Original Article

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

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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.

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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 biorganic 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.
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 hydrolysed by modifying the method of Kurtanoglu and Kurbanoğlu [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 (KH₂PO₄ 5 g/l, yeast extract 4 g/l, MgSO₄ 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 erlenmeyr flask. The control medium composition was as follows (g/l): glucose 40, citric acid 2.1, NH₄NO₃ 1.14, KH₂PO₄ 2.87, MgCl₂ 0.5, Na₂SO₄ 0.09, H₂BO₃ 0.0006, FeCl₃ 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 dinitrosalicylic 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 H₂SO₄. 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, 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 all the 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 identified 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 g/l) increased xanthan gum and biomass production compared to the control medium. Xanthan gum production was higher in the presence of CFP than without CFP (Table 1). The biomass production and pyruvate content increased significantly with the addition of CFP. This result shows the importance of CFP in the production of xanthan gum.

Table 1

| Isolates              | Biomass (g/L) | Xanthan gum (g/L) | Pyruvate (%) |
|----------------------|--------------|------------------|--------------|
| *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          |
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,
researchers reported that presence of ram horn hydrolysate increases the pyruvulation 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 H₂O₂ [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 (trypotide 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].

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.

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Conflicts of interest

No conflict of interest declared.

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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.
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