International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

Advanced in the Production of L-Ascorbic Acid using Biotechnological Processes

Samar Wagih Abdel-Salam [1,*], Mohamed Helmy Abdel-Aziz [1], Mohamed Abdel-Razik [1], Zakaria Ahmed Mattar [2], Amany Badr-Eldeen Abdel-Aziz [2] and Rabab Refaat Makharitta [1]

[1] *Suez Canal University, Faculty of Science, Botany and Microbiology Department, Ismailia 41522, Egypt.*
[2] *National Centre of Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt.*

International Journal of Science and Research Archive, 2024, 13(02), 546–556

Publication history: Received on 28 September 2024; revised on 03 November 2024; accepted on 06 November 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.13.2.2147

Abstract

Over the past decade there has been increasing demand to develop alternatives to the Reichstein process, a largely chemical synthesis by which the majority of vitamin C (L-ascorbic acid) is produced. Many studies improve the biotransformation of Reichstein intermediate, but no natural bacterial strain is capable of catalyzing the biosynthesis of intermediates in single step fermentation. The study aimed to Isolate and identify bacterial strains have the ability to produce Ascorbic acid and detect their ability to be cultivated on plant wastes as substrate. In this study a total of Seventy-five different bacterial colonies of acetic acid bacteria were isolated from eight different samples. The most potent isolate was identified by 16S rRNA and phylogenetic tree relationship showed that the isolated strain was novel and named *Gluconobacter oxydans st SW* with accession number OP429626. The optimal levels of different nutritional and cultural variables were reached. The data revealed that the yield of ascorbic acid was increased from 7.17 g/l to 10.348 g/l. The effect of low doses of gamma radiation was tested. For *Gluconobacter oxydans st. SW,* the highest ascorbic acid yield was obtained was 20.480 g/l at activation radiation dose 1.2 kGy compared with 10.34 g/l from the parent isolate at the optimum fermentation conditions. It was observed that the radiated *Gluconobacter oxydans st SW* can adapt to the waste's hydrolysate more than the un-radiated one. The highest yield of ascorbic acid (21.2 g/l) was obtained from fermentation broth containing 70% supplemented hydrolsate and 30% synthetic fermentation broth.

Keywords: Vitamin C; Sorbitol; Acetic acid bacteria; Gamma radiation; Co-culture

1. Introduction

Vitamin C, called L-ascorbic acid (L-AA), holds significant importance for humans and other mammals due to their inability to synthesize it endogenously [1]. Since its discovery and isolation, L-AA has been widely utilized in diverse industries such as medicines, food and beverage, cosmetics, and feed [2]. L-AA prevents human scurvy and is crucial in detoxifying and removing free radicals [3]. Additionally, it may facilitate the delivery of active substances to the body [4].

During the pandemic, an escalated desire for vitamin C supplements was observed. The potential capability of vitamin C supplements to inhibit viruses such as herpes simplex influenza and poliovirus [5] made them a viable option for addressing the minor symptoms of COVID-19 [6].

Currently, three process technologies are used to produce vitamin C: the Reichstein process, two-step fermentation, and one-step fermentation [7]. The chemical synthesis known as the Reichstein process dominates the current L-AA production. This process included one microbial biotransformation step and six chemical synthesis steps [8]. This process has been used for over 50 years, and further productivity improvement is difficult. The process is energy-intensive, requires hazardous conditions, and poses challenges with waste disposal [9]. Therefore, processes involving

* Corresponding author: Samar Wagih Abdel-Salam

microbial biotransformation, which produce microbiologically derived L-AA, may be deemed natural and attractive due to its lower cost and much fewer ecological problems [10].

Acetic acid bacteria (AAB) known as the oxidative bacteria, have great biotechnological importance as they are able to oxidize a wide range of sugars, sugar alcohols, and sugar acids. This biotransformation is very useful for medicine and food industry. The researchers studied various microorganisms, such as bacteria, yeast, and filamentous fungi, to determine their capacity to produce vitamin C. They used different substances, such as D-sorbitol, L-sorbose, L-sorbose, or D-glucose, as substrates. The microorganisms could produce either vitamin C or its precursor, 2-ketogulonic acid. This information is based on studies conducted by [11]. Further, the production and optimization of L-ascorbic acid by fermentation method were investigated using optimized nutritional and cultural conditions. Conventional methods of mutagenesis, like ultraviolet and gamma irradiation, have been applied to breed DHA-overproducing strains [12].

By gamma radiation, a mutant strain could be obtained that could tolerate a high concentration of substrate and perform excellent production capacity Gamma mutation in irradiated bacterial strains was evaluated based on the high biomass yield and increasing substrate transformation rate [13].

The technological process is influenced by the amount of the starting inoculum, medium composition, and biosynthesis conditions (pH and temperature) [11].

These economic factors gave rise to tremendous interest in using alternative processes. As a result, microbiological biotransformation using reasonable raw materials got the focus of particular attention. The modern fermentation processes, as well as cell-free bio-catalysis systems combined with recent innovations in biochemistry and molecular biology, have raised the chances of the development of a succeeding process.

Lignocellulose is the earth's most abundant and least expensive biomass [14]. Egypt produces more than thirty-five million tons of agricultural waste annually. However, only 12% of this amount is recycled, while millions of other tons are disposed of by burning or dumping them in canals and drains. The process of chemical production from lignocellulosic biomass includes three significant steps: pretreatment, hydrolysis, and fermentation [15].

Radiation-induced degradation of agricultural wastes, such breakdown enhances the production yields of glucose and other sugar alcohols, such as sorbitol, upon subsequent enzymatic hydrolysis or acid hydrolysis [16].

2. Materials and Methods

2.1. Preparation of samples

Eight sources including flowers (apple and pear) collected from farm near Fayoum governorate, fruits purchased from the local market (apple, pear, dry date and fig), rose and Honey were used in this study. Ten grams of each sample were homogenized in sterile 0.85% sodium chloride solution. Series of serial dilutions up to 10^{-6} were carried out. Final dilutions (10^{-5} and 10^{-6}) diluted with saline solution for each source were inoculated in enrichment broth medium. Incubation was carried out at 30 °C for one week. After1, 3, 5 days of incubation, one milliliter of the two dilutions of enrichment broth was streaked on plates containing specific media for acetic acid bacteria.

Media used for isolation of acetic acid bacteria: Three types of specific media were used for isolation of Acetic acid bacteria Carr medium**,** CAAR medium [17] and Potato agar medium [18].The plates were in triplicates and incubated for 3-5 days at 30 °C. The colonies with clear zone and yellow color (indicate acid production) were selected as Acetic Acid bacteria.

2.2. Screening of Ascorbic Acid production in fermentation broth by 2,4-Dinitrophenylhydrazine

One standard *Gluconobacter* strain (*Gluconobacter oxydans* 3024) was obtained from Cairo MERCIN, Faculty of Agriculture, Ain shams University, Cairo, Egypt. This method was described by Khan *et al.*, 2006 [19] to determine total ascorbic acid content in the sample by determination of both forms (ascorbic acid and oxidized form, dehydroascorbic acid) using bromine water to oxidize ascorbic acid to dehydroascorbic acid which employ coupling reaction with 2,4-Dinitrophenylhydrazine.The color produced was measured spectrophotometrically at 540 nm.

2.3. Identification of the selected Acetic Acid bacteria isolates

The isolates showed high ascorbic acid production were identified by VITEC test and most potent sample confirmed by 16s rRNA sequencing.

2.4. Metabolic optimization for enhancement of Ascorbic Acid production by *Gluconobacter oxydans*

Optimization of bacterial growth and fermentation conditions were occurred by one factor at a time (OFAT) by varying one factor and other factors kept constant [20]. Different nutritional and physiological parameters were tested as Incubation temperature (ranged from 28, 30 and 35°C), different incubation periods (24, 48 and 72 hours), initial pH of the fermentation medium (adjusted with 0.1 N NaOH or 0.1 N HCl to different pH degrees ranging from 4.5 to 8.5,). The effect of inoculum size studied by inoculating the fermentation medium flasks by different volumes (1, 3, 5, 7 ml) of seed broth culture (OD_{600} = 0.5). Inoculum age of seed culture with different ages (24, 48 and72 hours) of the tested bacteria was studied. Effect of different carbon sources [20] was studied by replacing sorbitol by different carbon sources (glucose, sucrose, lactose and mannitol). All carbon sources were used at 8% concentration (w/v). The effect of supplementation by nitrogen sources (organic as beef extract and peptone at 1% concentration and inorganic as sodium nitrate and potassium dihydrogen orthophosphate) on L-ascorbic acid production was studied. Aeration rate was studied by varying the volume of the medium in the flasks. Three levels of medium volumes were used to stimulate different aeration conditions (50, 100 and 150 ml) in 250 ml flasks. Each experimental factor occurred in triplicates, all other factors kept at optimum level.

- **Biological improvement of L-ascorbic acid production by combination between *Gluconobacter oxydans* and *Bacillus subtilis* in fermentation broth:** Co-culture fermentation system composed of *Gluconobacter oxydans* and *Bacillus subtilis* was carried out by serial dilution method [21]. *Bacillus subtilis* was inoculated in companion broth medium which contained (g/l): urea 1, glucose 2, and yeast 5. The inoculated broth was incubated at 30 °C for 24 hours. After the end of incubation period, series of serial dilutions (from 10^{-1} to 10^{-4}) were carried out to broth medium (B) using sterile distilled water, then mixed with seed culture inoculated by *Gluconobacter oxydans* in equal volume (1.5 ml from each one) and cultured for another 18 hours, then 3 ml of the mixture of *Gluconobacter oxydans* and *Bacillus subtilis* culture inoculated in the fermentation broth medium flasks (50 ml) [11].
- **Physical improvement by Gamma irradiation**: The average dose rate of gamma radiation source was 1 kGy/60 min at the time of the experiment which used for giving the activation doses for the tested isolates. Seed broth medium tubes (5 ml) were inoculated with the tested strains (*Gluconobacter oxydans*, and positive control sample) and incubated for 24 hours. The tubes (three replicates) were subjected to various low gamma doses (0.1, 0.3, 0.5, 0.7, 0.9, 1.2 and 1.5 kGy), then inoculated in fermentation broth medium and incubated at 30°C for 48 hours.

2.5. Fermentation with hemi-cellulosic hydrolysate from plant wastes for l-ascorbic acid production

Agricultural wastes for different fruits were collected from Botany department garden- Faculty of science – Suez Canal University. Leaves of five plants were collected including apricot *(Prunus armeniaca*), Guava (*Psidium guajava*), Pelargonium, Rose (*Rosa damascena)* and Mango (*Mangifera indica).*

2.6. Direct production of sorbitol from agricultural wastes cellulosic materials

Wastes samples were exposed to some chemical and physical hydrolytic treatments. Sorbitol content in the resulting hydrolysate was determined. Heat treatment carried out in autoclave at high temperature and pressure for 30min using water in ratio 10ml/g of solid wastes [22]. Alkali treatment for wastes samples was occurred with 30% of 4% NaOH, thereafter the material was hydrolyzed in an autoclave at 130 °C for 30 min and then filtered with muslin filtration. The pH was adjusted to 7 with 0.1N HCl. Dilute acid treatment by treating samples(water / waste ratio 10 ml/1g) with H_2SO_4 (0.7% w/v) and autoclaved at 121 °C for 30 minutes. The hydrolysate was then filtered. The hydrolysates obtained from the different treatments were concentrated at 70 °C to increase the initial sorbitol content.

Determination of sorbitol in wastes hydrolysate: Sorbitol was determined by colorimetric method described by **Tomasskovics *et al,* 2014** to select the agricultural waste with high sorbitol content under the appropriate treatment [23].

- **Gamma radiation treatment for waste sample:** After sorbitol assessment in all collected waste samples, mango leaves founded as the highest sample in sorbitol content and selected for further gamma radiation treatment. Samples are air-dried for 3 days. Air-dried mango leaves were subjected to three different high doses of gamma radiation (25, 50 and 100 kGy). Radiated samples were directly hydrolyzed by dilute acid and sorbitol content was estimated [24].

- **Bacterial strain adaptation for fermentation with wastes hydrolysate:** Un-irradiated *Gluconobacter oxydans st SW* strain, gamma radiated *Gluconobacter oxydans st SW* were adapted for fermentation with hydrolysate from radiated and un-radiated waste sample with the highest sorbitol content as substrate. The hydrolysate was adjusted to desired initial sorbitol level, then supplemented with the other ingredients of fermentation broth medium. Seed broth of bacterial strains were inoculated in flasks containing 50 ml of fermentation broth which composed of supplemented hydrolysate and synthetic broth medium in different proportions for more adaptation of bacterial strain to grow on waste hydrolysate.

2.7. Statistical analysis

All experiments were repeated independently three times. Data are expressed as mean values with standard error of derivations (± SE). Data were analyzed using t-test to assess significance ($P< 0.05$) using Microsoft Excel and GraphPad Prism 8. Software.

3. Results

3.1. Screening of ascorbic acid production

Tweenty-four *Gluconobacter sp.* isolates were tested for their ability to produce ascorbic acid. Quantitative screening by 2, 4-Dinitrophenylhydrazine data recorded in **Table (1)** showed produced ascorbic acid concentration ranged between (0.743 g/l) and (7.37 g/l) in fermentation broth after 48 hours of incubation. The isolate P.AF.4 showed the highest ascorbic acid concentration (7.37 g/l) which was highly more than produced by control sample (1.2 g/l).

Table 1 Quantitative screening of Ascorbic acid concentration in g/l determined by 2, 4-Dinitrophenylhydrazine

Isolate code	Ascorbic acid produced in g/l	
	24 hours	**48 hours**
Control	0.844	1.204
P.AF.9	0.707	3.197
CA.AF.7	0.556	3.499
P.AF.4	3.398	7.376
CR.AF.6	1.355	4.197
CA.Pch.3	2.477	3.197
P.PF.10	1.513	3.096
CR.AF.7	1.197	2.168
CA.AF.4	2.894	5.225
P.A.6	2.736	3.204
P.A.8	2.096	2.635
CA.AF.6	3.499	5.398
Cr.PF.2	1.643	2.398
CA.AF.5	2.693	3.643
CA.AF.8	3.398	4.139
P.H.3	3.707	5.794
P.PF.2	0.146	2.484
CA.AF.9	2.585	4.319
CR.PF.4	3.002	4.333
P.AF.8	0.844	0.909

P.A.7	0.276	1.959
P.FA.6	4.499	5.233
CR.P.4	0.096	3.197
P.A.2	0.736	0.743
P.PF.2	0.348	0.751

P: Potato agar; AF: Apple flower; A: Apple fruit; CA: CAAR medium; PF: Pear flower; P: Pear fruit; CR: Caar medium; H: Honey; Pch: Peach

Phenotypic identification was confirmed by molecular identification of the most potent isolate using 16S rRNA and gene sequencing. Phylogenetic tree was constructed to obtain the relationship between the isolate and described reference acetic acid strain **Fig. (1).** Evolutionary analyses were conducted in MEGA X. The phylogenetic tree indicated the isolate has similarity (S ≥ 94%) with the strains found in GenBank. The sequence was deposited in GeBank with accession number (OP429626) and named *Gluconobacter oxydans strain SW (OP429626).*

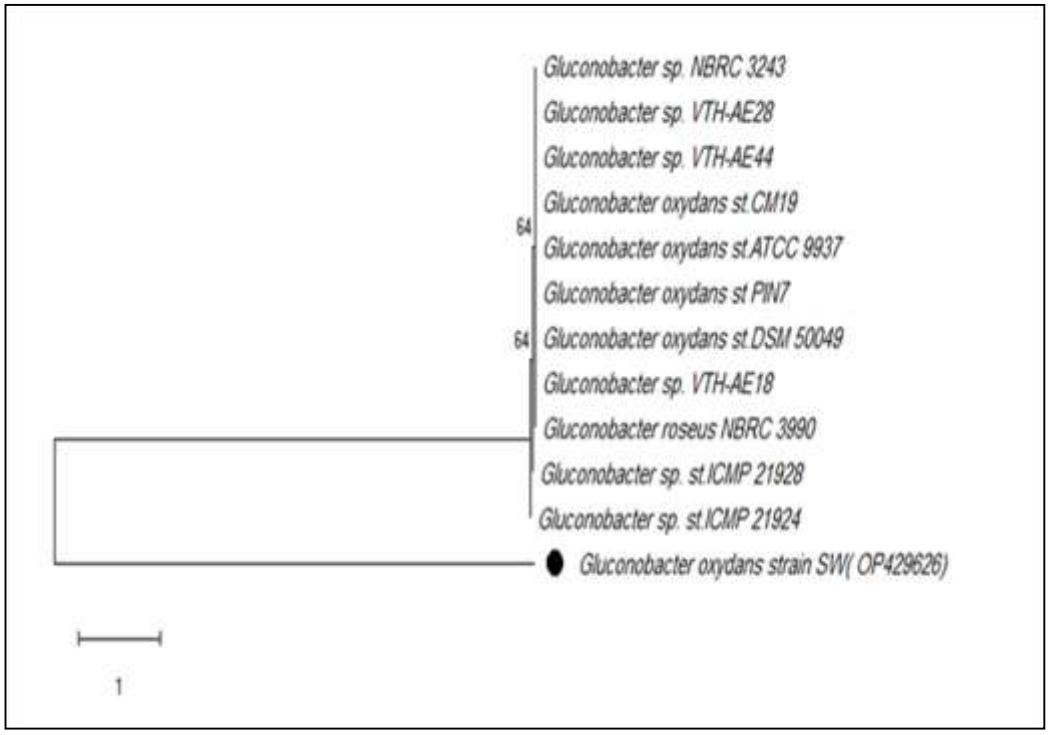

Figure 1 Molecular phylogenetic relationships between bacterial 16S rRNA gene sequences from isolates and close related (S ≥ 94%) sequences in the GenBank database. Using the Neighbor-joining method

3.2. Metabolic optimization for enhancement of Ascorbic Acid production by *Gluconobacter oxydans st SW*

Cultural parameters for L-ascorbic acid production were studied. In determining the optimum incubation temperature, the maximum yield of ascorbic acid concentration was revealed at 30±1 °C as the optimum incubation temperature (7.17 g/l).

Effect of incubation period on selected isolate productivity was studied. The concentration of ascorbic acid increased by increasing incubation period until reach the optimum at 48 hours (7.51 g/l) and then decreased gradually at 72 hours of incubation (4.95 g/l) at the optimum temperature**.**

Influence of initial pH was studied. The optimum initial pH value was 7.5 for tested strain with maximum ascorbic acid production (7.887 g/l)**.**

Inoculum size is also has effect on L-ascorbic acid production**.** The results showed that the ascorbic acid concentration produced by *Gluconobacter oxydans st SW* increased by increasing the inoculum size and reach the optimum (8.096 g/l) at inoculum size 5 ml/50 ml, but the lowest concentration was (1.398 g/l) at inoculum size 1 ml/50 ml.

Various inoculum ages of seed cultures were inoculated in flasks containing 50 ml of fermentation broth and incubated according to optimum conditions. The data showed that seed culture inoculum with 24 hours age was the best inoculum preparation for ascorbic acid concentration (8.254 g/l).

Different aeration levels were studied. The data revealed that the highest ascorbic acid concentration (10.348 g/l) obtained under aerobic incubation condition.

Nutritional parameters for L-ascorbic acid production were studied. The data showed that the selected bacterial isolate can grow at different carbon sources as the sole carbon source. The highest yield of ascorbic acid achieved by culturing on sorbitol as sole carbon source followed by sucrose then lactose (9.98, 6.542, 6.45 g/l) respectively. The lowest concentrations were 3.39 and 1.72 g/l from glucose and mannitol respectively**.**

The data revealed that organic nitrogen sources were more suitable than inorganic nitrogen sources to be utilized by selected strains for ascorbic acid production**.** Out of the tested organic nitrogen sources, yeast extract achieved the highest yield of ascorbic acid production (10.06 g/l)

3.3. Biological improvement by Combination between *Gluconobacter oxydans* and *Bacillus subtilis* in fermentation broth

Under the optimum nutritional and cultural conditions revealed from the previous experiments, *Gluconobacter oxydans st SW* was co-cultured with *Bacillus subtilis* sample. The yield increased from 10.348 to 14.2 g/l under the optimum fermentation conditions. It is noticed that the high dilution of *Bacillus subtilis* culture still can enhance ascorbic acid production rather than mono seed culture after 48 hours of fermentation (12.72 and 11.12 g/l from co-culture 10^{-2} and 10^{-3} verse 10.348 g/l from mono culture seed medium).Table (2)

Table 2 Time course of ascorbic acid production by *Gluconobacter oxydans st SW* and mutant strains (Co-cultured with different dilutions of *Bacillus subtilis)*

Time	**Ascorbic acid concentration (g/l)**				
	Gluconobacter oxydans st SW	**Mutant strain (Co-cultured with dilutions of *Bacillus subtilis)***			
		10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-4}**
0	0	0	0	0	0
18	0.624	0.968	0.831	0.792	0.751
24	3.4	4.301	3.596	2.261	1.160
48	10.34	14.192	12.725	11.128	3.128

3.4. Physical improvement by Gamma irradiation

The data showed that ascorbic acid concentration obtained from irradiated isolate was significantly different compared with parent isolates. For *Gluconobacter oxydans st.SW,* the highest ascorbic acid yield obtained was 20.480 g/l at activation radiation dose equal 1.2 kGy in comparison with 10.34 g/l from the parent isolate at the optimum fermentation conditions. It is noticed from the recorded data that ascorbic acid concentration and the productivity increased gradually by increasing activation dose to reach the optimum at 1.2 kGy, then the production decreased at higher gamma radiation doses. **Fig. (2)**

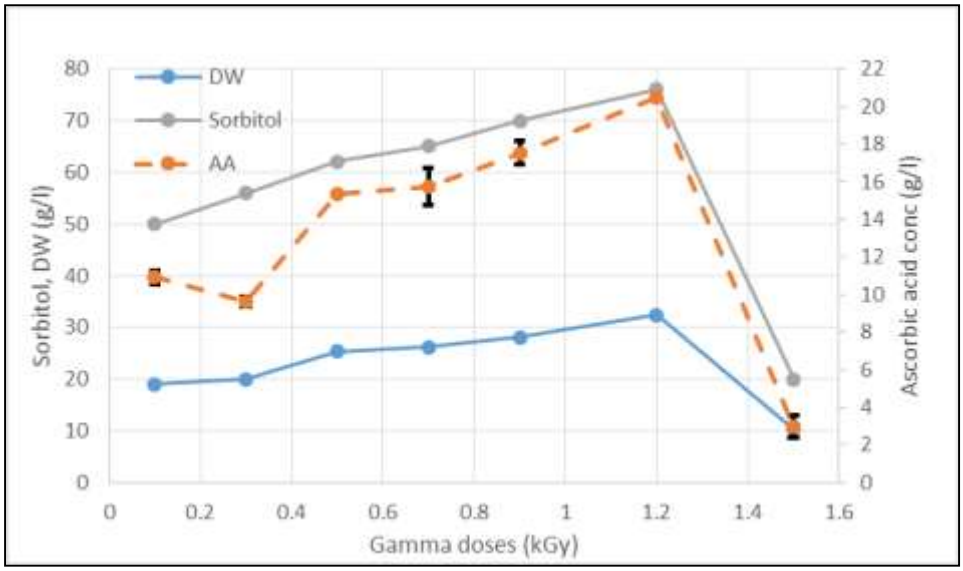

Figure 2 Effect of different gamma radiation activation doses on ascorbic acid production by *Gluconobacter oxydans st.SW*

3.5. Direct production of sorbitol from agricultural wastes cellulosic materials

Table 3 Sorbitol content in different plant wastes with physical and chemical treatment

Plant waste (100 g)	Sorbitol content (g/100 g)			
	Untreated	Treatment		
		Heat	Alkali	Dilute acid
Apricot	5.05	6.8	7.1	10.2
Guava	8.6	9.3	9.5	12.1
Pelargonium	14.2	15.6	14.8	16.3
Rose	9.5	10.2	11.4	16.2
Mango	16.57	16.8	16.72	18.79

The waste samples (100 g from each sample) were hydrolyzed to demonstrate sorbitol content in each waste sample. The results of sorbitol content in different plant wastes with and without physical and chemical treatment showed that Mango leaves have the highest sorbitol content followed by Pelargonium leaves (16.57 g and 14.2 g) respectively before treatment. Apricot leaves has the lowest sorbitol content from all selected wastes .Table (3).

3.6. Gamma radiation treatment for waste samples

Table 4 Effect of high doses of gamma radiation on sorbitol obtained from dry mango leaves

Radiation dose (kGy)	Sorbitol (g/100 g) of radiated wastes
0.00	18.79
25	21.2
50	25.3
100	30

Gamma radiation had significant effect on waste degradation as the sorbitol content derived from Mango leaves increased by increasing the radiation dose up till 30 g/100 g of radiated wastes at 100 kGy radiation dose compared to 18.79 g/100 g from un-radiated wastes **Table (4).**

3.7. Fermentation with hemi-cellulosic hydrolysate from plant wastes for l-ascorbic acid production

For un –radiated *Gluconobacter oxydans st SW* It is observed from data illustrated in **Fig.(3)** that the highest ascorbic acid concentration (10.81 g/l) obtained from fermentation broth contained 50% hydrolysate from radiated wastes and 50% synthetic broth medium followed by fermentation broth contained 30% hydrolsate from radiated wastes and 70% synthetic broth medium (10.51 g/l). It is clearly observed that *Gluconobacter oxydans st SW* lack the ability to adapt to the fermentation broth containing waste hydrolysat only as the produced ascorbic acid was the lowest (1.54 g/l).

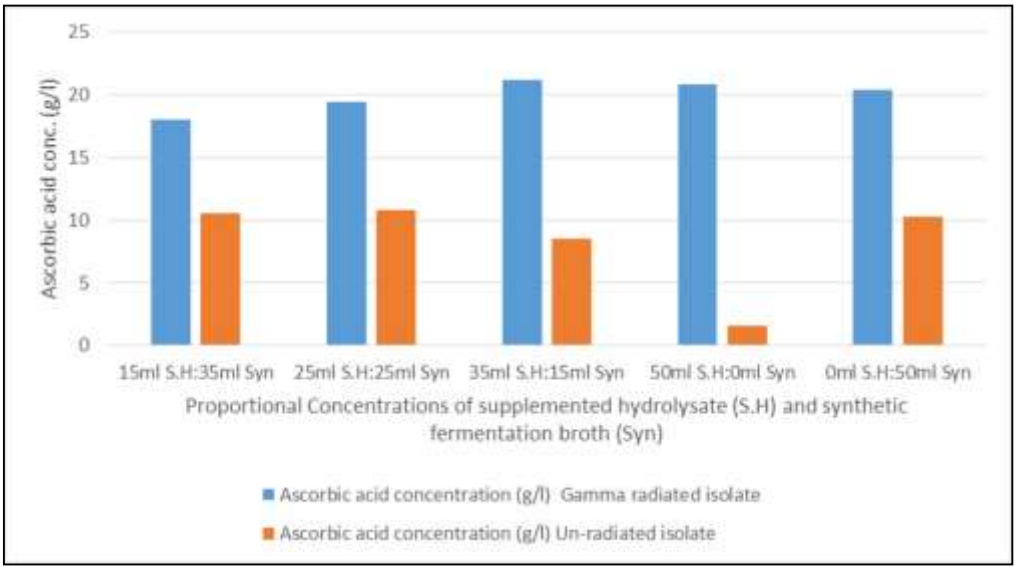

Figure 3 Ascorbic acid production by Gamma radiated and un-radiated *Gluconobacter oxydans st SW* from supplemented waste hydrolysate (S.H) and synthetic fermentation broth (Syn)

It was observed that the radiated *Gluconobacter oxydans st SW* can adapt to the wastes hydrolysate more than the un-radiated one. The highest yield of ascorbic acid (21.2 g/l) was obtained from fermentation broth containing 70% supplemented hydrolsate and 30% synthetic fermentation broth followed by yield obtained from culturing on 100% waste hydrolysate from radiated wastes (19.8 g/l).

4. Discussion

L-Ascorbic Acid (L-AA, also referred to as vitamin C) is an essential vitamin and antioxidant in humans, primates and some other animals, other mammals as they cannot synthesize this vitamin [25, 30].

In this study a total of Twenty-four *Gluconobacter sp.* isolates which were resulted from purification on Carr medium were tested for their ability to produce ascorbic acid quantitavely [31]. Results revealed that after the screening of 24 isolates, seven isolates recorded had high ascorbic acid production ranging between 5.19 to 7.23 g/l. This yield was higher than that recorded by Sugisawa *et al*., 2005 [26] as they reported *Ketogulonicigenium vulgare* DSM 4025 can produce 0.090 g/l from 8% sorbitol and 1.37 g/l from 0.5% sorbosone after 24 hours of fermentation [32].

The optimal levels of different nutritional and cultural variables were reached. The analysis of the data of the cultural variables revealed that the high yield obtained at 30 °C in neutral medium (pH 7.5) inoculated with 24 hours inoculum age after 48 hours of incubation. The yield was increased from 7.1 to 8.2 g/l. During bacterial fermentation, the carbon source acts as a building block for cellular structure as well as energy source. In our study for *Gluconobacter oxydans st SW* the highest yield of ascorbic acid achieved by culturing on sorbitol as sole carbon source followed by sucrose then lactose (9.98, 6.542, 6.45 g/l) respectively.

In studying the effect of Combination between *Gluconobacter oxydans* and *Bacillus subtilis* in fermentation broth for enhancement of ascorbic acid production, the results approve the hypothesis that combination of *Gluconobacter oxydans*

with *Bacillus subtilis* in fermentation broth as seed inoculum can increase ascorbic acid production. The yield increased from 10.34 to14.19 g/l under the optimum fermentation conditions. Fang *et al.,* (2021) suggested that *Bacillus subtilis* can provide some nutrients as serine, glycine, threonine, proline, nicotinic acid, and biotin which were key nutrients support the growth of *Gluconobacter oxydans*. In the same pattern *Gluconobacter oxydans* can stimulate the sporulation of *Bacillus subtilis* causing cell lysis and releasing of nutrients in the fermentation system [27].

Activation doses of gamma radiation can enhance ascorbic acid production by *Gluconobacter oxydans.* Our results showed that the high yield was obtained at 1.2 kGy (20.48 g/l) as the yield increased by approximately 2 folds. It was observed that there was dose- dependent decrease in ascorbic acid yield after 1.2 kGy, it was actually due to the decrease in survival rate of *Gluconobacter oxydans* at high doses. Some studies improve hypothesis that gamma irradiation mutation can activate the expression of sorbitol dehydrogenase enzyme that led to increasing the yield of products as 6-(N-hydroxyethyl)-amino-6-deoxy-α-Lsorbofuranose (Miglitol) [14].

Fermentation with hemi-cellulosic hydrolysate from plant wastes for ascorbic acid production were studied. The waste samples (100 g from each sample) were hydrolyzed to demonstrate sorbitol content in each waste sample. Sorbitol in the hydrolysate was measured. Mango leaves showed the highest sorbitol content. Through observing the different methods of treatment it was clear that the highest waste degradation and sorbitol content achieved by dilute acid hydrolysis. These results were in agreement with results reported by Lenihan *et al*., (2010) as the sugar yield was found to be the maximum by acid hydrolysis of biomass across all the variable conditions of temperature and acid concentration [28].

The effect of high gamma radiation doses on mango leaves degradation was studied. Radiation pretreatment of lignocellulosic, before acid or alkali hydrolysis, facilitated the degradation of the material and could be used as a step in the processes leading to the isolation of cellulose from biomass [29]. Our study gave evidence that gamma radiation had significant effect on waste degradation as the sorbitol content increased by increasing the radiation dose up till 30 g/100 g of radiated wastes at 100 kGy radiation dose compared to 18.79 g/100 g from un-radiated wastes.

Fortunately, radiated *Gluconobacter oxydans st SW* can adapt to the wastes hydrolysate more than the un-radiated one. The highest yield of ascorbic acid (21.2 g/l) was obtained from fermentation broth containing 70% supplemented hydrolsate and 30% synthetic fermentation broth followed by yield obtained from culturing on 100% waste hydrolysate from radiated wastes (19.8 g/l).

5. Conclusion

This study underscores the effectiveness of using plant wastes as substrate for L-ascorbic acid production by biotechnological processes. Acetic acid bacteria have metabolic potential by partial oxidation of carbohydrates releasing the corresponding products as organic acid. *Gluconobacter oxydans* shows high ability for L-ascorbic acid production using sorbitol as substrate. The observations of this study give very promising evidence that irradiation treatment for plant wastes and activation of bacterial strain has positive effect as L-ascorbic acid production increased by two folds in fermentation broth optimized by cultural and nutritional parameters. Keeping in view the economic value and nutritive potential of these plant wastes, our results give evidence that using agricultural wastes as substrates has great potential in clean biotechnological approaches due to environmentally friendly nature, easy availability, low cost and sustainability.

Compliance with ethical standards

Acknowledgments

All acknowledgments (if any) should be included at the very end of the paper before the references and may include supporting grants, presentations, and so forth.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Bechara N, Flood VM, Gunton JE. (2022) A Systematic Review on the Role of Vitamin C in Tissue Healing. Antioxidants.; 11(8):1605.

[2] Malik, M.; Narwal, V.; Pundir, C.S. (2022) Ascorbic acid biosensing methods: A review. Process. Biochem., 118, 11–23.

[3] Eggersdorfer, M., Laudert, D., Letinois, U., McClymont, T.,Medlock, J., Netscher, T., and Bonrath, W. (2012) One hundred years of vitamins—a success story of the natural sciences. Angew. Chem. Int. Ed., 51 (52), 12960–12990.

[4] Caritá, A.C.; Fonseca-Santos, B.; Shultz, J.D.; Michniak-Kohn, B.;Chorilli, M.; Leonardi, G.R. (2020) Vitamin C: One compound, several uses. Advances for delivery, efficiency and stability. Nanomed. Nanotechnol. Biol. Med., 24, 102–117.

[5] Mousavi, S.; Bereswill, S.; Heimesaat, M.M. (2019) Immunomodulatory and antimicrobial effects of vitamin C. Eur. J. Microbiol. Immunol., 9, 73–79.

[6] Hiedra, R.; Lo, K.B.; Elbashabsheh, M.; Gul, F.; Wright, R.M.; Albano, J.; Azmaiparashvili, Z.; Apontec, G.P. (2020) The use of IV vitamin C for patients with COVID-19: A case series. Expert Rev. Anti Infect. Ther., 18, 1259–1261.

[7] Zhou, M.; Bi, Y.; Ding, M.; Yuan, Y. (2021) One-Step Biosynthesis of Vitamin C in Saccharomyces cerevisiae. Front. Microbiol., 12, 643472.

[8] Mandlaa, M. (2014) *Study of Mechanism and Application of 2-Keto-L-gulonic Acid Fermentation Based on Ecological Relationships of Co-Culture*, University of Chinese Academy of Sciences, Beijing, pp. 1–5

[9] Hancock RD, Galpin JR, Viola R (2000) Biosynthesis of L-ascorbic acid (vitamin C) by Saccharomyces cerevisiae. FEMS Microbiol. Lett. 186: 245-250.

[10] Yang, W.; Xu, H. (2016) Industrial Fermentation of Vitamin C. In Industrial Biotechnology of Vitamins, Biopigments, and Antioxidants; Vandamme, E.J., Revuelta, J.L., Eds.; Wiley: Weinheim, Germany,; pp. 161–192.

[11] Yang, W., Han, L., Mandlaa, M., Chen, H., Jiang, M., Zhang, Z., and Xu, H. (2013). Spaceflight-induced enhancement of 2-keto-L-gulonic acid production by a mixed culture of Ketogulonigenium vulgare and bacillus thuringiensis. Letters in Applied Microbiology, 57(1), 54-62.

[12] Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. (2017) Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study. Chest. Jun; 151(6):1229-1238. doi: 10.1016/j.chest.2016.11.036. Epub 2016 Dec 6. PMID: 27940189.

[13] Liu, L., Pohnert, G., and Wei, D. (2016). Extracellular metabolites from industrial Microalgae and their biotechnological potential. Marine Drugs, 14(10), 191.

[14] Liu, L., Pohnert, G., and Wei, D. (2016). Extracellular metabolites from industrial Microalgae and their biotechnological potential. Marine Drugs, 14(10), 191.

[15] Yamaguchi, A., Sato, O., Mimura, N., and Shirai, M. (2016). Catalytic production of sugar alcohols from lignocellulosic biomass. *Catalysis Today*, *265*, 199-202.

[16] Tissot, C., Grdanovska, S., Barkatt, A., Silverman, J., and Al-Sheikhly, M. (2013). On the mechanisms of the radiation-induced degradation of cellulosic substances. Radiation Physics and Chemistry, 84, 185-190.

[17] Romero-Cortes, V., Robles-Olvera, G. Rodriguez, J. and Ramírez, M.(2012). Isolation and characterization of acetic acid bacteria in cocoa fermentation. African Journal of Microbiology Research, 6(2). , pp. 339-347.

[18] Kanlaya, K. and Wasu, P. (2009). Isolation of acetic acid bacteria from honey. Maejo International Journal of Science and Technology, 3(01), 71-76.

[19] Khan, F., Ahmad, S.R., and Kronfi li, E. (2006). γ-Radiation induced changes in the physical and chemical properties of lignocellulose. Biomacromolecules, 7, 2303-2309.

[20] Dnyaneshwar, V., Bhand, Anand, V., and Patwardhan, F,. (2015). Statistical optimization of L-ascorbic acid production by Xanthomonas campestris MTCC 2286 using sucrose as a low cost carbon source. Journal Biochemical Technology , 6(1): 922-928.

[21] Zhang, Y.-he, Lin, J.-ye, Bai, L., Huang, M., Chen, H.-quan, Yao, S., and Lyu, S.-xia. (2018). Antioxidant capacities of bacillus endophyticus st-1 and ketogulonicigenium vulgare 25B-1 in vitamin C fermentation. Biotechnology & Biotechnological Equipment, 32(3), 628–637.

[22] Paida, V., Kersten, S., Van der Ham, A., and Brilman, D. (2019). A two-step approach to the hydrothermal gasification of carbohydrate-rich wastes: Process design and economic evaluation. International Journal of Hydrogen Energy, 44(47), 25524-25541.

[23] Tomasskovics, B., Horváth, V., Balla, J., Örsi, F., and Szarka, A. (2014). Determination of sorbitol in the presence of high amount of mannitol from biological samples. Periodica Polytechnica Chemical Engineering, 58(1), 1

[24] Chung, B.Y., Lee, J.T., Bai, H.-W., Kim, U.-J., Bae, H.-J., Wi, S.G., and Cho, J.-Y. (2012). Enhanced enzymatic hydrolysis of poplar bark by combined use of gamma ray and dilute acid for bioethanol production. Radiat. Phys. Chem., 81, 1003-1007.

[25] Camarena, V., and Wang, G. (2016). The epigenetic role of vitamin C in health and disease. Cellular and Molecular Life Sciences, 73(8), 1645-1658.

[26] Sugisawa, T., Miyazaki, T., and Hoshino, T., (2005). Microbial production of l-ascorbic acid from d-sorbitol, l-sorbose, l-gulose, and lsorbosone by Ketogulonicigenium vulgare DSM 4025. Biosci. Biotechnol. Biochem. 69, 659–662.

[27] Fang, J., Wan, H., Zeng, W., Li, J., Chen, J., and Zhou, J. (2021). Transcriptome analysis of Gluconobacter oxydans WSH-003 exposed to elevated 2-keto-L-gulonic acid reveals the responses to osmotic and oxidative stress. Applied Biochemistry and Biotechnology, 193(1),128-141.

[28] Lenihan, P., Orozco, A., O'Neill, E., Ahmad, M., Rooney, D., and Walker, G. (2010). Dilute acid hydrolysis of lignocellulosic biomass. Chemical Engineering Journal, 156(2), 395-403.

[29] Chen, D., Liang, F., Feng, D., Du, F., Zhao, G., Liu, H., and Xian, M. (2016) A. Sustainable utilization of lignocellulose: Preparation of furan derivatives from carbohydrate biomass by bifunctional lignosulfonate-based catalysts. Catalysis Communications, 84, 159-162.

[30] Caritá, A.C.; Fonseca-Santos, B.; Shultz, J.D.; Michniak-Kohn, B.;Chorilli, M.; Leonardi, G.R. (2020) Vitamin C: One compound, several uses.Advances for delivery, efficiency and stability. Nanomed. Nanotechnol.Biol. Med., 24, 102–117.

[31] Bremus, C. Herrmann, U.; Bringer-Meyer, S.; and Sahm, H. (2006). The use of microorganisms in L-ascorbic acid production. J. Biotechnol. 124, 196–205.

[32] Supawade S, Warinda S, and Wasu P (2007). Comparison of three enrichment broths for the isolation of thermotolerant acetic acid bacteria from flowers and fruits. Research Journal of Microbiology, 2(10), 792-795.