# Review of major sources of agarase, purification, method, categories, and application

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**ABSRACT - Microbes in soil are abundant, and they can be employed for a range of tasks ranging from decomposition to antibiotic synthesis. Agar-agar, a polysaccharide recovered from the sea, is a useful polysaccharide that may be used in a variety of ways after being degraded by bacteria. The goal of this research was to find bacteria that produced the agarase enzyme. Morphological and biochemical characterization of a wide range of soil sources. Agarolytic bacteria were identified from a seawater source in this investigation. The isolates grew in a media that contained solely agar as a carbon source. Lugol's solution was used to visualize agarase activity. It was determined using the DNSA approach. The organisms created reducing sugars, according to the findings. The impact of several parameters was also investigated.**

**Keywords: agar, agarase, agarolytic,**

## I. INTODUCTION

Since Gran (1902) discovered a marine organism (*Bacillus gelaticus*) that liquefied seaweed agar using an external enzyme, agar digesting bacteria have been known. Over twenty agar-digesting organisms have now been identified, mostly belonging to the genera *Vibrio*, *Cytophaga*, *Agarbacterium* Several, Bacterium, *Achromobacter*, and *Flavobacterium.* [1,2]species of agarase enzyme-producing bacteria have been isolated from various sources and have been found to degrade agar by creating extracellular or intracellular enzymes.[3,4] Several bacterial strains from marine environments and other sources can degrade agar-agar. Agarolytic bacteria are found in abundance in coastal and estuary environments; however, they are not solely autochthonous in the marine environment, as evidenced by reports that they can also be found in freshwater, sewage, and soil. [5,6]

Agar is a polymer made of galactose sugar subunits. It is found in the cell walls of algae, mostly Gelidium and Gracilaria, as a structural carbohydrate. Its calcium salt or a mixture of its calcium salt and magnesium salt gives it its main structural support. Agar is made up of two main parts: Agarose (70%) and Agaropectin (30%). Agarose is the gelling part of agar, and Agaropectin is the non-gelling part.[49] Most of the time, agar is used to make growth media for microorganisms more solid. Agar is firmer and stronger, and most bacteria find it hard to break it down. Those who want to ruin it can do so in three ways: The first way is to break up the double helix structure without letting the polymer break down. This small amount of activity doesn't show up on the agar, but it can be seen when the gel and iodine don't mix to make a dark brown colour. [2]

Agar is a biopolymer that is common in red algae and is a component of their cell walls. It's a polysaccharide made up of agarase and agaropectin, with agarose being the neutral agar fraction with a linear chain of 3-0-linked -d-galactopyranose and 4-0linked 3,6-anhydrous L-Galactose residues. [7] The primary component of the cell wall of several Rhodophyta algae species is agar. Agrophytes are the general name for these algae.[8] Agarases are divided into two types based on their cleavage patterns: α-agarase (EC 3.2.1.158) and β-agarase (EC 3.2.1.81). [9]

Because these agarases can break down agar or agarose into oligosaccharides, it is used a lot in the food, cosmetics, and medical industries. Neoagarooligosaccharides, which are made when agar breaks down, stop bacteria from growing and slow down the rate at which starch breaks down. Also, neoagarobiose (NA2) is a rare compound that makes melanoma cells more moist and makes them lighter [23]. The polysaccharide fractions made from marine algae by b-agarase also stimulate macrophages and are good sources of physiologically functional foods that are protective and boost the immune system [3,50]. Agarases are also used to break down the cell walls of marine algae in order to get at bioactive substances that are easily broken down and to make protoplasts [23,24,51].

## II. AGAR

For hundreds of years, agar has been used as food. During the seventeenth and eighteenth centuries, it was exported from Japan to various Asian countries. Because of its stabilizing and gelling properties, agar is now used in a wide range of applications. Most of the time, agar has been used in microbiological media because it is hard for microorganisms to break down and because it forms clear, stable, and firm gels. It is a food additive that is Generally Recognized as Safe (GRAS) and is used in icings, glazes, processed cheese, jelly candies, and marshmallows. [10]

.*Source of agar*

Agar is a phycocolloid derived from the cell walls of a family of red algae known as Rhodophyceae, which includes *Gelidium* and *Gracilaria*. *Gelidium* is the ideal source for agar production, although it is difficult to culture and has a limited

natural resource compared to Gracilaria, which is commercially grown in numerous nations and areas. As a result of its ease of harvesting and cultivation, Gracilaria has become a significant source of agar production.[11]

*Structure of agar*

Agar is made up of two polysaccharides known as agarose and agaropectin. [12] Agarose's core structure is made up of repeating units of β-D-galactose and 3,6-anhydrous-α-L-galactose (3,6-AG) with few changes and few sulphate esters. Agaropectin contains the same basic disaccharide-repeating units as agarose, but some of the hydroxyl groups of 3,6-anhydrous-α-L-galactose residues have been replaced with sulfoxy or methoxy and pyruvate residues. [13] Agarose has a molecular mass of more than 100,000 Daltons and a sulphate concentration of less than 0.15 percent. Agaropectin has a molecular mass of fewer than 20,000 Daltons and a substantially greater sulphate concentration of 5% to 8%.[27,28]

Agar is made up of a mixture of agarose and agaropectin in different amounts, depending on what the raw material was. The most agaropectin is found in Gracilaria, then Porphyra, and finally Gelidium**.**

## III. AGARASE

*Source of agarase*

Agarase has been extracted from a variety of different sources like sewage sludge compost, [29] plant, [30]soil [31,32,33,34]greenhouse soil, [35] rhizosphere soil, [36] rhizosphere of spinach,[37]black sand,[38] marine algae. [15] *Alteromonas sp.*[39,3,40,15] *Pseudomonas sp.*[41,42,43], *Vibrio sp*[18,20,44], *Cytophaga sp*,[45] *Agarivorans sp.*[46] *Thalassomonas sp.*[47] *Pseudoalteromonas sp.*[19] *Bacillus sp.*[4] *Acinetobacter sp.*[22] among others, have been found to produce agarase. Gram-negative bacteria make up all of the microorganisms. Except for a few agarases that are produced intracellularly, the majority of agarases are produced extracellularly. [8]

*Cleavage pattern of agarase enzyme*

The enzymatic breakdown of agar done by two types of agarases, α-agarases and β-agarases and they differbased on pattern of hydrolysis of the substrates. The α-agarase cleaves the α-L-(1,3) linkages of agarose,produce oligosaccharides of the agarobiose series with3,6-anhydro-L-galactopyranose at the reducing end,whereas, the β-agarases cleaves the β-D-(1,4) linkages of agarose, produce neoagaro-oligosaccharides with D-galactopyranoside residues at the reducing end. [48]

*Agarase activity*

Qualitative and quantitative tests were used to detect the presence of the agarase enzyme in the isolated bacterial cultures.

*Qualitative assay*

The culture plates were flooded with Lugol's iodine solution (1 g Iodine, 2 g KI, 100 mL distilled water), which stains polysaccharides of agar to a dark brown hue but not degraded oligosaccharide. [15,16]

*Quantitative assay*

The approach can be used to detect reducing sugars that are produced as a result of agar degradation. [17] The DNSA technique cannot identify agar since it is a polysaccharide. However, when an organism degrades agar, a huge amount of oligosaccharides with galactose at one end are formed, which may be detected using the DNSA approach. The standard was D-galactose. [8]

*Optimization*

Check various parameter pH, temperature, agar concentration, NaCl. [25,26]

## IV. ISOLATION AND PURIFICATION

The agarase separation and dialysis were carried out according to the protocol provided. [18] Magnetic beads were used to stir about 50 ml of crude enzyme generated by the potent strain. A 50 mL solution of 80% ammonium sulphate was added to this and slowly stirred for roughly an hour. The precipitate was allowed to develop for 24 hours a 4℃. The solution was then centrifuged for 10 minutes at 4℃ at 4,000 rpm. For 24 hours, the precipitate formed following ammonium sulphate precipitation was dialyzed against phosphate-buffered saline (pH-7) using a dialysis membrane. The buffer was modified on a regular basis. The dialysate was then examined using the dinitrosalicylic acid technique for agarase assay. [15] Several agarases have been purified using an ammonium sulphate fractionation process followed by anion exchange chromatography and gel filtration chromatography. [3,18,15,20,21,4,22]

## V. APPLICATION

Agarase enzyme is a food additive and gelling agent that is used to extract DNA from agarose gels.[8]It is anti-oxidant in nature.[15]when it comes to cosmetics and moisturizers,[23]It's also used as an immune booster in the field of medicine.[24]

## VI. CONCLUSION

we conclude that agarases are significant enzymes that have been isolated and cloned spontaneously from a wide range of bacteria. They are divided into two categories, each with more than four families capable of producing oligosaccharides with varying DP values. A few agarases have a high specific activity, great temperature and pH stability, and other qualities that reveal their vast range of possible uses.

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