Isolation of Species Specific Bacteria from Raw Nature for Porcine Gelatin Detection

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Abstract
The ability of gelatinase bacteria to hydrolyze gelatin makes them widely distributed and may have an impact on the safety and quality of gelatin manufacture. A study was conducted to find out specific species of gelatinase bacteria in 14 samples from selected sources using the agar isolation method through 1, 3 and 5% porcine gelatin. The samples are chosen from various types of soil, animal skin and water. The selection is made in the event of gelatinase activity on the agar surface. Findings were obtained with the 10 most active gelatinase bacteria characterized through culture morphology which were then isolated to see their sensitivity in the detection of Halal gelatin.

Keywords: Gelatin, Bacteria, Gelatinase, Porcine detection

1.0 Introduction
Gelatin is a special and unique hydrocolloid product, meeting the wide range of functions and requirements for various applications in the industry, including the food industry as a leading foaming agent, thickener, plasticizer, emulsifier, moisture retainer, texture improver and binding agent. Gelatin is obtained from collagen derivatives in the skin and extracting gelatin from animal bones [1]. Halal verification techniques on

gelatin products are very important due to the high demand from the food industry for halal certification applications that meet Halal standards and Halal integrity. However, until now, there has not been any research done to see the potential of microorganisms in determining the existence of pork gelatin in food. The isolation of microorganisms that can hydrolyze pig gelatin is not impossible because of its ability to produce gelatinase. The authentication of materials based on DNA techniques has been detailed through scientific studies [2]. After that, DNA detection methods were further developed through PCR (Polymerase chain reaction) amplification of the mitochondrial b-cytochrome gene and 12 rDNA in food which has been successfully documented [3,4,5,6] and then further developed with the method of determining meat species using the same analysis [7]. However, in this study, we suggest the use of specific species of gelatinase bacteria that are able to detect the presence of pig gelatin in gelatin agar. Gelatinase bacteria from various sources, whether soil or animal skin, that have the potential to react specifically to pork gelatin will be scanned in their entirety and isolated.

2.0 Methodology

Bacterial enrichment was performed to obtain a total count for the tested samples. For this purpose, 10 types of soil, 3 animal skins and 1 water sample were used as study samples where gelatinase bacteria could be found, which would then be totaled. All tests were performed in serial dilutions for bacterial isolation on minimum agar plates mixed with 1,3 and 5% porcine gelatin. The summation method also involves plate count agar (PCA) to detect the presence of aerobic bacterial colonies. Preparation of inorganic starch salt media agar [8] was also prepared to detect the possible presence of actinomycete bacteria. All media were adjusted to pH 7.2 and autoclaved for 15 minutes for sterilization. Finally, trypticase soy agar (TSA) plates were prepared at pH 5.5 and incubated at 50°C for 24 hours for the purpose of isolating thermophilic and acidophilic bacteria.

This extracellular gelatinase activity is identified through the color difference due to the decomposition of gelatin [9], after

the surface of the agar plate is flooded with mercury chloride reagent [10] or trichloroacetic acid solution, TCA [11]. Selected colonies will be isolated through the subculture method, where the working culture is prepared in slan agar, while the main stock is stored for a long time in 20% glycerol at a temperature of -200°C.

3.0 Results and Discussion

Gelatin hydrolysis proves that some microorganisms are able to produce an extracellular enzyme called gelatinase that can act to degrade this protein into amino acids. In scanning 14 samples from inoculation of various mediums, there were 10 best gelatinase bacteria produced in this gelatin decomposition process, where some could produce decomposition enzymes as early as 3 hours after incubation. In the species-specific gelatinase determination test for pig gelatin, it was found that most of the positive results obtained were on growth medium mixed with low peptone (aerobic low peptone ALP) [12] at a concentration of 3% w/w gelatin mixed media. due to its ability to provide a very clear visual halo zone around the colony to distinguish gelatinase positive or negative.

In addition, the composition of this mixture is also seen to reduce the active movement of the colony which can interfere with the test results. In the meantime, the presence of actinomycetes was seen to give a too strong response in hydrolyzing gelatin media, without showing any difference in terms of color density so that its specificity to pig gelatin became difficult to detect. The count of the presence of microorganisms is shown graphically in Figure 1 which gives the highest count for colonies obtained from pig soil and cow skin which is 1.0 x 106 cfu/ml on 3 % gelatin media, while the lowest value was recorded at 2.0 x 105cfu/ml for wet soil and pig habitat soil. Several types of soil have been tested for sampling as shown in Figure 1.

The morphological state of bacterial cells that have hydrolyzed gelatin is shown in Figure 2 and Table 1. Hydrolyzed gelatin is one of the six amino acids that comprise collagen, despite being an incomplete protein. It's unclear how collagen would

work to provide healthy connective tissue, bones, and skin without it. The benefits of hydrolyzed gelatin are confined to its user-friendliness, rather than significant modifications to its composition.

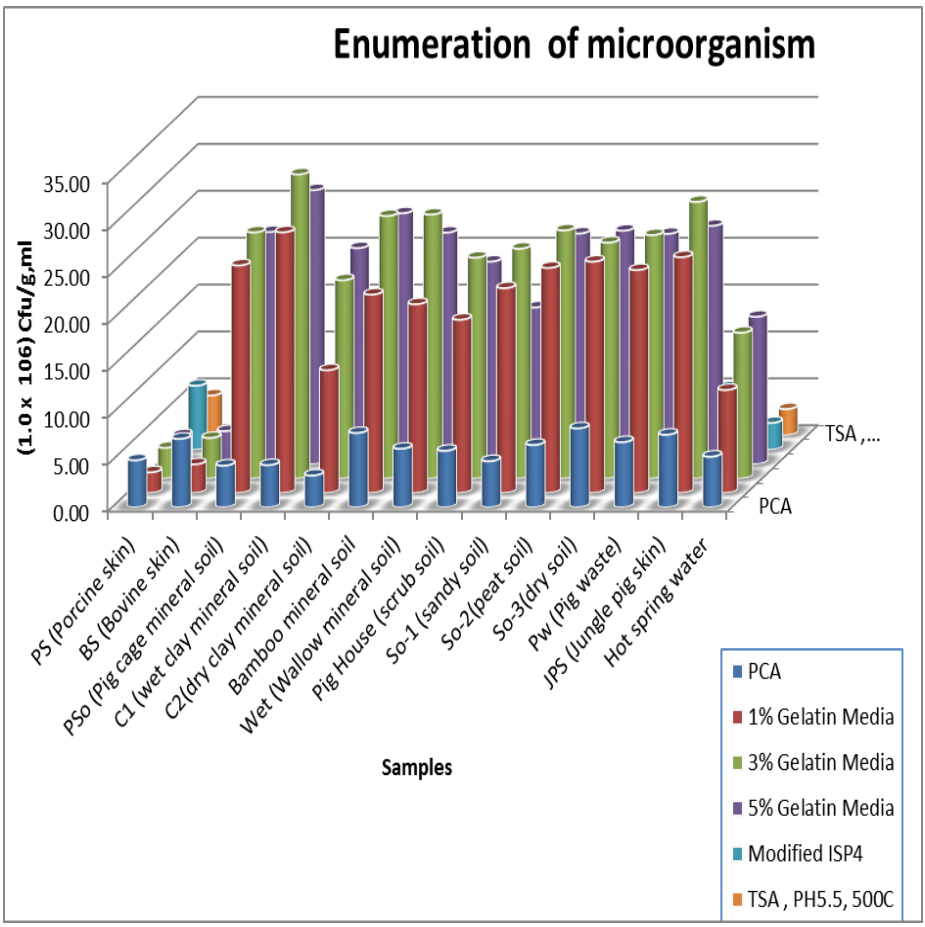

Figure 1: Count of microorganisms in the sample

Gelatinase enzyme genes are not present in every species of bacteria. Gelatinase can only be produced by bacteria that carry the gelatinase gene, which allows them to break down gelatin and use it as a source of nutrients. Since bacteria may be distinguished between those that hydrolyze and those that do not, the gelatin hydrolysis test is helpful in distinguishing and classifying different types of bacteria. The purpose of the gelatin hydrolysis test is to ascertain whether a certain bacterial species hydrolyzes gelatin, which indicates that it

possesses the gelatinase gene and generates the gelatinase enzyme.

(a) (b)

Figure 2: Gelatinase activity (a) clear zone (b) mild zone formed around the bacterial sample isolated on a gelatin agar plate

Table 1: Shows the bacterial strains that were isolated based on the sample source from which they were found and their morphological state

Strain	Sample source	Culture Morphology				
		Growth	Shape	Elevation surface	Cell volume	Pigment
G1	hot water	slow	round	flat	very low	white
G2	hot water	mediu m	round	convex	height	dark white
G3	hot water	fast	round	convex	height	yellow
G4	clay	slow	wide spot	Flat	very low	faded white
G5	clay	slow	spreading branched	convex	medium	dark brown
G6	common ground	mediu m	spreading branched	Flat	medium	brown
G7	hot water	fast	spreading branched	convex	big	white
G8	pig habitat	mediu m	uneven	convex	height	orange
G9	hot water	fast	uneven	convex	big	faded white
G10	pig land	slow	uneven	flat	very low	faded white

The results of this test show the potential of microorganisms in detecting pork gelatin through a certain scale (strong/weak), which can then show the sensitivity of these bacteria in Halal verification. In the global food business, halal certification holds significant value. It resolves worries about food safety, hygiene, and quality in addition to guaranteeing adherence to Islamic dietary requirements.

4.0 Conclusion

This study shows that gelatinase bacteria obtained from various areas can be isolated through the activity of bacteria that appear on the surface of gelatin agar, this in turn helps researchers develop a tool that can detect Halal verification and then obtained Halal certification to assure conformity with Islamic dietary restrictions.

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Author Contributions

M.F. Lamri: Original idea of study, writing mentor; **S. A. Mutalib:** Original draft Preparation, paper guider and validation. **M. A. Ghani:** Data curation, validation, supervision; **A. Abdullah:** Validation, writing-reviewing and editing.

Conflicts of Interest

The manuscript has not been published elsewhere and is not under consideration by other journals. All authors have approved the review, agree with its submission and declare no conflict of interest on the manuscript.

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