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Biosynthesis, extraction, purification of postbiotic from probiotic isolate

Nada E. Taha [1], Amr A. El-Waseif [2], Mervat G. Hassan [1], Sabah A. Abo El-Maaty[1]

[1]Botany and Microbiology Dept., Faculty of Science, Banha University, Egypt.
[2] Botany and Microbiology Dept., Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.

Abstract

The most effective microbes for society are probiotics. Exopolysaccharide (EPS) from probiotics, which has a range of biological activities, has enormous promise in the areas of health, chemical material production, and cosmetics. Examine the process of producing, extracting, and purifying postbiotic (probiotic EPS) after identifying probiotic isolates that are capable of producing EPS. Using MRS, 32 bacterial isolates were examined. Nine isolates lacked EPS production in the liquid MRS, while 23 isolates demonstrated EPS production. The broth culture's ultimate pH changed from 6.5 to 4.0–5.0. Two volumes of 100% ethanol were used to precipitate the EPS. Deionized water was used to dissolve the EPS, which was then dialyzed against deionized water and freeze-dried. The purification phase involves the removal of proteins using 10% (w/v) trichloroacetic acid at a final pH of 6.8, the purified freeze-dried was measured at 470 mg/L. The culture measured the dry weight of the cells and the dry weight of the EPS before calculating the yield coefficient, or $Y_{P/X}$ (0.39 g / g). Our findings indicate that the isolates are a promising source of probiotics that could be used in the future to produce probiotic products.

Keywords: *Probiotics, postbiotic, exopolysaccharide, precipitation, purification.*

1. Introduction

Probiotics have been shown to be effective fermenting agents and are widely used in biotechnology, traditional dairy products, medicines, and fermentation processes (1). Exopolysaccharides (EPS) produced by probiotics have received special attention recently since they are "generally regarded as safe (GRAS)" and contain bioactive properties that prevent disease and enhance human health (2). With a wide range of biological activities, probiotics, also known as postbiotics or EPSs, have great potential in the fields of health, beauty care, and chemical material production. Some of these functions include immune system improvement, antioxidation, plastic film production, prevention of intestinal microbial infections, enhancement of the intestinal microbiological environment, and antiulcer properties (3). In the food sector, postbiotics offer many great functional qualities. These include enhancing the flavor and rheological qualities of fermented meals and bread crumbs, as well as enhancing the softness of baked goods (4).

Microbial exopolysaccharides, which mostly consist of mucus polysaccharides and capsular polysaccharides, are secondary metabolites that are discharged into the extracellular environment during the growth and metabolism of bacteria (5). Postbiotics are categorized as either homopolysaccharides, which are composed of a single kind of monosaccharide, or heteropolysaccharides, which are composed of two or more types of monosaccharides, such as fructose, mannose, arabinose, rhamnose, xylose, and glucose (6). The structural makeup and physicochemical characteristics of postbiotics vary greatly due to their diverse variety of sources, and these variations convey distinct biological activities (7).

Probiotic-produced postbiotics are the focus of more and more research. Probiotics have the ability to make EPSs, which may be used as safe supplements to enhance the viscosity and texture of naturally fermented milk products as well as as antioxidant, emulsifying, and stabilizing agents (8). Furthermore, it has been proposed that these biopolymers could help the consumer's health. According to certain research, these postbiotics may have anti-tumoral and immunostimulatory properties. Usually, oligosaccharide units are repeated to create these exopolysaccharides. Depending on whether they include several forms of sugar, they are classified as either homopolysaccharides or

heteropolysaccharides. The characteristics mentioned above are all related to the chemical structure of these molecules. The identification of these carbohydrate connections is therefore crucial. According to Jolly et al., the biological and technological characteristics of EPS are influenced by the sugar content, molecular weight, sugar connections, presence of repetitive units, and replacements. Therefore, it is essential to characterize new EPS chemically in order to forecast their possible use (9).

After screening for probiotic isolates to identify those with the capacity to produce EPS, the research moves on to the generation, extraction, and purification of postbiotic (probiotic EPS).

2. Materials and Methods

2.1. Screening of postbiotic production

Following the screening of 32 probiotic bacterial isolates, the selection process was conducted. The isolates were grown and incubated separately for 24 hours at 30 °C in a 20 ml test tube with 10 ml of MRS liquid medium; peptone, 10.0; potassium hydrogen phosphate, 2.0; sodium acetate trihydrate, 5.0; triammonium citrate, 2.0; magnesium sulfate heptahydrate, 0.2; magnesium sulfate tetrahydrate, 0.05 at pH 6.2. (10).

2.2. Production, extraction and purification of the EPS

With slight modifications, the EPS was extracted and purified in accordance with Cerning et al. 1994 (11). To deactivate enzymes that might be able to break down polymers, the growing culture was heated to 100 °C for five minutes. The cells were then extracted using centrifugation at 8000 rpm for five minutes at 4 °C. Two volumes of 100% ethanol were used to precipitate the EPS. The resulting precipitate was collected by centrifugation at 8000 rpm for 20 minutes after standing overnight at 4 °C. Deionized water was used to dissolve the EPS, which was then dialyzed against it for 24 hours at 4 °C before being freeze-dried. As a purification stage, the freeze-dried powder was treated in 10% (w/v) trichloroacetic acid to extract the proteins. The supernatant was dialyzed at 4 °C against deionized water for 5 days and freeze-dried. These preparations were referred to as purified EPS and were stored at 4 °C. The culture was determining cells dry weight (CDW) and EPS dry weight then calculate the yield coefficient. $Y_{P/X}$ (EPS g / cell dry weight g) .

3. Results and discussion

Figure 1 depicts the approximately 32 bacterial isolates that were used for screening and were found in probiotic

habitat sources. Characteristics of isolates often matched those of probiotics. MRS was used to filter these isolates. Nine isolates did not produce any EPS, but twenty-three isolates did in liquid MRS. EPS production was categorized as (+) low, (++) moderate, and (+++) high. Table 1 also shows the cell dry weight. The broth culture's final pH, which began at 6.5, changed to 4.0–5.0. The isolate with code NE 13 produced the most probiotic EPS (postbiotic).

In 2023, Lu et al. employed 48 LAB strains that had been kept in the lab to screen for high exopolysaccharide synthesis. Of those strains, 18 were found to be prospective high EPS producers and were further screened using liquid fermentation to measure the EPS yield (12).

Table 1: Screening for selecting of the most potent EPS producer isolate

Isolate code	EPS	CDW	Final pH
NE 1	-	+	5.0
NE 2	-	+	4.0
NE 3	+	+	4.5
NE 4	-	++	4.2
NE 5	-	+	4.5
NE 6	-	+++	4.0
NE 7	+	+	5.0
NE 8	+	+	5.0
NE 9	+	+	4.0
NE 10	+	++	4.5
NE 11	+	++	4.5
NE 12	+	++	4.5
NE 13	+++	++	4.0
NE 14	++	++	5.0
NE 15	+	++	4.2
NE 16	++	+	4.5
NE 17	++	+	4.5
NE 18	++	+	4.0
NE 19	-	++	4.0
NE 20	+	++	4.2
NE 21	+	++	5.0
NE 22	-	+++	5.0
NE 23	++	++	5.0
NE 24	++	++	4.0

NE 25	++	++	4.0
NE 26	-	++	4.0
NE 27	++	+	4.5
NE 28	+	+	4.5
NE 29	-	+++	5.0
NE 30	++	++	5.0
NE 31	+	++	4.2
NE 32	++	+	4.5

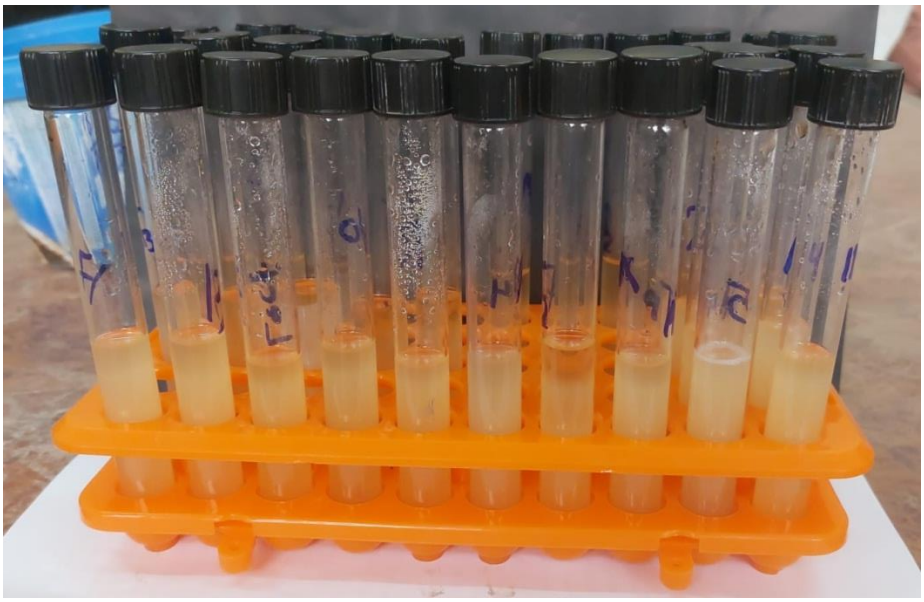


Figure 1: Screening for selection EPS producer depending on yield production in MRS

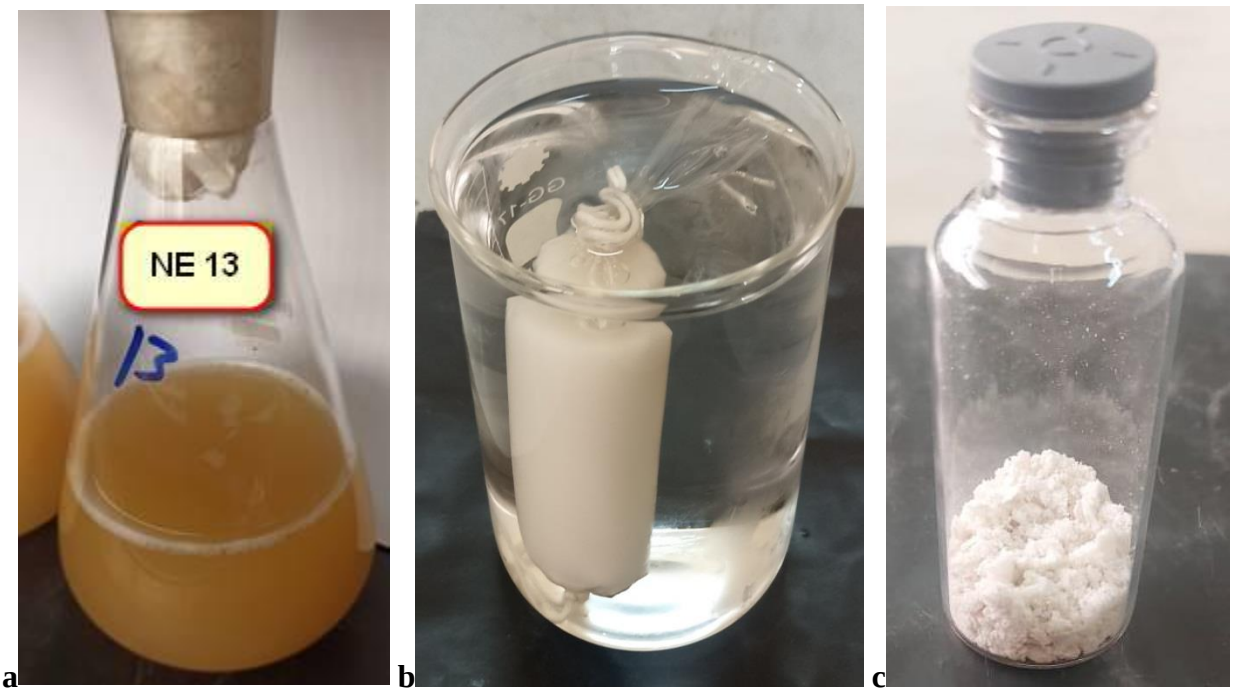
4. Production, extraction and purification of the EPS

In order to inactivate enzymes that might be capable of breaking down polymers, the growing culture was heated to 100 °C for five minutes, as indicated in table 2 and figure 2. The cells were then extracted using centrifugation. Absolute ethanol was utilized to precipitate the EPS. In order to determine the final pH of 4.5, the EPS was dissolved in deionized water, dialyzed against deionized water, and crude EPS freeze-dried with a reported 1410 mg/L and cell dry weight of 1.2 g/L. As a purification step, proteins were extracted from the freeze-dried powder by dissolving it in trichloroacetic acid (TCA). After five days of dialyzing against deionized water, the filtered, freeze-dried supernatant had a final pH of 6.8 and 470 mg/L. Cell dry weight and EPS dry weight were measured in the culture, and the yield coefficient, $Y_{P/X}$ (0.39 g / g), was then calculated.

Due to its significance in possible industrial applications, the majority of current EPS research focuses on the isolation and characterization of microbial EPS (13). However, a significant barrier to the widespread use of EPS is its high production costs. Under non-optimized circumstances, the majority of known lactic acid bacteria (LAB) strains that produce EPS such as *Lactobacillus fermentans* (0.75–0.85 g/L), *Lactobacillus brevis* (0.35 g/L), and *Lactobacillus plantarum* (0.14–0.4 g/L) produce less than 1.0 g/L (14). 34 LAB strains were identified from saliva by Peng et al. in 2022. The ropy trait is present in eight strains in all. With an EPS output of 0.603 mg/mL, Strain O17 was the most productive of them (15).

Table 2: Production and purification of postbiotic EPS yield

Sample	EPS mg/L	Cell dry weight g/L	pH	$Y_{P/X}$
Crude	1410	1.2	4.5	1.16
Purified	470	1.2	6.8	0.39



a **b** **c**

Figure 2: a- production culture of isolate NE 13 after 24h incubation; b- purification step dialysis after TCA; c- final pure lyophilized postbiotic EPS yield 470 mg/L.

5. Conclusion

The most beneficial microbes for society are probiotics. Postbiotic production holds enormous promise for the health sector. In liquid MRS, 23 isolates produced EPS. Two volumes of 100% ethanol were used to precipitate the EPS. Purified freeze-dried probiotics were found to have 470 mg/L, which suggested that they could be used to further process probiotic products.

6. References

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