MASTER CLASS 3
XANTHAN GUM PRODUCTION

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Introduction

Xanthan gum (XG) is an important polysaccharide produced by specific bacteria, *Xanthomonas* spp. offering superior properties like excellent viscosity, stability across varying conditions, and versatility for use in numerous industries such as pharmaceuticals, food, cosmetics, medical, and textiles [1]. The production and applications of XG are being redefined through innovative research. It has attracted industry attention because of its significant properties, such as biocompatibility, biodegradability, non-toxicity, and renewability [2].

The structure of XG comprises of repeating pentasaccharide units with β-D-glucose units forming a central chain and trisaccharide side chains containing D-glucuronic acid and D-mannose units with acetyl and pyruvate groups [3] as shown in ***figure 1***. The acetyl and the pyruvate groups are responsible for the stability of the gum, whiles the acetyl groups stabilize the structure, the pyruvate group disrupts it [4].

The major producers are in China and Austria, with the food industry utilizing 65% of the global production. However, factors like bacterial strains and fermentation conditions impact the properties of the gum. Therefore, genetically engineered species of *Xanthomonas* spp. are being used more recently as well as the use of wastes products to improve gum production and to cut down on the cost of production [5], [6].

The production of xanthan gum under laboratory conditions represents a fascinating area of study, enabling scientists to delve into the controlled synthesis and optimization of this biopolymer. This intricate process involves the cultivation of specific microorganisms, such as *Xanthomonas campestris*, and the meticulous manipulation of environmental factors to yield high-quality xanthan gum. Exploring the methodologies, parameters, and advancements in laboratory-based xanthan gum production not only contributes to a deeper understanding of microbial biopolymers but also paves the way for enhanced production techniques with broader industrial applications. This work sets the stage for a comprehensive exploration into the intricacies and innovations surrounding xanthan gum production within controlled laboratory settings.

The main aim of this Master class is to produce xanthan gum using the bacteria strain *X. campestris* B6720, glucose as main carbon source under laboratory conditions.

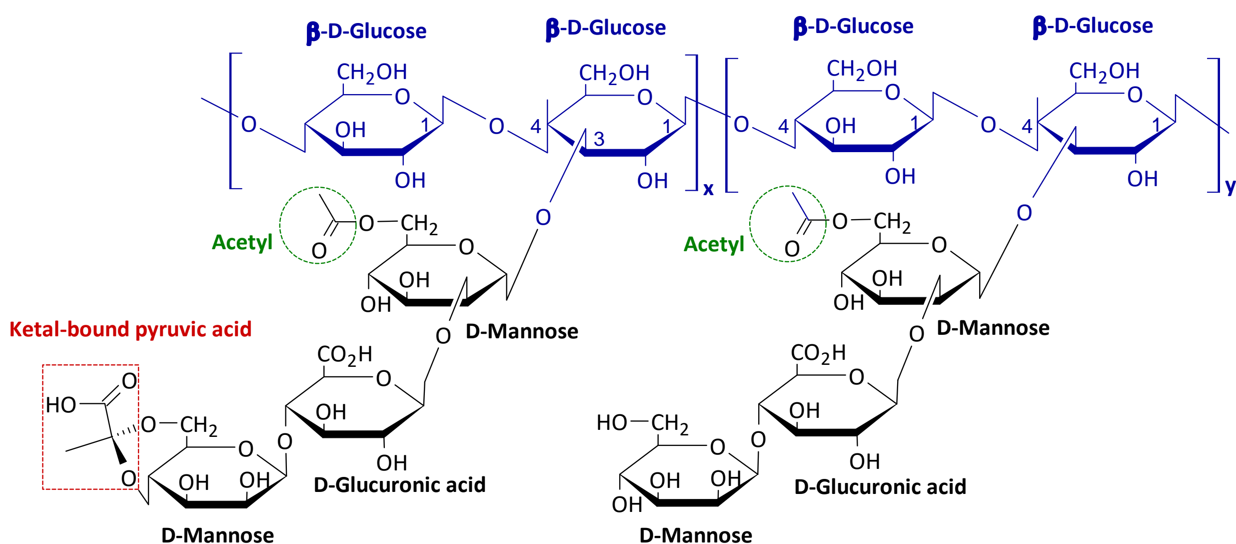

Figure 1. Xanthan gum structure

Materials and methods

The bacteria *Xanthomonas campestris* B6720 strain was purchased from Bioresources Center All-Russian Collection of Industrial Microorganism (BRC VKPM), National Research Center (Moscow, Russia).

Chemicals and reagents

1. Glucose
2. Yeast extract
3. Peptone
4. Malt extract
5. Magnesium Sulphate ($MgSO_4.7H_2O$)
6. Potassium Hydrogen Phosphate (K_2HPO_4)
7. Potassium dihydrogen Phosphate (KH_2PO_4)
8. Agar
9. Isopropanol

Production of xanthan gum

Rehydration of bacteria cells

1. Bacteria cells were rehydrated for 15 minutes in malt extract (1.7 g), peptone (1.0 g), and sterilized distilled water (100 ml) as shown in ***table 1***.
2. A 1 ml aliquot of the rehydrated cell mixture was plated on a glucose-yeast extract agar plate (glucose 2 g, yeast extract 1 g, peptone 1 g agar 1.7 g, and distilled water 100 ml).
3. The cultures were incubated at 28 ^{o}C for 48 hours.
4. Active growing bacteria is then obtained and ready for pre-inoculum preparations.

Pre-inoculum preparations

1. Pre-inoculation media for biosynthesis was prepared using Glucose, Yeast extract, Magnesium Sulphate ($MgSO_4.7H_2O$), Potassium Hydrogen Phosphate (K_2HPO_4), and Potassium dihydrogen (KH_2PO_4) Phosphate as shown in ***table 1***.

2. The media was sterilized using autoclave and about 50 ml was poured into a conical flask under sterile condition.
3. A loop of the bacteria culture from the agar plates of the two was dissolved in the conical flask containing the media.
4. The prepared culture was then incubated at 28 ^{O}C for 24 hours at 250 rpm, using Biosan Orbital Sharker-Incubator ES-20.
5. The pre-inoculum is then obtained for xanthan production.

Xanthan gum production

1. Biosynthesis media is prepared as shown in ***table 1***.
2. The media was sterilized using autoclave and distributed to 250 ml conical flasks under sterile conditions, about 100 ml in each flask.
3. About 5 ml of the pre-inoculated biosynthesis media was transferred into a 250 ml conical flask containing 100 ml of biosynthesis medium under sterile condition.
4. The resulting media was incubated on a shaker 250 rpm at 28 ^{O}C for 72 hours using Biosan Orbital Sharker-Incubator ES-20.
5. The culture broth is then ready for xanthan gum recovery.

Table1. Media compositions

Media contents	*Medium types*			
	rehydration	Bacteria growth	Pre-Biosynthesis	Biosynthesis
Glucose	1.7 g	2.0 g	0.2 g	2.0 g
Yeast extract	-	1.0 g	0.03 g	0.3 g
Peptone	1.0 g	1.0 g	-	-
Agar	-	1.7 g	-	-
$MgSO_4.7H_2O$	-		0.001 g	0.01 g
K_2HPO_4	-		0.02 g	0.2 g
KH_2PO_4	-		0.02 g	0.2 g
Distilled water	100 ml	100 ml	10 ml	100 ml

Xanthan gum recovery

1. The culture broth was transferred into 50 ml falcon tubes and centrifuged at 5000 rpm for 40 min to remove cells (biomass).
2. The gum contained in the supernatant was precipitated using isopropanol in the ratio 1: 3.
3. The sediment containing the cell was mixed with water (washed) and centrifuged at 5000 rpm for 20 mins.
4. The water was discarded, and the cells dried in oven at 50 ^{O}C for 48 hours and the weight of the dried biomass obtained.
5. The isopropanol precipitated of the gum were centrifuged at 5000 rpm for 20 mins and the process repeated for two times.

6. The solvent was then discarded, and the precipitate (gum) was dried at 50 ^{O}C for 48 hours in a hot air oven and the weight obtained.
7. The gum was then milled using mortar and pestle.

The general production of xanthan gum is represented in ***figure 2*** and in picture ***figure 3.***

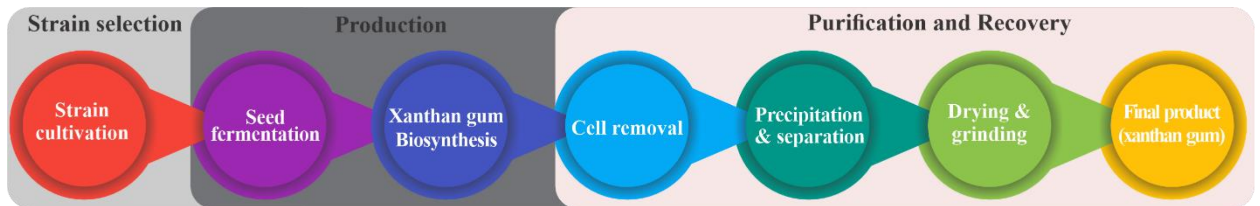

Figure 2. Flow chart of xanthan gum production

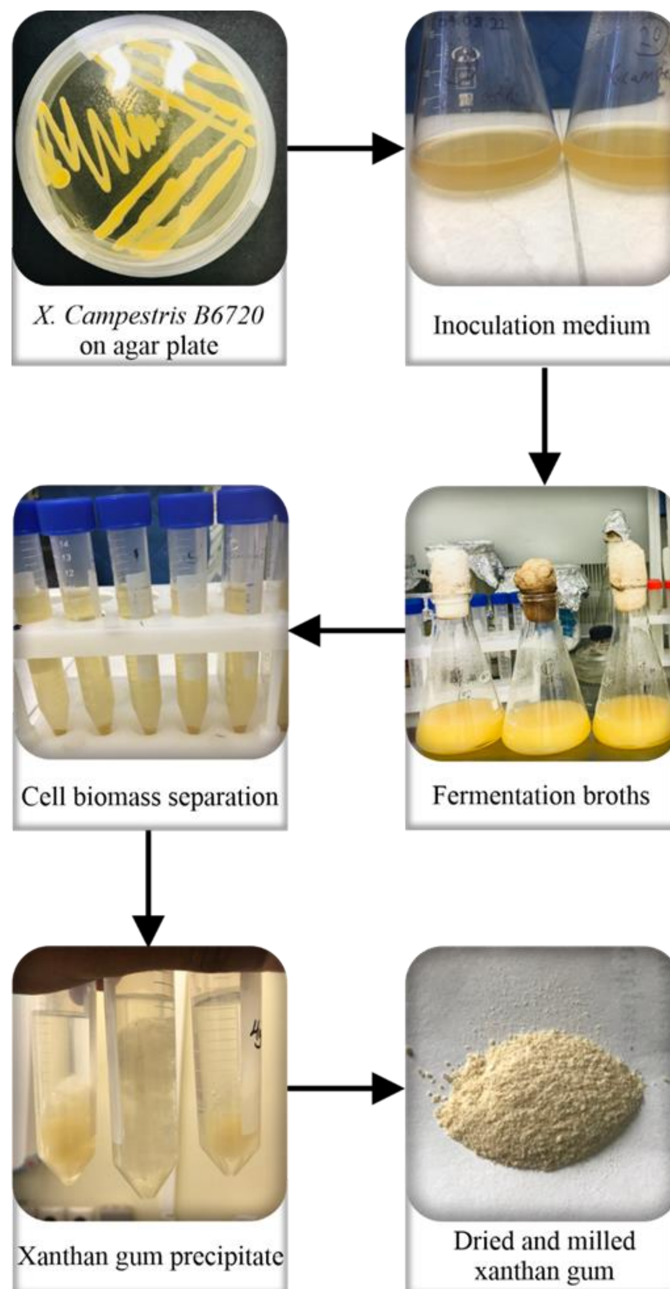

Figure 3. Xanthan gum production in picture

Conclusions

In conclusion, the production of xanthan gum under laboratory conditions represents a vital avenue for both scientific exploration and industrial applications. Through meticulous cultivation of microorganisms and precise manipulation of environmental factors, researchers can optimize the synthesis of this polysaccharide, unlocking its full potential across diverse industries. The methodologies developed in laboratories not only contribute to the fundamental understanding of microbial biopolymers but also pave the way for scalable, efficient, and sustainable production methods on an industrial scale. As technology advances and scientific knowledge expands, further innovations in laboratory-based xanthan gum production will likely continue to refine this process, offering new possibilities for improving product quality, functionality, and applicability in various sectors. The continuous pursuit of enhancing xanthan gum production under controlled conditions promises to sustain its relevance and impact across numerous fields in the foreseeable future.

References

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