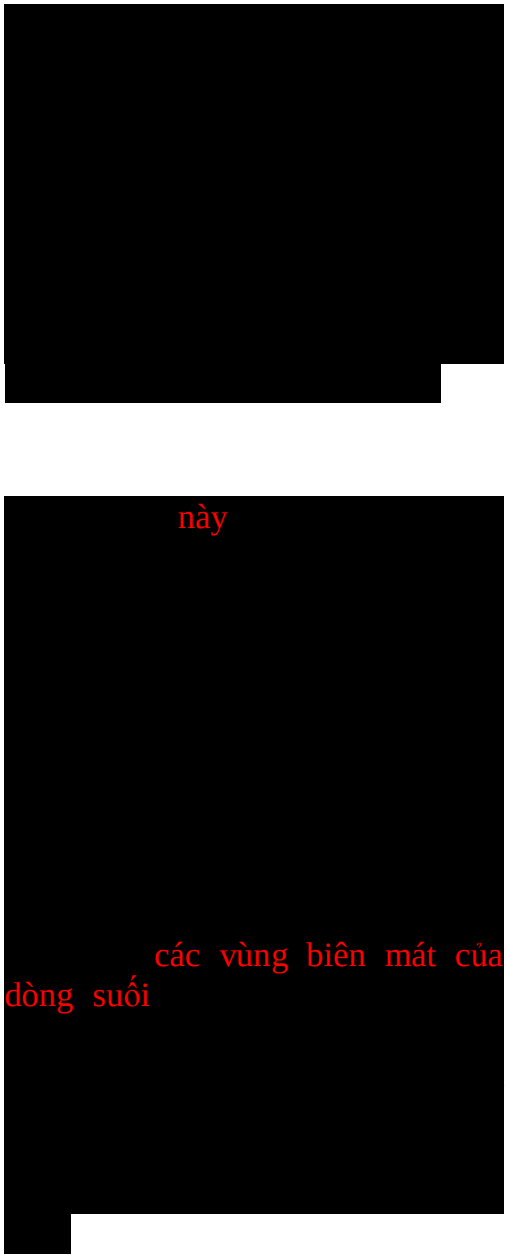
(việc, ở chổ)

10 Sigma units per ml. lacking catalase. Many of these points have been touched on only briefly here and are expanded upon in the accompanying paper (20), but they are mentioned to confirm that the Oregon strain is Beggiatoa sp. Clone 75-2a appears different from 30 of the 32 strains of Strohl and Larkin in that it grows well on acetate-supplemented mineral medium. In that respect, it is more like the strains of Pringsheim (23). Clone 75-2a also grew anaerobically under restricted conditions (20), but this may not be unique to this strain. The one unique feature of the Oregon strain is its previously mentioned ability to deposit s° granules from thiosulfate as well as sulfide. As previously stated, the taxonomy of Beggia- toa species is based entirely on cell width. Filaments of 1 to 2 jum in width are classified as B.leptomitiformis and those 2.5 to 4 fxm are B.alba (17). From the results presented, it is clear that clone 75-2a falls into or between these categories, depending on the growth medium. However, since the cells grown on 0.25% yeast extract were very abnormal in shape and growth was obviously inhibited, the matter can be somewhat simplified.	đã Dòng vô tính có vẻ khác với trong số Về khía cạnh này hoàn toàn dựa trên Các sợi có độ rộng từ 1 đến 2 micro mét xếp vào loại đã trình bày phụ thuộc vào bởi vì chiết xuất	

Since the other width determinations of clone 75-2a fall in the range of B. leptomiti-formis or between the two species, the species name leptomitiformis will be applied. This is done with the stipulation that other characteristics may ultimately prove much more useful than cell width in the taxonomy of Beggiatoa species.

Although this strain was isolated from the margin of a hot spring where the source temper-atures rose to the 50° c range, it is clear that a 37° c temperature optimum for growth rate and a 40.5° c upper limit (Fig. 3) make clone 75-2a a mesophile (2). If one can generalize from this clone, the parent Beggiatoa population was sur-viving only in the cooler margins of the spring. These findings differ slightly from the previous observation that 45°c was the upper tempera-ture limit for one strain of Beggiatoa sp., with good growth still obtained at 41°c (13).

In spite of the fact that Beggiatoa species has frequently been characterized as having poor growth on defined simple carbon sources (10, 26), the maximum value of the specific growth rate (ju\ base e) of 0.16 per h (doubling time = 4.3 h) makes it a relatively rapid growing heter-



otroph, although it is clearly not as fecund as some bacteria growing on simple organic media (19).

Catalase and complex media. It is clear that, although Beggiatoa sp. can grow on yeast extract as its sole source of organic carbon com-pounds, the growth yields were extremely poor (Figė 5). If one takes the generally accepted figure of 55 to 60% for the percentage of a utilizable carbon source converted to cell material (21), with the remainder being oxidized for energy, this implies that only about 2% of the total dry weight of yeast extract is useful to Beggiatoa sp. A general analysis of yeast extract indicates that about 8% of its total dry weight is composed of carbohydrates and about 43% is composed of amino acids (1). Thus, it is unlikely that Beggiatoa has any broad ability to utilize either of these classes of organic compounds.

Though the addition of catalase does have a slight stimulatory influence on yeast extract-supported growth yields, this increment exists even at zero yeast extract concentration and is fairly constant over the range of yeast extract tested (Fig. 5). Thus, as measured by yields, the interaction between yeast extract and catalase appears strictly additive. Because of its low ac-

tivity, impurities in the C-10 catalase added (10 mg/liter) could have accounted for the yield increment (2 to 3 mg/liter). Additionally, it appears that there is only an additive interaction between yield on yeast ex-tract plus catalase on the one hand and acetate on the other (Table 3). Combining this with the above results implies that growth yield on medium containing acetate, yeast extract, and cat- alase is simply the sum of yields on the three separate components. The fifth entry in the second line of Table 3 represents the yield of clone 75-2a on the “best growth” medium of Burton and Morita (3). For theừ strain of Beggiatoa sp., they claimed a 24-fold enhancement of yield on acetate + yeast extract medium when catalase was added. The results of this study argue against any synergistic effect of catalase (Fig. 5 and Table 3). Other studies (12, 30) have included catalase in organic media because it enhanced Beggiatoa growth in enrichments or maximized most-probable-number determina-tions. The beneficial effects of catalase detected in these studies may reflect different levels or types of organic contaminants in other catalase preparations. Since the question of growth yields was

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not quantitatively assessed in these other studies, the enhancements may also be only in rates and not final yieldsÊ It has been shown that catalase greatly shortens the growth lag of a Beggiatoa inoculum in yeast extract medium and that it may increase growth rate compared with an equivalent noncatalase medium (20). These rate-related factors may be responsible for the inclusion of catalase in the preferred media of the other studies. The very pronounced enhancement noted by Burton and Morita is difficult to reconcile with any explanation except for that of strain differences.

The inability of clone 75-2a to utilize the amino acids or sugars tested (Table 3) either singly or together with acetate is consistent with the low growth yields from yeast extract. Lack of ability in Beggiatoa species to utilize sugars has been pointed to repeatedly by others (3, 23, 26). Amino acids have been reported to enhance growth (22,23) and to have no influence (10,26).

Speculations on enzymology. That clone 75-2a grows well only on acetate or compounds which are frequent metabolic precursors of acetate (pyruvate, lactate, and ethanol) points strongly to the operation of the tricarboxylic acid (15). This is in

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contrast to the findings of Burton et al. (4). They argued against the operation of the tricarboxylic acid cycle in theừ Beggiatoa sp. strain because they failed to detect a number of key enzymes of the cycle and because they could not detect C02 evolution. Theừ strain of Beggiatoa sp. appeared to be capable of metabolizing acetate in the presence of yeast extract and catalase, but could not grow on acetate alone.

Several lines of evidence support the idea that clone 75-2a has a functional tricarboxylic acid cycle which results in CỚ2 evolution. Support for the existence of a functional tricarboxylic acid cycle comes from the previously discussed ability to grow only on acetate or its immediate precursors. Additionally, all tricarboxylic acid cycle intermediates tested, with the exception of citrate, greatly enhanced growth on acetate, and no non-tricarboxylic acid compounds tried had a similar influence. Other studies have shown citrate to be inhibitory to some Beggiatoa spp. strains, and it has been postulated that this is due to overchelation of essential divalent cations by citrate (4).

Studies using fluoroacetate—a known inhibi-tor of the tricarboxylic acid cycle (14)—

phát hiện số enzyme quan trọng của chu trình và bởi vì họ không thể

also gave evidence that the cycle was employed by Beg- giatoa in aerobic growth. The oxygen consump-tion rates of Beggiatoa sp. (harvested from ex-ponential growth phase and resuspended in acetate-containing medium) were diminished by 90% upon the addition of 1 mM fluoroacetate. In other procaryotes, where additional evidence supports the existence of functional tricarboxylic acid cycles, fluoroacetate at similar concentrations has produced corresponding reductions in oxygen uptake (9, 28). Evidence of complete oxidation of substrates (to C02) by clone 75-2a is somewhat indirect. Payne (21) has compiled a table of molar growth yields for a number of aerobically grown heter- otrophs utilizing a variety of organic substrates, and all entries from that study referring to growth on lactate, acetate, pyruvate, or ethanol, have been included in Table 3. Hydroge- nomonas and Pseudomonas species are known to contain functional tricarboxylic acid cycles (8). The similarities of yields of clone 75-2a grown on acetate or lactate with yields for these species are very great and indicate equally effi-cient metabolic pathways in Beggiatoa species. The ability of clone 75-2a to	giảm lại thành quá trình Như chúng ta đã biết, Khả năng phát triển tốt của dòng vô	

grow well on acetate as the sole carbon source also argues for the operation of the glyoxylate cycle (15). The glyoxylate cycle employs isocitrate lyase and malate synthase (Fig. 6), and it renews the C4 compounds of the tricarboxylic acid cycle de-pleted by biosynthesis. Growth of clone 75-2a with ethanol as the sole carbon source presum-ably proceeds by oxidation of ethanol to acetate.

On the other hand, growth on pyruvate may involve substantial enzymatic changes. Esche-richia coliy when grown on pyruvate as the sole source of cell carbon and energy, converts py-ruvate to acetyl-coenzyme A (CoA), which then passes through the tricarboxylic acid cycle and generates energyĖ However, pyruvate may also be converted to phosphoenolpyruvate (PEP), which is known to be a strong inhibitor of isocit-rate lyase, an essential enzyme of the glyoxylate cycle. Thus, E. coli would not be able to grow on pyruvate alone except with the existence of another anaplerotic sequence to renew the C4 compounds used for biosynthesis (15). This alternate sequence employs PEP-carboxylase, which condenses PEP with C02 to form oxaloac- etate (Fig. 6).

It seemed possible that the inability of clone 75-2a to grow

tính 75-2a

giải thích

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on pyruvate (except in the presence of traces of yeast extract) was the result of uncoupled respùration of pyruvate. However, it was found that several days of incubation of Beggiatoa sp. in pyruvate-containing medium before the addition of yeast extract did not di-minish the final yield (D. c. Nelson, Ph.D. thesis, University of Oregon, Eugene, 1979). This argues strongly against the idea that, in the absence of yeast extract, pyruvate was consumed but not effectively coupled to growth. Assuming that the basic enzymes of clone 75-2a are similar to those of E. coli, the inability of Beggiatoa sp. to grow with pyruvate as the sole carbon source could be explained by one of the following: (i) the absence of PEP-carboxylase (or its inhibition by pyruvate) and inhibition of the glyoxylate cycle by PEP; (ii) the absence of the enzyme which converts pyruvate to acetyl-CoA (pyruvate dehydrogenase complex); (iii) the inability of Beggiatoa sp. to take up pyruvate.

The relief provided by traces of yeast extract (Table 2 and Fig. 4) and growth on lactate (Table 2) rule out explanation (ii) above. The mechanism of yeast extract relief may be to reverse some inhibition by pyruvate or PEP, or

pyruvate bị tiêu thụ nhưng

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it may be to facilitate pyruvate uptake. Since lactate is presumably utilized via con-version to pyruvate, the ability of clone 75-2a to grow on lactate in the absence of yeast extract (Fig. 6) seems at odds with the absence of py-ruvate-supported growth. If, however, only a small quantity of pyruvate exists as an inter-mediate in the conversion of lactate to acetyl- CoA, then explanation (i) for pyruvate inhibition is still possible. This is based on the assumption that the glyoxylate cycle would still be operable if only traces of PEP were present. Addition of pyruvate to lactate-containing medium suppressed the growth rate of clone 75- 2a severely (in preliminary experiments) when compared with the rates in lactate controls. However, the yield on pyruvate + lactate eventually reached at least the level of lactate-grown controls. This result does not clearly differentiate between hypotheses (i) and (iii) for lack of pyruvate-supported growth. Either of these hypotheses would have to be embellished to account for the pyruvate + lactate results, and further experimentation is necessary. E. coli can grow on malate or other tricarbox-ylic acid cycle intermediates as the sole source	[redacted] của dòng 75 – 2a về chấp nhận được một lượng nhỏ hiện diện Tuy nhiên, cuối cùng, sản lượng trên pyruvate + lactate ít nhất đạt đến mức kiểm soát tăng trưởng lactate hoàn thiện thêm [redacted]	

of carbon. When doing so, it utilizes the “malic enzyme” (11, 25) in converting malate to pyruvate (Fig. 6). The ability of the tricarboxylic acid cycle intermediates to enhance growth in clone 75-2a and theù inability to support growth singly suggest either the lack of the malic enzyme or inhibition of growth by the pyruvate produced by this enzyme. Preliminary experiments indi-cate that traces (10 to 20 mg/liter) of yeast extract, unlike the case with pyruvate, do not allow growth on malate. This would argue that the malic enzyme is lacking in Beggiatoa sp. clone 75-2a. However, a definite answer must surely await an explanation of the pyruvate ± yeast extract phenomenon. Though clone 75-2a showed good growth yields on some organic compounds, the ex-tremely limited number and variety of these substrates make it an atypical heterotroph. The failure of carbohydrates and amino acids to stimulate growth could reflect metabolic limitations or the inability to transport these compounds into the cell. In either case, the organic substrate restriction raises questions about the ability of Beggiatoa species to compete as an aerobic het- erotroph. If the limitations are inherent in carbon	làm như thế enzyme malic axit tăng cường quá trình tăng trưởng sự không có khả năng hỗ trợ được tạo ra bởi enzyme này , khác với trường hợp của pyruvate, chứng tỏ rằng Việc không thể sự nền cạnh tranh với vai trò là	

metabolism rather than transport, they may represent a necessary consequence of a more general metabolic flexibility which may include mixotrophy or autotrophy.	cho chúng ta thấy rằng cần một quá trình trao đổi chất linh hoạt hơn, quá trình đó có thể bao gồm dinh dưỡng kép và tự dưỡng.	