**Could soyabean be an alternative to peptone & beef extract?**

**Title of Paper :** Could soyabean be an alternative to peptone & beef extract?

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**Abstract:** Aim of this research is to look for an alternative for Beef extract and peptone that are commonly employed in nutrient media in Microbiology and biotechnology laboratories. Focus is on growing microbes of commercial importance and microbes that serve as model systems for research like Aspergillus niger, Escherichia coli and other fungal species and strains of a few Gram negative and Gram positive bacteria. Hence an attempt was made in the present study to use soyabean as nitrogen source instead of beef-extract and peptone. Growth response of various microorganisms was recorded and tabulated.

**Keywords**: Sabaroud’s media., Bhaktivedanta Medium (1,2,3,4), Bhaktivedanta Liquid (1,2,3), Soyabean, Nutrient Agar medium, Nutrient broth.

**Introduction**: I always felt guilty, when I had to use Beef-extract or peptone for any of our Microbiology and Biotechnology experiments. No matter who in our Laboratory prepared it. I knew for sure that sometime ago, a non-violent, harmless domestic and motherly creature was slaughtered to get the Beef-extract or peptone (animal source). Hence I used to think was it not possible to prepare a purely 100% vegetarian nutrient media for micro-organisms. Such that we could grow micro-organisms without harming any other macro-creature which essentially has developed consciousness and cries when it feels pain.

**Research Question**: Is it possible to create a 100% vegetarian media for microbes without using any animal source?

Is there any research done already in this regard?

**Answer:** Already research was done and the following website gives details about vegetarian media.

<http://www.academia.edu/4980897/Optimization_of_an_effective_growth_medium_for_culturing_probiotic_bacteria_for_applications_in_strict_vegetarian_food_products>

But the organism that was cultured was Lactobacillus lactis using standard MRS culture medium. Hence I devised experiements to grow Aspergillus niger, Eschericia Coli, Fusarium, cladosporium, curvularia, on different composition of media like – Sabaroud’s media but with out peptone or beef extract , Nutrient Agar and nutrient broth using Soyabean as nitrogen source to grow the above mentioned organisms. And started the experiments in this direction.

Any nutrient media will have the following composition:

**Macro Nutrients**

* Carbon Source
* Nitrogen Source

**Micro Nutrients**

* Vitamins
* Hormones
* Minerals

**Review of Literature:**

**Hypothesis:** Most of the microbiologists use Beef-extract and peptone obtained from animal source majorly as Nitrogen source and source for other micronutrients. My logic was to use Soyabean which was very rich in Proteins (hence Nitrogen source) and its composition is depicted in the following table 1:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table1: Nutrient content of Soybean | | | | |
| STAPLE: | [Soybean](http://en.wikipedia.org/wiki/Soybean) |  | STAPLE: | [Soybean](http://en.wikipedia.org/wiki/Soybean) |
| Component (per 100g portion) | Amount |  | Component (per 100g portion) | Amount |
| Water (g) | 68 |  | [Vitamin C (mg)](http://en.wikipedia.org/wiki/Vitamin_C) | 29 |
| Energy (kJ) | 615 |  | [Thiamin (mg)](http://en.wikipedia.org/wiki/Thiamin) | 0.44 |
| [Protein (g)](http://en.wikipedia.org/wiki/Protein_(nutrient)) | 13 |  | [Riboflavin (mg)](http://en.wikipedia.org/wiki/Riboflavin) | 0.18 |
| [Fat (g)](http://en.wikipedia.org/wiki/Fat) | 6.8 |  | [Niacin (mg)](http://en.wikipedia.org/wiki/Niacin) | 1.65 |
| [Carbohydrates (g)](http://en.wikipedia.org/wiki/Carbohydrate) | 11 |  | [Pantothenic acid (mg)](http://en.wikipedia.org/wiki/Pantothenic_acid) | 0.15 |
| [Fiber (g)](http://en.wikipedia.org/wiki/Dietary_fiber) | 4.2 |  | [Vitamin B6 (mg)](http://en.wikipedia.org/wiki/Vitamin_B6) | 0.07 |
| [Sugar (g)](http://en.wikipedia.org/wiki/Sugar) | 0 |  | [Folate Total (μg)](http://en.wikipedia.org/wiki/Folate) | 165 |
| [Calcium (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 197 |  | [Vitamin A (IU)](http://en.wikipedia.org/wiki/Vitamin_A) | 180 |
| [Iron (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 3.55 |  | [Vitamin E, alpha-tocopherol (mg)](http://en.wikipedia.org/wiki/Vitamin_E) | 0 |
| [Magnesium (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 65 |  | [Vitamin K1 (μg)](http://en.wikipedia.org/wiki/Vitamin_K1) | 0 |
| [Phosphorus (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 194 |  | [Beta-carotene (μg)](http://en.wikipedia.org/wiki/Beta-carotene) | 0 |
| [Potassium (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 620 |  | Lutein+zeaxanthin (μg) | 0 |
| [Sodium (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 15 |  | [Saturated fatty acids (g)](http://en.wikipedia.org/wiki/Saturated_fat) | 0.79 |
| [Zinc (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 0.99 |  | [Monounsaturated fatty acids (g)](http://en.wikipedia.org/wiki/Monounsaturated_fat) | 1.28 |
| [Copper (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 0.13 |  | [Polyunsaturated fatty acids (g)](http://en.wikipedia.org/wiki/Polyunsaturated_fat) | 3.2 |
| [Manganese (mg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 0.55 |  |  |  |
| [Selenium (μg)](http://en.wikipedia.org/wiki/Dietary_mineral) | 1.5 |  |  |  |

http://en.wikipedia.org/wiki/Soybean

**Materials & Methods:**

**Name of all organisms:** Aspergillus niger, Eschericia Coli, Fusarium, Cladosporium, Curvularia, Trichoderma, Pseudomonas, Staphyllococcus.

**Name of all media components:** Dextrose, Soyabean, Agar, Lactose, Sucrose, Sodium chloride, Distilled water.

**Media Composition:  
(Table 2)**

|  |
| --- |
| **Bhakti-Vedanta Medium 1:**  (Sabaroud’s media without peptone)  Dextrose – 40g  Soyabean(meal maker –crushed)-10g  Agar – 15g  Distilled water- 1000ml  pH : 6.5 |
| **Bhakti-Vedanta Medium 2:**  (Sabaroud’s media without peptone & using sucrose instead of Dextrose)  Sucrose – 10g  Soyabean(meal maker –crushed)-2.5g  Agar – 3.1g  Distilled water- 250ml  pH : 6.5 |
| **Bhakti-Vedanta Medium 3: (for growing E.coli)**  Lactose -5g/L  Soyabean(meal maker –crushed)-8g/L  Agar – 15g  Distilled water- 1000ml  pH : 6.7 |
| **Bhakti-Vedanta Medium 4: (for growing Pseudomonas)**  Soyabean(meal maker –crushed)-6g/L  NaCl-5g/L  Agar – 15g/L  Distilled water- 1000ml  pH : 7.0 |
| **Bhakti-Vedanta liquid 1**  (Sabaroud’s media without peptone – Liquid medium)  Dextrose – 10g  Soyabean(meal maker –crushed)-2.5g  Distilled water- 250ml  pH : 6.5 |
| **Bhakti-Vedanta liquid 2**  Soyabean(meal maker –crushed)-7.5g  Distilled water- 200ml  pH : 6.5 |

**Description:** All the ingredients of the nutrient medium were weighed according to the proportion as mentioned above for respective medium. Dry Soyabean chunks availiable in local market was crushed and made in powder form and weighed. All components were mixed with required amount of distilled water. The pH levels of the medium was adjusted. Agar was added after desired pH level was achieved only for preparing the solid media.

**Experiment protocol:** The respective medium was weighed according to the composition of the medium mentioned in the above table. And thoroughly washed & cotton plugged conical flask and wrapped petri-plates were autoclaved along with media conical flasks. After autoclaving the media was poured in respective pertri-plates and conical flask. The respective micro-organism was inoculated from already available stock cultures in laboratory. The fungal cultures were inoculated at room temperature and bacterial cultures were incubated in a incubator. Cultures were observed at periodic intervals and their growth was recorded and tabulated. Similar set of experiments were repeated 3 times.

**Pictorical representation of Experiment protocol:**

(Table 3)

|  |  |
| --- | --- |
| **Picture-1** |  |
| **Picture -2** |  |
| **Picture -3** |  |
| **Picture -4** |  |
| **Picture -5** |  |
| **Picture -6** |  |

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**(Table 4: Observation of bacterial and fungal colonies):**

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| --- | --- |
|  | **Picture -7: Cloudy appearance of Aspergillus niger growth on second day after inoculation.** |
|  | **Picture -8: Appearnace of Fusarium on second day after inoculation.** |
|  | **Picture -9: Fusarium growth on third day after inoculation** on Bhakti-Vedanta medium1 |
|  | **Picture -10:** Fusarium growth on Bhakti-Vedanta medium1 in with A. niger after 10 days of growth |
|  | **Picture -11:** Appearance of Gram positive bacterial colony on second day after inoculation which was contaminated by Aspergillus niger (Rear view) |
|  | **Picture -12:** Appearance of Gram positive bacterial colony on second day after inoculation which was contaminated by Aspergillus niger(Front view). |
|  | **Picture -13:** Cladosporium colony contaminated by Aspergillus niger as observed on second day after inoculation. |
|  | **Picture -14:** Colonies of Cladosporium contaminated by A. niger after 7days. |
|  | **Picture -15:** Cloudy appearance of Aspergillus niger after 48hours in Bhaktivedanta liquid2. |
|  | **Picture -16:** Growth of Aspergillus niger after 72hours in Bhaktivedanta liquid2. |
|  | **Picture -17:** Growth of Aspergillus niger after 12 days in Bhaktivedanta liquid2. |
|  | **Picture -18:** Growth of Gram negative bacilli colonies on Bhaktivedanta Medium 1 after 1day of inoculation. |
|  | **Picture -19:** Growth of Gram negative bacilli on Bhaktivedanta medium (2 days after inoculation). |
|  | **Picture -20:** Growth of Gram negative bacilli on Bhaktivedanta medium (3 days after inoculation) |
|  | **Picture -21:** Gram negative bacilli under 100x microscope grown on Bhaktivedanta medium1. |
|  | **Picture -22:** Mild growth of pseudomonas colonies on BhaktiVedanta medium 4 (2 days after inoculation). |
|  | **Picture -23:** Pseudomonas colonies after 5 days from inoculation in a conical flask |
|  | **Picture -24:** Pseudomonas colonies after 5 days of inoculation in a slant test tube |
|  | **Picture -25:** Trichoderma growth after 6 days of incubation at room temperature. |
|  | **Picture -26:** Escherichia coli colony growing on Bhaktivedanta medium 2 with sucrose as carbon source. |
|  | **Picture -27:** Escherichia coli colony growing on Bhaktivedanta medium4 growing in a slant test tube after 5 days of incubation. |
|  | **Picture -28:** Gram positive bacilli growing in a slant test tube on Bhaktivedanta medium4 after 5 days of incubation. |
|  | **Picture -29:** Slants of Eschericia coli, pseudomonas, staphylococcus and Gram positive Bacillig grown on Bhaktivedanta medium 4. |
|  | **Picture -30:** Pseudomonas colony grown on Bhaktivedanta medium 4 after 5 days of incubation. |
|  | **Picture -31:** Eschericia coli grown on Bhaktivedanta medium 3 after 5 days of incubation |
|  | **Picture-32:** Gram positive bacilli spores under 97x microscope. |
|  | **Picture-33:** Escherichia coli under 97x microscope grown on Bhaktivedanta medium. |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1000ml Conical flask** | **500ml conical flask** | **250ml conical flask** | **250ml conical flask** | **Petriplate** | **Petriplate** | **Petriplate** | **Petriplate** |
| **Volume** | **400ml** | **180ml** | **150ml** | **150ml** | **30ml** | **30ml** | **30ml** | **30ml** |
| **Day** | **A. Niger with Bhaktivedanta Media 1** | **A. Niger with Bhaktivedanta Media 1** | **A. Niger Bhaktivedanta Media 1** | **Fusarium Bhaktivedanta Media 1** | **Fusarium Bhaktivedanta Media 1** | **Cladosporium Bhaktivedanta Media 1** | **Gram positive bacilli** | **Gram positive bacilli** |
| **1** | - | - | - | - | - | - | - | - |
| **2** | cloudy white appearance around black spores | cloudy white appearance around black spores | cloudy white appearance around black spores | No apparent colonies | No apparent colonies | No apparent colonies | No apparent colonies | No apparent colonies |
| **3** | A. Niger colonies prominently visible on the Surface of solid media | A. Niger colonies prominently visible on the Surface of solid media | A. Niger colonies prominently visible on the Surface of solid media | White colony appearance towards the periphery | Apparent growth but there is contamination | a pie shaped white growth towards the periphery | Growth of bacterial Colony with | A Niger colonies predominantly apparent |
| **4** | Profuse growth of A.Niger | Profuse growth of A.Niger | Profuse growth of A.Niger | Widespread growth of Fusarium with 3 colonies of A. Niger | Growth of Fusarium with contamination | Growth of Colonies | Apparent bacterial colony | Profuse growth of A.Niger |
| **5** | A. Niger continue show growth | A. Niger continue show growth | A. Niger continue show growth | Continued growth of Fusarium with contamination of A. Niger | Continued growth of Fusarium with contamination of A. Niger | Cladosporium & A. Niger having an antagonistic relationship | Bacterial Colony visible with contamination | Bacterial Colony visible with contamination |
|  |  |  |  |  |  |  |  |  |

**Table 5:**

**Table 6:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Petriplate** | **Petriplate** | **Slant test tube** | **Slant test tube** | **Slant test tube** | **Slant test tube** |
| **Volume** | **30ml** | **30ml** | **18ml** | **18ml** | **18ml** | **18ml** |
| **Day** | **Eschericia coli on Bhaktivedanta medium2** | **Eschericia coli on Bhaktivedanta medium3** | **Eschericia coli on Bhaktivedanta medium4** | **Pseudomonas on Bhaktivedanta medium4** | **Staphylococcus on Bhaktivedanta medium4** | **Gram positive bacilli on Bhaktivedanta medium4** |
| **1** | - | - | - | - | - | - |
| **2** | mild growth | mild growth | mild growth | - | - | - |
| **3** | Profuse growth | Profuse growth | mild growth | mild growh | mild growh | mild growh |
| **4** | Profuse growth | Profuse growth | mild growth | mild growh | mild growh | mild growh |

**Result:**  Aspergillus niger, Eschericia Coli, Pseudomonas, staphylococcus, trichoderma, fusarium, and cladosporium can be grown on 100% vegetarian media without peptone and beef extract using soyabean as nitrogen source.

**References:**

<http://www.academia.edu/4980897/Optimization_of_an_effective_growth_medium_for_culturing_probiotic_bacteria_for_applications_in_strict_vegetarian_food_products>

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