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Single cell protein production by soil *Raoutella ornithinolytica* incubated on waste potato, paper and corn cob products.

*****Oqbah Abdul Halim Abdul Jabbar Al-Hadithi, *****Dhafer Fakhri Al-Rawi
******Mohammed Qais Abed Al-Ani

* College of Education for Pure Sciences, **College of Science, Anbar University, Iraq.

ABSTRACT

Al-Hadithi OAH, Al-Rawi DF, Abed Al-Ani MQ., Single cell protein production by soil Raoutella ornithinolytica incubated on waste potato, paper and corn cob media, Onl J Vet Res., 22 (12):1137-1144, 2018. We describe single cell protein (SCP) production by *Raoutella ornithinolytica* bacteria isolated from soil and cultured on potato, paper and corncob starch waste residue. We identified 3 strains (A1-3) of *R. ornithinolytica* by colony characteristics, microscopy and Kliger, urease, citrate, indole, catalalase, APUI 20, Vitek2 and motility tests finding that A1 was most productive. By inoculating 0.5ml/100ml *R. ornithinolytica* A1 on 10g/100 ml potato waste media for 72 hours at 50C and pH 8.5 we obtained a SCP with 24.4% protein, carbohydrate 24,6%, fat 17,9%, ash 21.8% with humidity of 88.6%, DNA 1.235% and RNA 1.012%. The SCP contained very high levels of essential amino acids. Essential and non-essential amino acids constituted 93.9 mg/100g. Copper constituted 0.002, phosphorus 3.90, zinc 0.00056, calcium 0.085, potassium 0.132 and sodium 3.86%.

Key words: single cell protein, *Raoutella ornithinolytica*

INTRODUCTION

Bacteria, yeasts, and fungal fungi have a high protein content of about (50%) of the dry weight [1], produced without the need for large areas of land, nor chemical fertilizers [2] and can produce single cell protein [3]. The production of single cell protein in the world was 2.0 x 106 tons per year used for food and feed production. In mid-1980, single-cell protein was used for direct consumption and food additive

[4]. We describe Single cell protein production by soil *Raoutella ornithinolytica* incubated on waste potato, paper and corn cob products.

MATERIALS AND METHODS

Two cm^3 soil samples from each of ten different locations 2-2.25 cm deep sites (Anbar, Iraq) were collected into sterile polyethylene bags and stored at 4°C. Bacteria were then isolated from soil by starch hydrolysis as described by Saito and Yamarnoto [5]. R*aoultella ornithinolytica* was identified as described by Bailey [6] by colony culture characteristics, microscopy by gram stain [7, 8] and Kliger, urease, citrate, indole, catalalase hemolysis and motility tests, API 20 NE and Vitek2 Compact (Biomeriux, France).

R*aoultella ornithinolytica* were grown in nutrient broth in 250 mL conical flasks shaken at 100 cycles/min at 30°C for 24 hours. To count bacteria, we used 100µL of 4^{th} and 5^{th} dilutions placed in petri dishes with nutrient agar medium NA incubated at 28 ± 2°C for 24 hours of 3 replicates. Number of colonies was determined from:

Number of cells/ml = number of colonies × Inverted dilution x 10

To determine bacterial amylase hydrolysis of starch, papyrus, potato residue and corn cob were oven dried at 70°C for 48 hours, grinded and powders sifted through a 40 mm mesh sieve, each mixed separately with 20g/L, equivalent to 2% of 0.2g KH2PO4, 0.2g CaCl2, 2.0g No3NH4, 0.5g MgSO4.7H2O, 0.2g MnSO4 g and 0.2g FeSO4 (g/L). The resultant powders were then sterilized by autoclaving and media was poured on Petri dishes cultured in a 1cm diameter circle incubated at a temperature of 2 ± 28 m.28 ± 2°C. Enzymatic hydrolysis of starch formed a white halo around bacterial colonies confirmed with iodine and measured (cm).

Liquid inoculum of bacteria were prepared in 250mL conical flasks containing 100 ml of liquid sterilizing medium cultured at $30C^{o}$ for 2h, repeated at stage of optimal production*, then* sterilized at $121C^{o}$. We added 1ml of bacterial inoculum to the medium incubated for 72 hours at 100 shakes/min to determine optimal conditions for production of SCP. We tested optimal SCP pH between 5 and 10, temperatures 30 to 55C, inoculum volumes 0.5 to 3ml/100ml production medium, starch media 1.25 to 10% and incubation time 24 to 120h. The resultant precipitate SCP was soaked in sterile gauze, washed with distilled water, dried at $60C^{o}$ for 24h, and weighed.

Protein in SCP was determined by Kjeldahl [9] as protein = nitrogen x 6.25, fat as described by Çevikkalp et al.[10], ash [9], amino acids as described by Folch et al. [11] with an Amino Acid Analyzer. DNA and RNA were determined as described by [12], carbohydrate [13] at 490nm with an optical absorption spectrometer, Cu, Fe, Pb, Zn, Ca, K, Na, P were determined by Atomic Absorption Spectrophotometer.

RESULTS

Results are shown in Tables 2-10 below

Table (2) Enzymatic hydrolysis by *Raoultella ornithinolytica* isolates (A1-3*)* incubated on (cm) starch waste products.

Carbon source	R. *ornithinolytica*	Hydrolysis (cm)
Potato	A1	**7.5**
	A2	**5.75**
	A3	**4.25**
Papyrus	A1	**4.75**
	A2	**2.75**
	A3	**3**
Corn cob	A1	**3.25**
	A2	**2.66**
	A3	**3.5**

Numbers in red denote greatest values.

Table (3) Conditions used to determine optimal production of single cell protein product produced by *Raoultella ornithinolytica* Strain A1 in potato residue.

	Units	**SCP Dry weight g/100 ml**	**LSD 0.05**
pH	4.5	0.5	0.0201
	5.5	1	
	6.5	1.5	
	7.5	2	
	8.5	2.5	
Temperature C^{o}	25	0.14	0.0256
	30	o.652	
	40	0.538	
	45	0.674	
	50	0.775	
	55	0.644	
Inoculum volume ml/ 100 ml	0.5	1.132	0.0846
	1	0,775	
	1.5	0.880	
	2	0.683	
	2.5	0.644	
	3	0.434	
Starchy residue (g)	1.25	1.206	0.0101
	2.5	1.993	
	5	1.132	
	7.5	4.148	
	10	5.209	
Incubation time (hour)	24	2.763	0.0038
	48	4.065	
	72	5.207	
	96	3.596	
	120	2.869	

Table 4. Composition of single cell protein product produced by *Raoultella ornithinolytica* Strain A1 in potato residue.

	Percentage
Protein	24.4
Carbohydrate	24.652
Fat	17.966
Ash	21.75
Moisture	88.06
DNA	1.235
RNA	1.012
DNA+RNA	2.247

Table 5. Metals and minerals detected in single cell protein product produced by *Raoultella ornithinolytica* Strain A1 in potato residue

	%
Fe	0.228
Cu	0.002
P	3.9
Zn	0.00056
Ca	0.085
K	0.132
Na	**3.866**

Table 6. Amino acids detected in single cell protein product produced by *Raoultella ornithinolytica* Strain A1 in potato residue.

Amino acid		**Concentration mg/100g**		
Essential Amino acids		**SCP**	**Egg**	**Wheat Straw**
1	Cysteine	3.956	2.2	0.32
2	Leucine	7.151	7.6	0.18
3	Isolucin	7.151	7.6	0.18
4	Methionine	27.249	3.1	0.28
5	Phenylalanine	2.195	5.4	0.26
6	Arginine	15.152	3.3	0.28
7	Valine	5.573	6.3	0.02
8	Threonine	10.367	3.7	0.04
Non-essential Amino acids		**SCP**	**Egg**	**Wheat straw**
9	Histidine	3.956	2.2	0.1
10	Glycine	5.113	3.1	0.06
11	Glutamic	4.943	11.2	0.05
12	Aspartic	1.160	9.1	0.13
Total concentration		93.966		

DISCUSSION

Eight *Raoultella ornithinolytica* isolates from different soil locations were grown on starch agar media to determine optimal amylase activity. Three strains of *R ornithinolytica* A1 to 3 as shown on Table 2 were tested. Greatest areas of hydrolysis were provided by strain A1 incubated on potato residue media.

Our bacterial isolates were identified as *Raoultella ornithinolytica* by morphology, culture, biochemistry, API20 and Vitek2 device, The colonies appeared creamy, yellowish, With a convex edge, rod shape, non-spore forming with a prominent polysaccharides capsule, gram-negative, hemolytic, lactose fermenter and positive for catalase, indole, citrate and urease. Others reported that 16 *Bacillus subtilis* isolates hydrolyzed starch efficiently *(14)* and that *Bacillus cereus* isolated from soils with organic residues had highest production of amylase (15). Garimella et al.(16) found that the *Rhodopsedomonas palustris* produced SCP with 70% crude protein.

We tested SCP production by *R ornithinolytica* A1 in waste materials and found that potato peel residue provided for the greatest hydrolysis of starch (Table 2) at pH 8.5. There were significant differences in SCP production related to pH ($P < 0.05$). it has been reported that pH of culture media changes during fermentation so pH should be maintained although microorganisms tolerate a wide range of pH [17] whereas others suggested that changes in pH can affect membrane permeability [18]. The authors [19] found that most microorganisms thrive at a wide range of pH except *Streptomyces hygroscopicus* which requires high pH.

Our results show differences ($P < 0.05$) between SCP production at 25, 45, 50 and 55 C^o, but none between 30 and 40ºC (Table 3). We found optimal SCP by *R ornithinolytica* at 50C yielding 0.775g/100ml media. Our findings confirm those of Bajpai [20] who report that 40-50C^o was optimal for amylase activity in *B. licheniformis* [21] and of Taran and Bakhtiyari [22] for hydrolysis of starch by alpha-amylase from *Streptomyces thermoriolaceus* at 50C^o. Others report SCP of 55.9% by *Haloarcula sp*. at 55 C^o.

As shown in Table 3, the least inoculum of 0.5ml bacteria produced most SCP (1.132g/100ml). This finding simulates Akindele and Fagade [23] who inoculated 0.5ml *Alcaligenes sp* with *Cellulomonas sp.* developed on cassava husks with banana peel producing 49.3% SCP. Others report similar results with 0.5ml [24]. Size of bacterial inoculum is one of the more important elements for SCP production [25]. We surmise that reduced SCP due to higher inoculum may be due to fermentation as described previously [26] or nutrient, lag phase or initiation of production [27].

We found that 10g potato peels provided highest SCP (5.207g/100ml) as shown in Table 3. Tanaka and Matsuno [28] and others suggested that SCP output depends on the amount of starch in waste materials or food [27]. Raw materials contribute between 60-70% of production cost [29]. Our findings are similar to others who produced ~47% SCP with 3 isolates of *Candida* spp in potato starch (30). Our results

showed differences ($P < 0.05$) in SCP production in 1.25, 2.5, 7.5 and 10g/100ml but none between 2.5 and 5g/100 ($P < 0.05$).

Effect of time of incubation time on SCP is shown in Table 3 with most at 72 hours providing 5.207g/100ml, which declined after. Declines in SCP biomass due to prolonged incubation may be due cell lysis with release of secondary metabolites during log phase growth [31]. Our results diverge from those of from El-Deek AA et al., [32] who reported greater SCP but only 14% yield after 96h incubation.

Our method with potato peel and *Raouttella ornithinolytica* yielded 24% SCP a higher level than that reported by Sun M et al (2017) who produced 13.5% SCP with *Bacillus subtilize, Aspirgillus niger* and *Candida tropica* with potato residue and wheat bran but lower than Liu B et al (2014) who produced 38.2% with *Bacillus licheniformis* with potato residue. Others report 27-30% SCP with *Bacillus* and *Saccharomyces cerevisiae* with glucose, fructose and potato residue [35],

Analysis of the SCP revealed 18% fat in our SCP, higher than reported previously (2.4%) (24). Ash in our SCP was 22%. Other report 32% ash using the same method and 5-6% higher than FAO limits (36) Humidity was 88.06%, higher than previous reports using *Saccharomyces cerevisiae* on potato husks with moisture of 85.51% [36].

The levels of DNA and RNA in our SCP were below the 5-7% limit so does require their removal. A major problem with SCP are high levels of nucleic acids, but not in ruminants which can convert nucleic acids to uric acid [37] The percentage of nucleic acids in the single cell protein may reach 25% 2% in fish 2.2% and in liver 4%. The obvious problem is that researchers found that the purine bases in which the nucleic acids are converted into the body during metabolism to uric acid [38]. As shown in Table 5 we found various metals and minerals with acceptable levelsof phosphorus sodim and potassium [39].

Amino acids in SCP additives used for animal feed include lysine, methionine and threonine whereas glutamic is used as flavor enhancer and aspartic in low calorie artificial sweetener [40, 41]. Of the essential amino acids, we found cysteine SCP levels higher than in egg or wheat straw, leucine, similar to egg but higher than wheat straw, methionine much higher than either and phenylalanine, arginine, valine and threonine higher than both as shown in Table 6. Non-essential amino acids histidine and glycine were higher in both whereas glutamic and aspartic were lower than in egg. Bacteria had more advantages than other groups, making them the first to produce single cell protein, with high protein content, high levels of essential amino acids and fewer nucleic acids (40).

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