Journal of Genetic Engineering and Biotechnology 16 (2018) 259–263

HOSTED BY

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Original Article

Valorisation of chicken feathers for xanthan gum production using *Xanthomonas campestris* MO-03

Murat Ozdal *, Esabi Basaran Kurbanoglu

Department of Biology, Faculty of Science, Ataturk University, Erzurum, Turkey

ARTICLE INFO

Article history:
Received 22 January 2018
Received in revised form 7 July 2018
Accepted 16 July 2018
Available online 24 July 2018

Keywords:
Chicken feather peptone
Polysaccharide
Pyruvate
Xanthomonas campestris
Xanthan gum

ABSTRACT

Xanthan gum is an important commercial polysaccharide produced by *Xanthomonas* species. In this study, xanthan production was investigated using a local isolate of *Xanthomonas campestris* MO-03 in medium containing various concentrations of chicken feather peptone (CFP) as an enhancer substrate. CFP was produced with a chemical process and its chemical composition was determined. The addition of CFP (1–8 g/l) increased the conversion of sugar to xanthan gum in comparison with the control medium, which did not contain additional supplements. The highest xanthan production (24.45 g/l) was found at the 6 g/l CFP containing control medium in 54 h. This value was 1.73 fold higher than that of control medium (14.12 g/l). Moreover, addition of CFP improved the composition of xanthan gum; the pyruvate content of xanthan was 3.86% (w/w), higher than that of the control (2.2%, w/w). The xanthan gum yield was also influenced by the type of organic nitrogen sources. As a conclusion, CFP was found to be a suitable substrate for xanthan gum production.

1. Introduction

Xanthan is a water-soluble hetero-exopolysaccharide produced by the Gram-negative plant pathogenic bacterium *Xanthomonas campestris*. Xanthan gum shows a wide range of applications such as suspending, stabilizing, thickening and emulsifying agent in the food, cosmetics, pharmaceutical, paper, paint, textile and oil industries [1–3]. Xanthan production is constantly increasing because of its numerous applications. It is estimated that the annual global production of xanthan gum is over 80.000 tonnes worth $400 million [4].

The production of xanthan gum has been shown to be influenced by many factors such as species type and environmental factors including dissolved oxygen level, media composition, temperature, pH and incubation time [1,5,6]. A cost reduction in xanthan gum production can be achieved by using inexpensive sources such as molasses [4], cheese whey [7], starch [8], kitchen waste [9], glycerol [10], coconut shell, passion fruit peel, corn straw and cobs [11] and jackfruit seed powder [12]. These materials have been used as a carbon source in submerged or solid state fermentations. Also, the type and concentration of nitrogen source affects xanthan fermentation [13]. Especially, organic nitrogen sources have been found to be better than inorganic nitrogen sources for xanthan production. Peptone [14], yeast extract, corn steep liquor [15] and ram horn peptone [13] have been used as organic nitrogen source in xanthan gum fermentations. Therefore, there is a need for cheap and available organic nitrogen sources.

Feathers are produced several million tons as a waste in poultry-processing plants. A large amount of feather waste is not recycled as required in nature and so it cause environmental pollution. About 10% of total chicken weight is feathers. Feathers are consist of approximately 90% protein composed of keratin thus can be a potential source of proteins and amino acids [16,17]. Considering these properties, feathers are cheap and available bioorganic waste to peptone production. Recently, the chicken feather peptone or hydrolysates have been used as a complex nitrogen source for the production of lactic acid [18], biosurfactant [19], polyhydroxyalkanoate [20] and citric acid [21].

In this study, it is the first time that CFP has been tested as an enhancer for *X. campestris* MO-03 to the production of xanthan. This study was aimed at the development of economical methods for higher yields of xanthan by suggesting the use of low cost raw material.

Peer review under responsibility of National Research Center, Egypt.
* Corresponding author.
E-mail address: mozdal@atauni.edu.tr (M. Ozdal).

https://doi.org/10.1016/j.jgeb.2018.07.005
1687-157X/

2. Material and methods

2.1. Hydrolysis of chicken feather

The chemicals used in this study were analytical grade and purchased from Sigma–Aldrich (St Louis, MO, USA) and Difco (Detroit, MI, USA). Chicken feathers were obtained from the Demircioglu Poultry Farm, Zonguldak, Turkey. Feathers were washed with deionized water and dried in oven at 60 °C. Dried feathers were cut into smaller pieces and then they were powdered with a blender. This material was hydrolysated by modifying the method of Kurbanoglu and Kurbanoglu [22], and the production process of CFP is shown in Fig. S1.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgeb.2018.07.005.

2.2. Isolation and identification of xanthan gum producing bacterium

Xanthomonas species were isolated from infected plants using Yeast Malt extract (YM) agar (KH_2PO_4 5 g/l, yeast extract 4 g/l, $MgSO_4$ 0.5 g/l, malt extract 2 g/l, glucose 10 g/l and agar 15 g/l, pH 7). The yellow-mucoid bacterial colonies were selected and maintained on Nutrient Agar (NA) slants. Bacterial isolates were identified by various tests, such as the Gram staining, catalase and oxidase tests, and morphology. Analysis of 16S rDNA was performed according to Gur et al. [23] for the best xanthan producing isolate.

2.3. Media

One loop of cells grown on YM agar plates for three days was used to inoculate a 250 ml flask containing 50 ml of YM broth. The shake flasks were incubated at 28 °C and 200 rpm for 24 h. Five milliliters of the inoculum culture were added to 100 ml production medium in a 500 ml erlen flask. The control medium composition was as follows (g/l): glucose 40, citric acid 2.1, NH_4NO_3 1.14, KH_2PO_4 2.87, $MgCl_2$ 0.5, Na_2SO_4 0.09, H_3BO_3 0.0006, $FeCl_3$ 0.020 and 0.03 ml/L concentrated HCl [13,24]. In order to determine the effects of CFP on the xanthan gum production, 0 (control medium, CM), 1–8 g/l CFP were added to the production medium, respectively. The pH was adjusted to 7.0 before autoclaving at 121 °C for 15 min. The culture temperature and agitation rate were maintained at 30 °C and 200 rpm, respectively. Later, CFP was compared with three commercial organic nitrogen sources (yeast extract, bacto peptone, and tryptone) at the concentration, which was determined as optimal for CFP.

2.4. Analytical methods

Total sugar, dry matter and ash contents of CFP were estimated by AOAC methods [25]. Nitrogen content was determined using a micro-kjeldahl apparatus (Labconco corporation, USA). Amino acids were analyzed using reverse-phase high performance liquid chromatography (C18 column, 3.9 mm × 15 cm). Crude fat content was measured with a Soxhlet apparatus using diethyl ether. At regular intervals (18 h) of fermentation, the microbial growth, residual sugar and xanthan gum were determined. For biomass estimation, cultures were harvested by centrifugation, washed twice with sterile distilled water and dried at 80 °C until constant weight was achieved. The dinitrosallicylic acid (DNS) method of Miller [26] was employed for residual sugar. Cell-free supernatant was mixed with ethanol (1:2 v/v) to precipitate the xanthan gum. The precipitated xanthan gum was separated and dried in oven at 90 °C until constant weight [13]. Pyruvate content of xanthan gum was determined by reaction with 2,4 dinitrophenylhydrazine according to Sloneker and Orentas [27].

2.5. Statistical analysis

Experiments were replicated three times in a randomized block design. The statistical analyses of the data were performed one-way analysis of variance (ANOVA). The level of significance was $P < 0.05$. All statistical analyses were performed using SPSS 15.0 software programme.

3. Results and discussion

3.1. Production and chemical analysis of CFP

As shown in Fig. S1, 100 g chicken feather was hydrolyzed with HCl and H_2SO_4. NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$ were used for the neutralization of hydrolysates. The main chemical composition of CFP is shown in Table S1. It was detected that CFP had high protein (56 g 100 g^{-1}), ash (41.5 g 100 g^{-1}), nitrogen (9 g 100 g^{-1}) and low fat (0.2 g 100 g^{-1}) contents. CFP contained all of amino acids, except methionine and tryptophan (destroyed by acid hydrolysis), at varying concentrations and was especially rich in alanine (3.758 g 100 g^{-1}), leucine (5.019 g 100 g^{-1}), glutamate (6.107 g 100 g^{-1}), glycine (5.453 g 100 g^{-1}), serine (4.250 g 100 g^{-1}) and proline (8.106 g 100 g^{-1}). As seen in Table S1, CFP was also rich especially in Ca, K, Mg, S and Na because of the hydrolysis processes. Similar results have been obtained in previous studies [18,19,21,22].

3.2. Isolation and identification of Xanthomonas isolates

The *Xanthomonas* sp. strains, plant pathogens, were isolated from the infected leaves of different plants. Primary identification of the *Xanthomonas* sp. strains (mucoid colonies) were conducted according to pigment production. Several species of bacteria contained identical pigments [28] and *X. campestris* produces yellow pigment called xanthomonadin [29]. *Xanthomonas* sp. strains were formed yellow pigmented slimy or mucoid colonies on YMA and NA plates. Morphological and classical tests showed that they were gram negative, aerobic, rod shaped, oxidase negative, catalase positive, mobile organisms. The isolated four cultures of *Xanthomonas* sp. were screened to obtain the best xanthan gum producer strain. Among the all strains, MO-03 produced maximum xanthan gum and biomass 14.45 g/l and 2.74 g/l, respectively. The xanthan gum from strain MO-02 had lower pyruvic acid content than other isolates (Table 1). The most xanthan producing strain, *Xanthomonas campestris* strain MO-03, was identificated according to 16S rDNA sequencing analysis. Finally, 1488 bp 16S rDNA sequence of the strain was BLAST searched and the sequence was deposited in GenBank with the accession number of KF939142.

3.3. Effect of CFP on xanthan gum production

Fig. 1 shows the effect of the adding CFP (1–8 g/l) to the control medium (CM) on xanthan fermentation. The addition of CFP (1–8

Table 1
Growth, xanthan production and pyruvate contents of xanthan by the isolates in control medium for 72 h.

Isolates	Biomass (g/L)	Xauthan gum (g/L)	Pyruvate (%, w/w)
Xanthomonas sp. MO-01	2.15	11.23	1.9
Xanthomonas sp. MO-02	2.32	7.65	1.5
***Xanthomonas* sp. MO-03**	**2.70**	**14.56**	**2.2**
Xanthomonas sp. MO-04	2.40	9.32	1.8

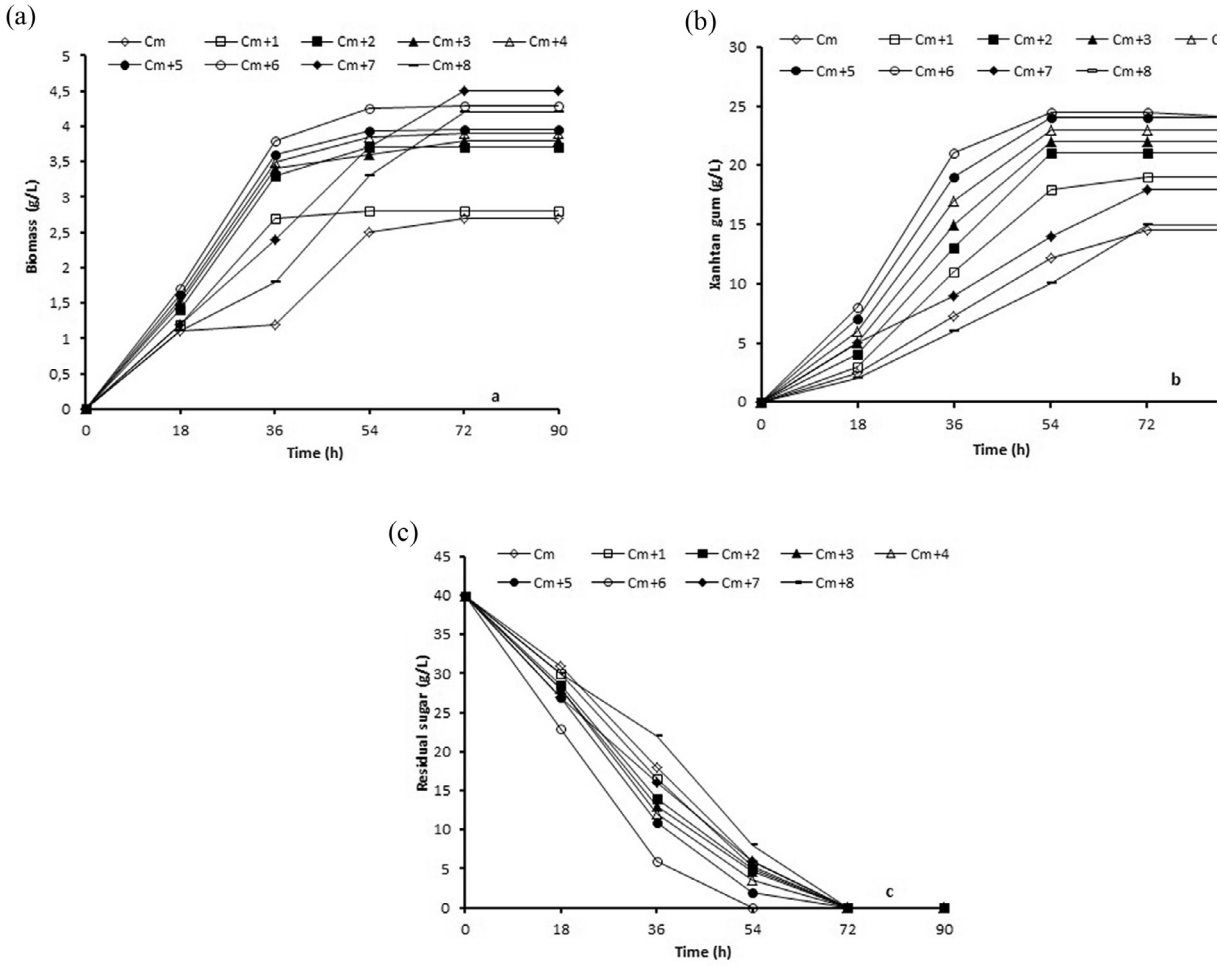

Fig. 1. Comparison of the effect of CFP in various concentrations for different incubation times: (a) biomass concentration, (b) xanthan concentration and (c) sugar utilization.

g/l) to the CM increased microbial biomass, sugar consumption and xanthan gum formation. As seen in Fig. 1a, addition of CFP at 7 g/l gave the highest biomass yield of 4.5 g/l at 90 h. However, *X. campestris* MO-03 produced a maximum of 24.45 g/l of xanthan gum at 54 h in the presence of 6 g/l CFP while the maximum xanthan concentration in the CM was 14.56 g/l at 72 h (Fig. 1b) with the complete depletion of sugar contents in these media (Fig. 1c). Increasing the concentration of CFP from 0 to 6 g/l in CM increased the xanthan gum yield from 12.2 g/l to 24.45 g/l for 54 h. This value is 2 fold higher as compared to CM. Xanthan gum yields based on sugar consumption were calculated to be 61.12% and 30.5% for the CM + 6 g/l CFP and CM, respectively. Obviously, the addition of CFP greatly stimulated the conversion of sugar to xanthan gum and also resulted in reduced fermentation time. The maximum xanthan gum production was obtained in the exponential growth phase (Fig. 1). Similar results were also confirmed by Faria et al. [4] and Savvides et al. [30]. In this study, it was determined that CFP concentration greater than 6 g/l CFP was inhibitory to xanthan production. This inhibitory effect may be due to high salt concentration, some toxic materials in CFP and, change of the C/N rate of culture medium [31–33].

It was reported that the presence of glutamate, aspartate, proline, hydroxyproline, threonine and alanine stimulated xanthan gum production [33]. Also, Murad et al. [14] found that several amino acids (cysteine, histidine, glycine and serine) were suitable for xanthan gum production. To produce xanthan gum, *X. campestris* needs several nutrients, including micronutrients (e.g. K, Fe, P, Mg, S and Ca salts) and macronutrients (C and N) [1,33]. The CFP was considered as an enhancer of xanthan gum production because of including these amino acids and minerals or salts.

The quality of xanthan depends on its pyruvate content [15]. Fig. 2 demonstrates that the pyruvate content of xanthan gum depends on concentration of the CFP. The pyruvate contents of xanthan gum measured at their maximum production times. There was a significant difference in pyruvate content of xanthan gum between the media ($P < 0.05$). It was found that the content of pyruvate increased in proportion to CFP concentration. Similarly,

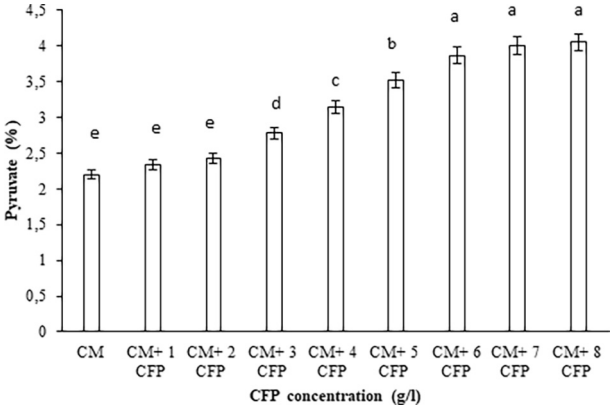

Fig. 2. Effect of the CFP on the pyruvate content of xanthan gum. Values with the same letter are not significant ($P < 0.05$).

researchers reported that presence of ram horn hydrolysate increases the pyruvilation degree [13]. But an increase in the initial inorganic nitrogen concentration reduces the amount of pyruvate [1,35,35]. Moreover, the extent of pyruvate of xanthan gum depends on the fermentation parameters such as media composition, oxygen, temperature, pH, incubation time and H_2O_2 [1,6,34].

3.4. Effect of organic nitrogen sources on production of xanthan

The type of nitrogen sources are very critical on growth and xanthan production by *X. campestris* [14,36]. Fig. 3 shows the marked stimulatory effect of organic nitrogen sources on xanthan synthesis. The results demonstrated that the maximum biomass yield was obtained with TP (4.88 g/l). Xanthan production (24.45 g/l) was the best when CFP was used as supplement nitrogen source, among all the organic nitrogen sources (tryptone TP, bacto peptone BP, and yeast extract YE) tested (Fig. 3). The least xanthan was obtained in the CM containing BP (17.9 g/l). This positive effect of CFP may be a result of high amino acid and mineral contents. Many researchers reported that the addition of organic nitrogen sources (yeast extract, ram horn hydrolyzate, peptone) to production medium promoted cell growth, and xanthan production [8,13,14,37].

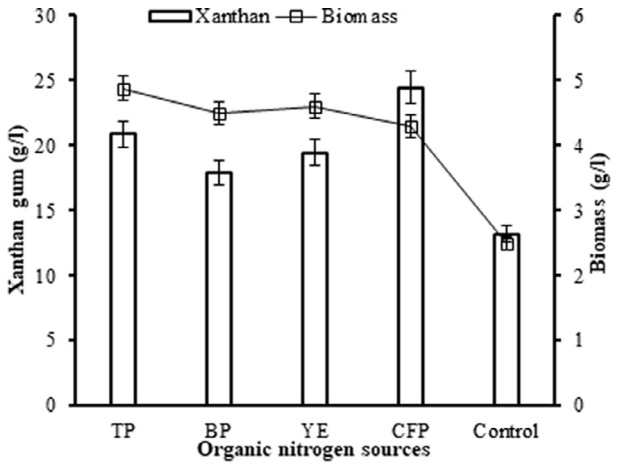

Fig. 3. Effect of the organic nitrogen sources on the xanthan gum and microbial biomass. Culture conditions: Initial pH 7.0, 200 rpm, 30 °C, 54 h. TP: Tryptone, BP: Bacto peptone, YE: Yeast extract, CFP: Chicken feather peptone.

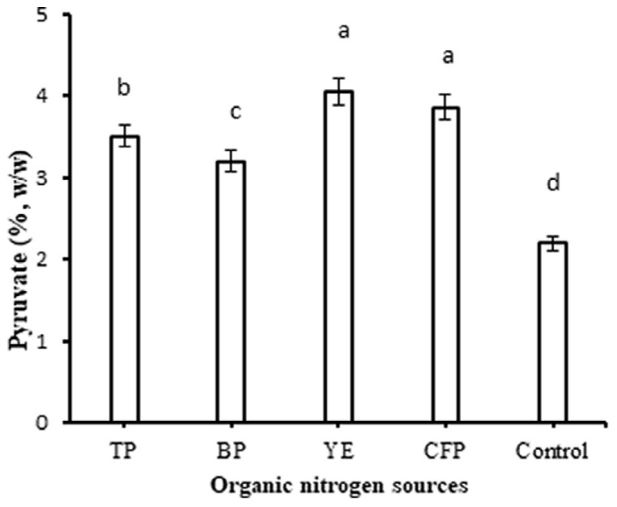

Fig. 4. Effect of the organic nitrogen sources on the pyruvate content of xanthan gum. Culture conditions: Initial pH 7.0, 200 rpm, 30 °C, 54 h. TP: Tryptone, BP: Bacto peptone, YE: Yeast extract, CFP: Chicken feather peptone.

As seen in Fig. 4, the addition of organic nitrogen sources significantly improved ($P > 0.05$) the quality of xanthan gum; the pyruvate content of xanthan was 3.2–4.05% (w/w), higher than that of the control medium (2.2%, w/w). The results showed that the maximum pyruvate yields were obtained with YE and CFP. Organic nitrogen sources were found to be good nitrogen sources for the production of xanthan gum with higher pyruvate contents [13,38]. As mentioned above, the stimulatory effect of these organic nitrogen sources may be due to the availability of soluble amino acids and minerals in the fermentation broth.

In conclusion, organic nitrogen sources are known to be better nitrogen sources for xanthan production compared to inorganic nitrogen sources. But, commercial organic nitrogen sources are expensive. Therefore, waste chicken feathers were converted to CFP through chemical processes. Chicken feathers are a renewable, inexpensive and easily available waste in Turkey and world. Utilization of CFP to produce xanthan gum appears to be economic. The xanthan synthesis was greater in the presence of CFP as compared with commercial organic nitrogen sources. The addition of the CFP greatly increased the bioconversion of sugar into xanthan by *X. campestris* MO-03. Consequently, CFP is an enhancer for xanthan gum production with high pyruvate content.

Acknowledgments

This research was supported by a grant from the research funds appropriated to Ataturk University, Erzurum, Turkey.

Conflicts of interest

No conflict of interest declared.

References

[1] Garcia-Ochoa F, Santos VE, Casas JA, Gomez E. Xanthan gum: Production, recovery, and properties. Biotechnol Adv 2000;18:549–79.
[2] Petri DF. Xanthan gum: a versatile biopolymer for biomedical and technological applications. J. Appl. Polym. Sci. 2015;132:42035.
[3] Kumar A, Rao KM, Han SS. Application of xanthan gum as polysaccharide in tissue engineering: a review. Carbohydr Polym 2017;180:128–44.
[4] Faria S, Vieira PA, Resende MM, França FP, Cardoso VL. A comparison between shaker and bioreactor performance based on the kinetic parameters of xanthan gum production. Appl Biochem Biotechnol 2009;156:45–58.
[5] Kalogiannis S, Iakovidou G, Liakopoulou-Kyriakides M, Kyriakidis DA, Skaracis GN. Optimization of xanthan gum production by *Xanthomonas campestris* grown in molasses. Process Biochem 2003;39:249–56.
[6] Cheng R, Lin L, Zhang Y. Hydrogen peroxide (H_2O_2) supply significantly improves xanthan gum production mediated by *Xanthomonas campestris* in vitro. J Ind Microbiol Biotechnol 2012;39:799–803.
[7] Niknezhad SV, Asadollahi MA, Zamani A, Biria D, Doostmohammadi M. Optimization of xanthan gum production using cheese whey and response surface methodology. Food Sci Biotechnol 2015;24:453–60.
[8] Bhatia SK, Kumar N, Bhatia RK. Stepwise bioprocess for exopolysaccharide production using potato starch as carbon source. 3 Biotech 2015;5:735–9.
[9] Li P, Li T, Zeng Y, Li X, Jiang X, Wang Y, Xie T, Zhang Y. Biosynthesis of xanthan gum by *Xanthomonas campestris* LRELP-1 using kitchen waste as the sole substrate. Carbohydr Polym 2016;151:684–91.
[10] Wang Z, Wu J, Gao MJ, Zhu L, Zhan XB. High production of xanthan gum by a glycerol-tolerant strain *Xanthomonas campestris* WXLB-006. Prep Biochem Biotechnol 2017;47:468–72.
[11] dos Santosa FP, Jra AMO, Nunesa TP, de Farias Silvab CE, de Souza Abud AK. Bioconversion of agro-industrial wastes into xanthan gum. Chem Eng Trans 2016;49:145–50.
[12] Katherine RF, Muthukumaran C, Sharmila G, Kumar NM, Tamilarasan K, Jaiganesh R. Xanthan gum production using jackfruit-seed-powder-based medium: optimization and characterization. Biotech 2017;7:248. 3.
[13] Kurbanoglu EB, Kurbanoglu NI. Ram horn hydrolysate as enhancer of xanthan production in batch culture of *Xanthomonas campestris* EBK-4 isolate. Process Biochem 2007;42:1146–9.
[14] Murad HA, Mohamed SH, Abu-El-Khair AG. Impact of amino acids, nitrogen source and buffering system on xanthan yield produced on hydrolyzed whey lactose. Biotechnology 2017;16:69–76.
[15] De Vuyst L, Vermeire A. Use of industrial medium components for xanthan production by*Xanthomonas campestris* NRRL-B-1459. Appl Microbiol Biotechnol 1994;42:187–91.

[16] Kamarudin NB, Sharma S, Gupta A, Kee CG, Chik SMSBT, Gupta R. Statistical investigation of extraction parameters of keratin from chicken feather using design-expert. 3 Biotechnology 2017;7:127.
[17] Maciel JL, Werlang PO, Daroit DJ, Brandelli A. Characterization of protein-rich hydrolysates produced through microbial conversion of waste feathers. Waste Biomass Valor 2017;8:1177–86.
[18] Taskin M, Esim N, Ortucu S. Efficient production of l-lactic acid from chicken feather protein hydrolysate and sugar beet molasses by the newly isolated *Rhizopus oryzae* TS-61. Food Bioprod Process 2012;90:773–9.
[19] Ozdal M, Gurkok S, Ozdal OG. Optimization of rhamnolipid production by *Pseudomonas aeruginosa* OG1 using waste frying oil and chicken feather peptone. Biotechnology 2017;7:117. 3.
[20] Benesova P, Kucera D, Marova I, Obruca S. Chicken feather hydrolysate as inexpensive complex nitrogen source for PHA production by *Cupriavidus necator* on waste frying oils. Lett Appl Microbiol 2017;65:182–8.
[21] Ozdal M, Kurbanoglu EB. Citric acid production by *Aspergillus niger* from agro-industrial by-products: molasses and chicken feather peptone. Waste Biomass Valor. 2018. doi: https://doi.org/10.1007/s12649-018-0240-y.
[22] Kurbanoglu EB, Kurbanoglu NI. Ram horn peptone as a source of citric acid production by *Aspergillus niger*, with a process. J Ind Microbiol Biotechnol 2004;31:289–94.
[23] Gur O, Ozdal M, Algur OF. Biodegradation of the synthetic pyrethroid insecticide α-cypermethrin by *Stenotrophomonas maltophilia* OG2. Turk J Biol 2014;38:684–9.
[24] Jana A, Ghosh P. Stimulation of xanthan production by *Xanthomonas campestris* using citric acid. World J Microbiol Biotechnol 1997;13:261–4.
[25] AOAC. Official methods of analysis. 13th ed. Washington, D.C.: Association of Official Agricultural Chemists; 1980.
[26] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426–8.
[27] Sloneker JH, Orentas DG. Pyruvic acid, a unique component of an exocellular bacterial polysaccharide. Nature 1962;104:478–9.
[28] Okay S, Ozdal M, Kurbanoğlu EB. Characterization, antifungal activity, and cell immobilization of a chitinase from *Serratia marcescens* MO-1. Turk J Biol 2013;37:639–44.
[29] Tuli HS, Chaudhary P, Beniwal V, Sharma AK. Microbial pigments as natural color sources: current trends and future perspectives. J Food Sci Technol 2014;52:4669–78.
[30] Savvides AL, Katsifas EA, Hatzinikolaou DG, Karagouni A. Xanthan production by *Xanthomonas campestris* using whey permeate medium. World J Microbiol Biotechnol 2012;28:2759–64.
[31] Kurbanoglu EB, Ozdal M, Ozdal OG, Algur OF. Enhanced production of prodigiosin by *Serratia marcescens* MO-1 using ram horn peptone. Braz J Microbiol 2015;46:631–7.
[32] Ozdal M, Gürkök S, Ozdal OG, Kurbanoğlu EB. Rhamnolipid production by *Pseudomonas aeruginosa* OG1 using waste frying oil and ram horn peptone. AIP Conf Proc 2017;1833:020102.
[33] Souw P, Demain AL. Nutritional studies on xanthan production by *Xanthomonas campestris* NRRL B1459. Appl Environ Microbiol 1979;37:1186–92.
[34] Davidson IW. Production of polysaccharide by *Xanthomonas campestris* in continuous culture. FEMS Microbiol Lett 1978;3:347–9.
[35] Candia JLF, Deckwer WD. Effect of the nitrogen source on pyruvate content and rheological properties of xanthan. Biotechnol Prog 1999;15:446–52.
[36] Palaniraj A, Jayaraman V. Production, recovery and applications of xanthan gum by *Xanthomonas campestris*. J Food Eng 2011;106:1–12.
[37] Carignatto CRR, Oliveira KSM, de Lima VMG, de Oliva Neto P. New culture medium to xanthan production by *Xanthomonas campestris pv. campestris*. Indian J Microbiol 2011;51:283–8.
[38] Cadmus MC, Knutson CA Jr, U.S. Patent No. 4. Washington, DC: U.S. Patent and Trademark Office; 1983. 394,447.